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MECHANISMS OF CENTRAL PRESSOR AND TACHYCARDIC EFFECTS OF PROSTAGLANDIN E_2 IN CONSCIOUS SHEEP

By

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ABSTRACT

MECHANISMS OF CENTRAL PRESSOR AND TACHYCARDIC EFFECTS OF PROSTAGLANDIN E_2 IN CONSCIOUS SHEEP

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Conscious, chronically-instrumented, mature female sheep were used to investigate the central cardiovascular effects of prostaglandin E_2 (PGE_2). Although PGE_2 is a potent vasodilator in the periphery, intracarotid (IC) administration of PGE_2, 10 ng/kg/min, causes blood pressure and heart rate to increase. These effects of IC PGE_2 are not due to direct action of PGE_2 at carotid sinus baroreceptors. The increase in blood pressure is caused by an increase in alpha-adrenergic tone in the periphery (Hull and Chimoskey 1984). The increase in heart rate is caused by a combination of cardiac beta-adrenergic activation and withdrawal of parasympathetic tone to the heart. In conscious sheep, IC PGE_2 (10 ng/kg/min or 100 ng/kg/min) has no effect on arterial P_{CO_2} or plasma renin activity (Hull and Chimoskey 1984), plasma osmolality, or plasma concentrations of sodium, epinephrine, norepinephrine, or vasopressin. Packed cell volume (PCV) is elevated by 100 ng/kg/min IC PGE_2, but not by 10 ng/kg/min IC PGE_2.

When PGE_2 is given into the lateral cerebral ventricles of conscious sheep, the maximum increase in blood pressure which can be obtained at any dose is less than that produced by 10 ng/kg/min IC PGE_2, and heart

rate is unchanged. The increase in blood pressure can be prevented by pretreatment with phentolamine in the presence of propranolol. Despite its less potent cardiovascular action, intracerebroventricular (IVT) PGE_2 has certain effects that are not observed during IC administration of PGE_2. These include pupillary constriction, vocalization, and production of a dry, hacking cough. At 300 ng/kg/min, IVT PGE_2 increases PCV and plasma vasopressin concentration, and decreases plasma sodium concentration. Plasma osmolality and catecholamines are unchanged.

Intracerebroventricular angiotensin II (AII), 50 ng/kg/min, increases blood pressure, total peripheral resistance, right atrial pressure, stroke volume, and plasma vasopressin concentration in conscious sheep. Blockade of the brain renin-angiotensin system with $[Sar^1Thr^8]AII$ (sarthran), 1000 ng/kg/min IVT, does not prevent the pressor responses to IC PGE_2 or IVT PGE_2. Conversely, prostaglandin synthesis blockade with indomethacin or flunixin meglumine does not alter the pressor response to IVT AII. Thus, central PGE_2 and AII act independently to increase blood pressure in conscious sheep.

Prostaglandin E_2 may participate in cardiovascular regulation through action within the central baroreflex pathway. Endogenous PGE_2 probably comes from cerebral vascular endothelial cells or is made locally in neural tissue; it is unlikely that it comes from cerebral spinal fluid.

To GUS

For whom I was too busy

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I. GENERAL INTRODUCTION

When given into the cerebral blood supply or into cerebral spinal fluid, prostaglandins of the E series (PGEs) cause blood pressure and heart rate to increase (Kaplan et al. 1969; Gyang et al. 1973; Andersson and Leksell 1975; Leksell 1976; Hoffman and Schmid 1979; Hoffman and Valigura 1979; Kondo et al. 1979; Hoffman et al. 1981; Skarnes et al. 1981; Takahashi and Bunag 1981b; Okuno et al. 1982; Wu and Wei 1982; Breuhaus and Chimoskey 1983; Hull and Chimoskey 1984). This observation has attracted attention for at least two reasons. First, the ability of PGEs to raise blood pressure is paradoxical, since PGEs are potent vasodilators when infused into the arterial inflow of most peripheral vascular beds (Moncada et al. 1980). Second, the increase in blood pressure is accompanied by tachycardia, not bradycardia, suggesting some form of interference with the baroreflex. Peripheral mechanisms by which centrally-administered PGEs raise blood pressure have been studied in a variety of species and will be discussed in the general literature review which follows.

This thesis extends what is known about peripheral changes in response to centrally-administered PGE_2 in conscious chronically-instrumented sheep, and then focuses on possible central mechanisms of the PGE_2 pressor response. The questions answered are divided by chapters, outlined below. Each chapter has its own introduction which reviews literature specific to that subject, a methods section which builds on methods introduced in previous chapters, a results section,

and a discussion specific to those results. A general discussion of the possible significance of PGE_2 in arterial pressure regulation is included in the summary and general conclusions.

Chapter 1 examines whether intracarotid PGE_2 increases blood pressure by direct action at carotid sinus baroreceptors.

Chapter 2 examines whether withdrawal of parasympathetic tone to the heart contributes to the increase in heart rate caused by intracarotid infusion of PGE_2.

Chapter 3 discusses development of a method of chronic catheterization of the cerebral ventricles for repeated infusions in conscious sheep and the use of a drinking response to intracerebroventricular (IVT) infusion of angiotensin II to verify proper catheter placement premortem. Changes in blood pressure, heart rate, plasma vasopressin and sodium concentrations, plasma osmolality, and packed cell volume during infusions of angiotensin II also are reported.

Chapter 4 compares hemodynamic and hormonal responses to intracarotid (IC) and IVT PGE_2.

Chapter 5 examines whether centally-administered PGE_2 raises blood pressure by causing central release of angiotensin II or whether centrally-administered angiotensin II raises blood pressure by stimulating central prostaglandin biosynthesis.

II. GENERAL LITERATURE REVIEW

A. Discovery of PGE

Prostaglandins were discovered by Goldblatt (1933, 1935) and von Euler (1936) in fluid from male accesory sex glands. Prostaglandin E was first isolated by Bergström and Sjövall (1960), and its structure was identified by Bergström, Ryhage, Samuelsson, and Sjövall in 1962. Prostaglandins are unsaturated fatty acid autacoids formed from arachidonic acid by a series of enzymatic reactions. The types of prostaglandins produced by a given tissue are determined by the terminal enzymes present in the tissue. Since most prostaglandins have very short half lives (on the order of seconds to minutes) they are not stored, but are produced as needed. Prostaglandins of the E series are metabolized rapidly by the lungs (Ferreira and Vane 1967; Moncada et al. 1980), making it possible to separate peripheral effects of PGE from central effects.

B. Pertinent Peripheral Actions of PGE

1. Effects on Vascular Smooth Muscle

Although there are some exceptions (primarily in the rat), in most species and in most vascular beds PGEs relax vascular smooth muscle. Bergström et al. (1964, 1965) and Carlson and Oro (1966) were the first to report that peripherally-administered PGE_1 decreases blood pressure and increases heart rate in man and in dog. The hypotensive

action of PGE_1 in the periphery was greater when given intra-arterially than when given intravenously.

In anesthetized dogs, Nakano and McCurdy (1967) showed that the tachycardia produced by intravenous PGE_1 is caused by reflex stimulation of the sympathetic nervous system in response to the decrease in blood pressure. Administration of PGE_1 into the arterial inflow of a variety of vascular beds increased flow in those beds without changing systemic blood pressure. These investigators concluded that PGE_1 lowers blood pressure by peripheral vasodilation caused by a direct action of PGE_1 on vascular smooth muscle. The depressor effect of PGE_1 is not prevented by atropine (von Euler 1936; Bergström et al. 1959), antihistamine (Bergström et al. 1959), propranolol (Carlson and Oro 1966; Nakano and Kusakari 1966), reserpine, or ganglionic blockade (Carlson and Oro 1966). In vitro studies have confirmed a direct relaxant action of PGEs on vascular smooth muscle (Strong and Bohr 1967).

In addition to their direct action to relax vascular smooth muscle, PGEs also affect vascular smooth muscle tone by modulating adrenergic activity. Although there is some variation by species and by tissue, in most tissues studied PGEs inhibit adrenergic neurotransmission by acting presynaptically to decrease norepinephrine release (Hedqvist 1970, 1976; Kadowitz 1972; Bergström et al. 1973; Frame and Hedqvist 1975; Malik and McGiff 1975; Malik 1978; Hillier and Templeton 1980). Prostaglandins of the E series also may act postsynaptically to attenuate pressor responses to infused norepinephrine (McGiff et al. 1972; Okuno et al. 1982).

2. Renal Effects

Acute intrarenal infusion of PGE_2 increases renal blood flow without changing systemic blood pressure, increases urine flow, and increases sodium excretion (Lee 1973). Chronic (7 days) intrarenal administration of PGE_2 in conscious unilaterally-nephrectomized dogs on a fixed sodium intake increases urine output and water intake without changing glomerular filtration rate, increases blood pressure, and increases sodium excretion despite increased plasma renin activity and increased plasma aldosterone concentration. The increase in blood pressure can be reversed by blockade of the renin-angiotensin system (Hockel and Cowley 1979, 1980).

There are several mechanisms by which renal PGE_2 increases sodium excretion. Prostaglandin E_2 increases renal sodium excretion directly by acting on renal tubular cells to inhibit sodium reabsorption (Kauker 1977; Stokes and Kokko 1977). Prostaglandin E_2 increases renal sodium excretion indirectly by changing intrarenal blood flow distribution (Feigen et al. 1976), by interfering with vasopressin action on the distal nephron (Grantham and Orloff 1968; Fulgraff and Brandenbush 1974) and by interactions with the renin-angiotensin and kallikrein-kinin systems (Mills et al. 1978).

C. Central Actions of PGE

1. Behavior

The first report of prostaglandins acting in the central nervous system appeared in 1964. Horton found that PGEs injected into the lateral cerebral ventricles of conscious cats cause sedation and stupor lasting as long as 24 hours and signs of catatonia lasting up to 4

hours. Moderate pupillary dilation occurred, although pupillary reflexes were normal. The doses of PGE used in this study were high (7-10 ug/kg). Intravenous injection of PGE into conscious chicks (10-400 ug/kg) also caused profound sedation. At the higher doses the chicks lost righting reflexes. Horton believed that these results in chicks were caused by a central action of PGEs, since the blood-brain barrier is not yet developed in chicks of that age.

2. Fever

Prostaglandins of the E series, but not PGAs or PGFs, cause hyperthermia accompanied by shivering, skin vasoconstriction, and piloerection when injected into the cerebral ventricles of conscious cats and rabbits (Milton and Wendlandt 1970 and 1971). The doses of PGEs used in these studies (10 ng - 10 ug) were lower than those used by Horton (1964). Signs of catatonia were not observed, although some of the cats were unresponsive to external stimuli and appeared stuporous while shivering vigorously. Body temperature began to rise within minutes after injection of PGE and this increase was not prevented by the cyclo-oxygenase inhibitor paracetamol. Similar results were observed in sheep by Hales et al. (1973). One hundred ug of PGE_1 or PGE_2 injected into the lateral ventricles of sheep exposed to cool, thermoneutral, or warm environments increased rectal temperature 0.5-1.2^{o} C. Shivering occurred in cool and thermoneutral environments. Panting was decreased in warm environments. Prostaglandins of the F series were less potent.

The studies of Milton and Wendlandt described above also provided early evidence for a role of endogenous prostaglandins in the production

of fever. In some experiments there was a late rise in body temperature within hours after injection of any substance tested (including isotonic saline). This late rise was prevented or reversed with paracetamol. Dey et al. (1974) confirmed that fevers which occur nonspecifically after perfusion of cat cerebral ventricles with various salt solutions are abolished by paracetamol or indomethacin, and are accompanied by increased PGE-like activity in cerebrospinal fluid.

The role of PGE as the mediator of fever produced by bacterial pyrogen or endotoxin has been studied in a variety of species (Feldberg and Gupta 1973; Feldberg et al. 1973; Feldberg and Milton 1973; Myers et al. 1974; Phillip-Dormston and Siegert 1974; Veale and Cooper 1974; Feldberg 1975; Skarnes and McCracken 1980; and Skarnes et al. 1981). Intracerebroventricular injection of endotoxin in conscious sheep produces a biphasic fever response (Skarnes et al. 1981). The initial, but not the secondary, rise in body temperature is preceded by an increase in systemic blood pressure accompanied by increased concentrations of PGE and PGF in jugular venous and carotid arterial blood. Pretreatment with indomethacin prevents the initial pressor effect of endotoxin as well as both phases of the fever response. In that same study, intracarotid infusions of PGE_2, 20–55 ng/kg/min, raised blood pressure (10–25% beginning 1–3 minutes after the start of infusion) and caused monophasic fevers (0.9–1.2^{o} C. beginning 3–5 minutes after the start of infusion).

3. Blood Pressure

The first evidence of a central pressor effect of PGE was reported by Kaplan et al. in 1969. Using cross-circulation procedures,

these investigators found that intracarotid PGE_1 increases blood pressure in neurally-intact vascularly-isolated trunks of recipient dogs with denervated carotid sinuses and carotid bodies. This increase in blood pressure was prevented by administration of the ganglionic blocking drug hexamethonium into the recipient dog's trunk. Blood pressure decreased in response to intracarotid PGE_1 in the donor dog. Blood pressure also decreased in recipient dog trunks with intact carotid sinuses and carotid bodies. The authors concluded that, in addition to its depressor action on vascular smooth muscle, PGE_1 acts in the brain to increase blood pressure and acts at the carotid sinus to decrease blood pressure.

Central pressor effects of PGE_1 since have been demonstrated by infusion into the vertebral arteries of chloralose-anesthetized cats (Gyang et al. 1973), by administration into cerebral spinal fluid of conscious goats (Andersson and Leksell 1975; Leksell 1976), and by intracarotid infusion in urethane-anesthetized rats (Rinchuse and Deubin 1976). More recently, PGE_2 also has been shown to cause centrally-mediated increases in blood pressure when given into cerebral spinal fluid of conscious rats (Hoffman and Schmid 1979; Kondo et al. 1979; Hoffman et al. 1981; Takahashi and Bunag 1981b; Okuno et al. 1982; Wu and Wei 1982) or into carotid blood of conscious sheep (Skarnes et al. 1981; Breuhaus and Chimoskey 1983; Hull and Chimoskey 1984), dogs, and calves (Hull and Chimoskey 1984). Note that pressor effects of centrally-administered PGE_2 have been demonstrated only in conscious animals; anesthesia inhibits this response (Vilhardt and Hedqvist 1970; Yamamoto et al. 1976; Takahashi and Bunag 1981b; Hull and Chimoskey 1984).

One exception to this last statement must be mentioned, since it is the only study in which PGE_2 has been injected into discrete hypothalamic nuclei. In this study (Feurstein et al. 1982), rats were prepared surgically 3-4 days before use with guide cannulae either into a lateral cerebral ventricle, into the dorsomedial nucleus of the hypothalamus, or into the posterior hypothalamic nucleus. On the day of the experiment the rats were anesthetized with halothane and oxygen only, and during injections of PGE_2 they were maintained on 0.8% halothane, which is below the concentration of halothane required for surgical anesthesia (Minimal Alveolar Concentration, MAC, for halothane is 0.95-1.1%: Eger 1974). Control blood pressures in these rats were not high (approximately 90 mmHg). Intracerebroventricular or intrahypothalamic injections of PGE_2 in these rats caused increases in blood pressure and heart rate.

Since centrally-administered PGEs cause fever, it is possible that their pressor effect is simply a by-product of peripheral vasoconstriction produced as part of the febrile response. It also is possible that the increase in body temperature results from or is augmented by an independent effect of PGE on central cardiovascular centers to increase total peripheral resistance. In studies designed to disassociate the blood pressure and body temperature responses to intracerebroventricular PGE_2 in conscious rats, Hoffman and Valigura (1979) proved that the PGE_2-induced increase in body temperature is not caused by peripheral vasoconstriction. Pretreatment with phenoxybenzamine prevented the pressor response but not the febrile response to PGE_2. The reverse study has not been done.

Although centrally-administered PGE_2 does cause a small increase in

cardiac output, the pressor response is mainly caused by an increase in total peripheral resistance (Breuhaus and Chimoskey 1983; Hull and Chimoskey 1984) mediated by increased sympathetic nerve activity (Takahashi and Bunag 1981b), and can be inhibited or reversed by alpha-adrenoceptor blockade (Hoffman and Schmid 1979; Okuno et al. 1982; Hull and Chimoskey 1984) or cervical section of the spinal cord (Takahashi and Bunag 1981b). Decreased blood flow in renal, mesenteric, and iliac beds contributes to the increase in total peripheral resistance (Hull and Chimoskey 1984).

The central pressor effect of PGE_2 is accompanied by tachycardia (Hoffman and Schmid 1979; Hoffman et al. 1981; Takahashi and Bunag 1981b; Feurstein et al. 1982; Okuno et al. 1982; Breuhaus and Chimoskey 1983; Hull and Chimoskey 1984), not bradycardia, suggesting that the baroreflex's ability to control blood pressure is interfered with. Hull and Chimoskey (1984) have shown that, over a limited range of blood pressure, the baroreflex is reset upward with no change in sensitivity. Further studies on the effect of PGE_2 on the baroreflex will be discussed in Chapter 1.

The PGE_2-induced tachycardia is inhibited, but not always abolished, by the beta-adrenoceptor antagonist propranolol (Hoffman and Schmid 1979; Hull and Chimoskey 1984). The increase in heart rate which remains in calves and sheep after adrenergic blockade may be caused by parasympathetic withdrawal; atropine can attenuate heart rate and blood pressure responses to intracerebroventricular PGE_2 in rats (Hoffman and Schmid 1979). This possibility will be examined in Chapter 2.

4. Circulating Hormones / Body Fluid Regulation

The response of circulating catecholamines to central administration of PGE_2 has been measured only in rats. In one study, peak plasma norepinephrine increased approximately 2.5X after intracerebroventricular or intrahypothalamic PGE_2. (Control values ranged from 186 to 294 pg/ml.) Plasma epinephrine did not change significantly (Feurstein et al. 1982).

In addition to measuring circulating catecholamines before and during intracerebroventricular injection of PGE_2 in conscious rats, Okuno et al. (1982) also assessed the effect of bilateral adrenalmedullectomy on the PGE_2 response. Control values of plasma norepinephrine (356 ± 40 pg/ml) and epinephrine (413 ± 90 pg/ml) in this study were slightly higher than those reported by Feurstein et al. (1982). Intracerebroventricular PGE_2 in intact rats increased plasma norepinephrine 2X and plasma epinehrine 2.6X. After adrenalmedullectomy, control and PGE_2-induced plasma norepinephrine levels were similar to those found before adrenalmedullectomy. Plasma epinephrine concentration decreased to 19 ± 6.6 pg/ml after adrenalmedullectomy, and did not change in response to intracerebroventricular PGE_2. Bilateral adrenalmedullectomy also prevented a PGE_2-induced rise in plasma renin activity (to be discussed next). Loss of epinephrine and renin responses to PGE_2 in these rats, however, had no effect on PGE_2-induced pressor responses. Whether there was any effect on the heart rate response was not discussed.

As alluded to above, centrally-administered PGE_2 increases plasma renin activity (Okuno et al. 1982; Hull and Chimoskey 1984), probably by renal beta-adrenoceptor stimulation from circulating epinephrine.

Bilateral renal denervation did not affect the renin response to centrally-administered PGE_2, propranolol attenuated it, and bilateral adrenalmedullectomy (which prevented the increase in plasma epinephrine) prevented the renin response (Okuno et al. 1982). Although this increase in plasma renin activity may influence long-term blood pressure control through fluid balance mechanisms, it does not mediate the acute pressor effect of centrally-administered PGE_2, since intravenous pretreatment with the angiotensin-converting enzyme inhibitor captopril does not change the PGE_2-induced increase in blood pressure in rats (Okuno et al. 1982) or in sheep (Hull and Chimoskey 1984).

Unlike changes in plasma renin activity, changes in plasma vasopressin concentration may participate in the pressor response to centrally-administered PGE_2. Hypophysectomy halved the pressor response to intracerebroventricular PGE_2 in rats (Takahashi and Bunag 1981b) and the vasopressin antagonist $d(CH_2)_5Tyr(Me)AVP$ attenuated this response (Okuno et al. 1982). In the latter study, alpha-adrenoceptor blockade alone only attenuated the PGE_2-induced pressor response; the combination of both vasopressin blockade and alpha-adrenoceptor blockade were necessary to abolish it. If vasopressin is released during intracarotid infusion of PGE_2 in conscious sheep or calves, it must not participate in the pressor responses, since adrenergic blockade alone completely abolishes the increases in blood pressure.

Early evidence for the release of vasopressin in response to centrally-administered PGEs was reported before vasopressin could be detected by radioimmunoassay. As early as 1970 Vilhardt and Hedqvist showed that intracarotid PGE_2 decreases urine volume and increases urine osmolality without changing blood pressure in anesthetized, water-loaded

rats. Hoffman and Schmid (1979) also reported an antidiuretic effect of intracerebroventricular PGE_2 in conscious water-loaded rats. Median emminence lesions abolished the PGE_2-induced antidiuretic responses without changing the cardiovascular responses.

In <u>in vitro</u> studies, Gagnon et al. (1973) demonstrated that PGE_2 can stimulate vasopressin release from isolated rat neurohypopheses. Vasopressin concentration was determined in this study by bioassay; blood pressure responses to extracts of the hypophyseal bathing medium were compared to pressor responses to synthetic vasopressin in anesthetized assay rats.

Rat bioassay also was used to measure increases in urinary concentration of vasopressin in response to intracerebroventricular infusions of PGE_1 in conscious, water-loaded goats (Leksell 1976). In this and in an earlier study (Andersson and Leksell 1975) PGE_1 also decreased urinary free water clearance, increased urinary sodium excretion, increased blood pressure, and weakly stimulated drinking behavior. Body temperature did not change.

Yamamoto et al. (1976) used radioimmunoassay to measure changes in plasma vasopressin concentration in response to ventriculocisternal perfusion with PGE_2 at two different rates in morphine-chloralose-urethane anesthetized dogs. Plasma vasopressin was unchanged at the lower dose. At the higher dose (152.6 ng/min or approximately 8-15 ng/kg/min), plasma vasopressin concentration doubled by ten minutes after the start of infusion and was increased 4.5X by thirty minutes after the start of infusion. There were no significant changes in arterial pressure, rectal temperature, plasma sodium or potassium concentrations, or plasma osmolality. Hematocrit decreased 9%, but a

similar decrease with time also occurred during vehicle perfusion. Despite the increase in plasma vasopressin concentration, there also were no significant changes in urinary volume, osmolar and free water clearances, or urinary excretion of sodium or potassium. Some of the negative results in this study may have been caused by anesthesia. Nevertheless, it should be noted that in this and in previous studies cited, it is possible for PGE_2 to increase plasma vasopressin concentration without reaching levels high enough to have a pressor effect.

In contrast to the last study, the contribution of vasopressin release to the pressor response of intracerebroventricular injections of PGE_2 in conscious rats has been discussed previously (Takahasi and Bunag 1981b; Okuno et al. 1982). Okuno et al. also measured plasma vasopressin concentrations by radioimmunoassay. Plasma vasopressin increased 11.6X from 2.5 $\pm$ 0.5 pg/ml to 29 $\pm$ 8 pg/ml. Plasma osmolality did not change.

D. Localization and Synthesis of PGE in Brain Tissue

1. PGE Stimulation of Central Neurons

Avanzino et al. (1966) examined the effects of prostaglandins on brain stem neurons from the medulla and caudal pons (0-7 mm rostral to the obex and within 3 mm from the midline) of decerebrate, unanesthetized cats. Applied by iontophoresis, PGE_1 excited 18.5% and inhibited 4% of the 341 spontaneously-firing neurons tested. Prostaglandin E_2 excited 27.5% of 69 neurons tested, but had no inhibitory effect. Although no attempt was made to correlate spontaneous nerve firing to body function, it is possible (due to the location of the neurons

tested) that at least some of them could have been involved in cardiovascular regulation. In any event, the study shows that PGEs can stimulate central neurons.

2. Endogenous Formation of Prostaglandins by Central Nervous Tissue

In order for endogenous PGEs to influence central cardiovascular regulation, they must be synthesized by brain tissue, by vascular tissue supplying the brain, or by blood cells, since prostaglandins are not stored and since PGEs are removed rapidly from blood and metabolized by the lungs (Moncada et al. 1980). In early in situ studies, perfusion fluid from cat cerebral cortex (Ramwell and Shaw 1966) and from frog spinal cord (Ramwell et al. 1966) contained material which stimulated smooth muscle and which was identified by thin-layer chromatography as a mixture of PGE and PGF. Direct electrical and peripheral nerve stimulation increased the release of this active material.

In vitro measurements of prostaglandins in various brain tissue preparations have not yielded consistent results, both in terms of absolute concentrations measured and in terms of distribution. This is probably due to many things, including variations in species, tissue collection and preparation, and assay techniques. All studies agree, however, that PGEs are present in central nervous tissue. Several of these studies are discussed below.

The concentration of PGF_{2alpha} in cat forebrain (rostral to the intercollicular level), whole chicken brain, and chicken spinal cord was approximately 10 ng/g wet weight tissue in one study (Horton and Main 1967). By comparison, PGE (probably PGE_1) was relatively low in cat forebrain (5 ng/g), but PGE_2 was relatively high in chicken brain and

spinal cord (100 ng/g and 400 ng/g respectively). The authors suggested that uneven distribution of prostaglandins in the central nervous system might indicate that prostaglandins have a role in the specializedfunction of the region where their concentrations are highest.

To determine the distribution of prostaglandins in the brain, Holmes and Horton (1968b) measured prostaglandins in homogenates of dog cortex, hippocampus, caudate nucleus, hypothalamus, cerebellum, medulla and pons, cortical white matter, and spinal cord. In whole brain, the following concentrations were found: PGE_1 35 ng/g, PGE_2 62 ng/g, PGF_{1alpha} 63 ng/g, and PGF_{2alpha} 56 ng/g. (All values are uncorrected for recoveries, which probably were somewhere between 65 and 80%.) All four prostaglandins also were found in approximately equal concentrations in all regions of the central nervous system tested separately, although the medulla and pons and the cortical white matter tended to contain less PGE.

Prostaglandins E_2 and F_{2alpha} also have been isolated from slices of rat cerebral cortex (Wolfe et al. 1976a and 1976b). These investigators showed that the trauma involved in collecting the brain tissue to be analyzed stimulates prostaglandin synthesis. When whole cerebral hemispheres from rats were frozen immediately in liquid nitrogen, levels of PGF_{2alpha} were very low (approximately 0.1 ng/g tissue) and PGE_2 was undetectable. Merely slicing the cortex increased prostaglandin synthesis approximately 8X. In incubated slices of cortex, synthesis of PGE and PGF increased almost linearly with respect to time for the first 60 minutes, and then decreased slightly. The ratio of PGF_{2alpha} formation to PGE_2 formation was 3.5. In incubated homogenates of

cortex, PGF_{2alpha} synthesis reached a maximum in 60 minutes, whereas PGE_2 formation peaked in 15 minutes. Formation of PGE_2 was much more rapid, and relatively more PGE_2 was formed, in homogenates than in slices; the ratio of PGF_{2alpha} to PGE_2 in homogenates was 2.1. Steady-state values of prostaglandin synthesis in homogenates and slices were on the order of 2-3 ng/g initial tissue weight for PGE_2 and 6-8 ng/g for PGF_{2alpha}.

Wolfe et al. (1976a and 1976b) also reported that prostaglandin synthesis in cat cortex is 3X greater than in rat cortex and that synthesis in human cortex is 2/3 less than in rat cortex. In all three species, synthesis of PGF_{2alpha} was greater than that of PGE_2, except in cat cerebellum. These investigators showed, however, that although brain tissue does not catabolize tritiated PGF_{2alpha} to any great extent (<10%), it can convert PGE_2 to PGF_{2alpha}, suggesting that it has PGE 9-keto reductase activity. Although this transformation may account for part of the preponderance of PGF_{2alpha} compared to PGE_2 in brain tissue, the authors considered this unlikely to be significant at the low level of prostaglandin synthesis occurring spontaneously.

Abdel-Halim and Änggård (1979) confirmed that brain tissue does not catabolize prostaglandins, but they found no evidence for interconversion. In contrast to the suggestion of Horton and Main that prostaglandin synthesis may vary in different regions of the brain, they showed that, although total amounts of prostaglandins varied, the relative amounts of prostaglandins in various brain regions studied (cortex, cerebellum, hippocampus, striatum, pons-medulla, amd nucleus accumbens) were constant. Relative prostaglandin concentrations varied by species. The main prostaglandin(s) produced by species were: rat- PGD_2, guinea

pig- PGD_2 and PGF_{2alpha}, rabbit- PGF_{2alpha}, and cat- PGE_2.

In a more recent study, Abdel-Halim et al. (1980) measured prostaglandin synthesis in homogenates of mouse, rat, and rabbit whole brain, cerebral cortex, and cerebral blood vessels (choroid plexus and internal cerebral vessels). After thirty minutes of incubation, PGE_2, PGF_{2alpha}, PGD_2, and 6-keto-PGF_{1alpha} (the stable metabolite of PGI_2) were detected in whole brains of all three species. In rat and mouse brain PGD_2 predominated (approximately 5 ng/g tissue), with levels of PGF_{2alpha}, and PGE_2 slightly lower. In rabbit brain PGF_{2alpha} (7.5 ng/g) and PGE_2 (6 ng/g) predominated. In rat cerebral blood vessels 6-keto-PGF_{1alpha} was by far the most abundant prostaglandin formed (15 ng/g). Prostaglandin F_{2alpha} also was formed, but PGE_2 and PGD_2 were below the level of detection.

In contrast to the study described above, Hagen et al. (1979) showed that exogenous administration of labelled arachidonic acid to bovine cerebral arteries results in synthesis of PGE_2, PGF_{2alpha}, and 6-keto-PGF_{1alpha} in approximately equal amounts. Differences in results between the two studies may be due to differences in species or in size of blood vessels incubated, or may reflect differences in spontaneous vs. stimulated production of prostaglandins. In this same study, unstimulated production of PGE_2 and PGF_{2alpha} also was measured and found to be equal, but unstimulated production of 6-keto-PGF_{1alpha} was not measured.

Quantitative interpretation of prostaglandin synthesis results after administration of exogenous precursors may be unreliable for several reasons. In the kidney, the products resulting from stimulation of prostaglandin synthesis by exogenously-administered arachidonic acid

differ from those produced in response to endogenous arachidonic acid release. Exogenous arachidonic acid causes formation of both PGE_2 and PGI_2, whereas endogenous arachidonic acid released by bradykinin forms only PGE_2 (Blasingham and Nasjletti 1979). Failure to isolate labelled prostaglandins in brain tissue after administration of labelled arachidonic acid (Coceani and Wolfe 1965; Pace-Asciak and Nashat 1976) has been attributed to dilution of the labelled arachidonic acid in a large pool of unlabelled endogenous arachidonic acid (Pace-Asciak 1976; Wolfe et al. 1976a, 1976b). As described above, Hagen et al. (1979) were able to detect labelled prostaglandins in extracts of incubation medium from cerebral arteries preincubated with labelled arachidonic acid, but most of the radioactivity measured was still associated with arachidonic acid; radioactivity associated with individual labelled prostaglandins was only about 5% of the total radioactivity for each prostaglandin. These investigators made no attempt to identify radioactive products within the tissue itself. Thus, interpretation of relative amounts of prostaglandins measured is still difficult. Similarly, the relative amounts of PGE to PGF formation in different areas of the brain reported by Pace-Asciak and Nashet (1976), in which the labelled endperoxides PGG_2 and PGH_2 were added to homogenates of rat brain, are opposite to the results of Wolfe et al., described previously. In the studies reported by Pace-Asciak and Nashet, relatively more PGE was formed by cerebral hemispheres than by whole brain, and relatively more PGF_{2alpha} was formed by cerebellum than by whole brain.

A variety of substances have been shown to stimulate synthesis of prostaglandins by brain tissue, most notably biogenic amines, including

norepinephrine, dopamine, adrenochrome, L-dopa, and 5-hydroxytryptamine. These substances mainly stimulate PGF_{2alpha} production, however, not PGE_2 (Ramwell et al. 1966; Wolfe et al. 1976a, 1976b). Analeptics also stimulate prostaglandin release from cat cerebral cortex (Ramwell and Shaw 1966) and ADP enhances formation of PGE_1 in rat brain (Abdull and McFarland 1972). Adenosine triphosphate has been shown to inhibit PGE_3 production (Abdull and McFarland 1972) or to have no effect on prostaglandin production (Wolfe et al. 1976a, 1976b).

Quinicrine inhibits PGE_2 formation, but not PGF_{2alpha}. Other substances which inhibit prostaglandin synthesis in brain tissue include (in order of potency) ketoprofen, indomethacin, prodilic acid, acetylsalycilic acid, paracetamol, and eicosatetraynoic acid (Wolfe et al. 1976a, 1976b).

Substances which have no effect on prostaglandin synthesis by brain tissue in vitro include reduced glutathione, hydroquinone, dibutyryl cAMP, bradykinin, histamine, morphine, and omission of glucose (Wolfe et el. 1976a, 1976b). It must be pointed out that these results were obtained in vitro and may not reflect the situation in vivo where availability of arachidonic acid is limited by other factors. This would be especially true for substances which alter prostaglandin synthesis by action on phosphlipase A activity and arachidonic acid release rather than by action on cyclo-oxygenase activity.

In summary, it is safe to say that PGD_2, PGE_2, PGF_{2alpha}, and 6-keto-PGF_{1alpha} (PGI_2) are synthesized by central nervous tissue. Six-keto-PGF_{1alpha} production is predominant in cerebral vasculature and choroid plexus. The actual concentrations of prostaglandins in brain tissue in vivo are probably much lower than the concentrations measured,

since trauma activates prostaglandin synthesis. Central nervous tissue does not catabolize prostaglandins; they probably are removed from the brain by venous blood and catabolized in the lungs.

3. Transport of Exogenous PGE into Central Nervous Tissue

In order for exogenously-administered PGE to affect neural function, it must get out of blood or cerebral spinal fluid and into brain tissue. Holmes and Horton (1968a) showed that, when tritiated PGE_1 is injected into cerebral spinal fluid or into cerebral blood at doses high enough to produce central nervous signs in cats and chicks, very little labelled PGE_1 is found in brain or spinal cord. When PGE_1 was injected into the lateral ventricles of cats, the largest amount of radioactivity was found in the liver, and this radioactivity no longer was associated with PGE_1, but probably with a metabolite. The kidneys and cerebral spinal fluid contained a moderate amount of radioactivity. The brain contained less than 5% of the injected radioactivity and this was distributed throughout, although there consistently was more radioactivity found in the midbrain, cerebellum, medulla, and pons. When 10 ug of tritiated PGE_1 was injected either into a vertebral artery or into a carotid artery of anesthetized cats, no area of the brain examined (forebrain, midbrain, cerebellum, or medulla and pons) contained radioactivity equivalent to as much as 0.1 ug PGE_1. Twenty-five percent of the injected activity was measured in venous blood in the first three minutes after injection. Since these cats had displayed central nervous signs (stupor, catatonia) before death, Holmes and Horton concluded that prostaglandins are active at extremely low concentrations.

The results reported by Holmes and Horton (1968a) are reasonable in view of present knowledge of prostaglandin transport and mechanisms of action. Bito (1972) has confirmed that brain tissue does not accumulate prostaglandins readily. Cell membranes in general are impermeable to prostaglandins, and uptake into cells is by carrier-mediated transport. In some tissues this may be passive (facillitated), but in some it is definitely active (Bito 1972; Bito and Baroody 1974; Bito 1975a, 1975b; Bito and Baroody 1975; Bito, Davson, and Hollingsworth 1976; Bito, Davson, and Salvador 1976; Bito, Baroody, and Reitz 1977; Syrota et al. 1982).

Choroid plexus has a great affinity for prostaglandins and can accumulate them against a concentration gradient (see references above). Bito has suggested that the choroid plexus acts as a sponge to absorb prostaglandins in order to protect the brain from potentially-damaging high levels of them (Bito 1972, 1975b; Bito et al. 1976). Presumably, then, the choroid plexus releases the prostaglandins into venous blood, in which they are returned to the lungs to be metabolized. This also requires transport of prostaglandins across the vascular endothelial membrane (Bito and Baroody 1975; Bito et al. 1977; Syrota et al. 1982). Thus, it is likely that most of the exogenous prostaglandins given into cerebral spinal fluid or into the cerebral arterial inflow are removed by cerebral venous blood, are metabolized by the lungs, and are excreted by the liver and kidneys.

In summary, exogenous prostaglandins must get out of cerebral capillary blood or cerebral spinal fluid and into brain extracellular fluid to act on neurons. This may occur in areas of the brain where the blood-brain-barrier is leaky or in areas of the brain that have the

specific prostaglandin carrier. Endogenous prostaglandins may be synthesized in nerve cell membranes and released directly into the extracellular space, or they may be synthesized by vascular endothelium (Gimbrone and Alexander 1975) or formed elements in the blood and enter the brain as do exogenous prostaglndins.

Once in the extracellular space, prostaglandins could act on specific membrane receptors. In tissues where the mechanism of prostaglandin action is known, prostaglandin effects are mediated by phosphonucleotide cyclase / cyclic nucleotide systems, and activation of these systems does not require entry of prostaglandins into the cells. This does not exclude the possibility that prostaglandins also could act intracellularly in cells which can transport them.

Prostaglandins may get from the extracellular fluid to venous blood by diffusion, bulk flow, facillitated diffusion, or active transport. Catabolism of prostaglandins occurs intracellularly in lung, liver, and kidney (Bito 1975a). Thus, metabolism of prostaglandins is compartmentalized so that they are synthesized within the cell membrane, they act extracellularly, and they are degraded intracellularly.

III. CHAPTER ONE

PGE_2 DOES NOT ACT AT THE CAROTID SINUS TO RAISE ARTERIAL PRESSURE IN CONSCIOUS SHEEP

INTRODUCTION

Prostaglandin E_2 (PGE_2) is a potent vasodilator when infused peripherally (Moncada et al. 1980). However, Hull and Chimoskey (1984) have shown that, when infused midcervically into the common carotid artery of conscious dogs, calves, and sheep at a dose of 10 ng/kg/min, PGE_2 causes blood pressure and heart rate to rise. The rise in blood pressure is mainly due to an increase in total peripheral resistance and is blocked by the alpha-adrenergic blocking agent phentolamine. There is no chemoreflex, but the baroreflex is reset to a higher level. Hull and Chimoskey postulated that the pressor effect of PGE_2 is mediated by PGE_2 acting centrally to stimulate the sympathetic nervous system.

This hypothesis, that PGE_2 acts centrally, is supported by work in rats (Kondo et al. 1979; Hoffman et al. 1981; Takahashi and Bunag 1981b; Okuno et al. 1982). These investigators independently have shown that intracerebroventricular (IVT) injections of PGE_2 into conscious or anesthetized rats increases blood pressure. Takahashi and Bunag showed that peripheral sympathetic nerve activity is increased, and Okuno et al. showed that the increase in blood pressure is decreased by phenoxybenzamine, indicating an alpha-adrenergic sympathetic mechanism. Additionally, Gyang et al. (1973) showed that blood pressure increases

when PGE_1 is injected into the vertebral artery of anesthetized cats. Note that with these two routes of injection which bypass the carotid sinus (i.e.: into the vertebral artery or IVT), PGE_2 also has a pressor effect.

Thus there is strong evidence that either blood-borne PGE_2 or PGE_2 given into cerebral spinal fluid causes a centrally mediated increase in blood pressure. However, there has been no work done to determine the role of the carotid sinus in the pressor response observed when PGE_2 is infused into the common carotid arteries of conscious sheep. This study was performed to determine the role of a direct action of PGE_2 on the carotid sinus in the pressor response observed when PGE_2 is infused into the common carotid artery of conscious sheep.

METHODS

Six mature female sheep ranging in weight from 48 to 66 kg were used for these experiments. The sheep were housed individually in the laboratory and allowed to acclimate to their surroundings and laboratory personnel. This was essential in order to ensure that the sheep were calm and unstressed during the experiments.

Instrumentation

Each sheep was anesthetized with intravenous sodium thiamylal, intubated, and maintained in a surgical anesthetic plane with halothane and oxygen. It was placed in dorsal recumbency and prepared for aseptic surgery. A midline incision, centered over the rami of the mandibles, was made in the neck. The right or left carotid sinus region (four sheep right, two sheep left) was exposed by careful dissection to

avoid damaging the parotid salivary gland, which was retracted laterally, and to avoid cutting any of the major nerves in this area (cranial nerves IX, X, and XII, the sympathetic trunk, the cranial laryngeal nerve, and various pharyngeal branches).

The carotid sinus region was identified by finding the lingual and occipital arteries. A fine nerve plexus could be seen in the adventitia of the carotid artery in this area. A small nerve, thought to be the carotid sinus nerve, usually could be identified going to this area. The carotid sinus nerve was cut and the adventitia was stripped from the carotid artery 360° over a 3 cm length extending from the external carotid artery just rostral to the origin of the lingual artery and caudally past the occipital artery to an area on the common carotid artery approximately 1 cm beyond where any small nerve fibers could be seen. This adventitial stripping also was carried up the occipital artery for approximately 1 cm, where the occipital artery often branched into 3-5 very small arteries. The surrounding tissues were packed off with sponges and 10% phenol was painted on the stripped area of the carotid artery.

The external carotid artery then was dissected as far as possible until it disappeared over the stylohyoid bone, resulting in a short length of artery (approximately 1.5 cm) that could be catheterized using the technique of Herd and Barger (1964). This catheter was non-occlusive and the tip was positioned beyond the origin of the lingual artery in order to ensure that it was rostral to the carotid sinus (Figure 1).

At the caudal end of the incision, the right and left common carotid arteries were exposed 12-14 cm proximad to the carotid sinuses and

non-occlusive catheters were implanted, again using the Herd-Barger technique (1964). Hydraulic occluders (Rhodes Medical Instruments, Woodland Hills, CA) were placed around each common carotid artery upstream to each catheter. An occluder size was chosen such that in the deflated state there was a loose fit and no pressure was placed on the arterial wall. When inflated, however, the common carotid artery was totally occluded. A jugular vein catheter also was implanted in this area.

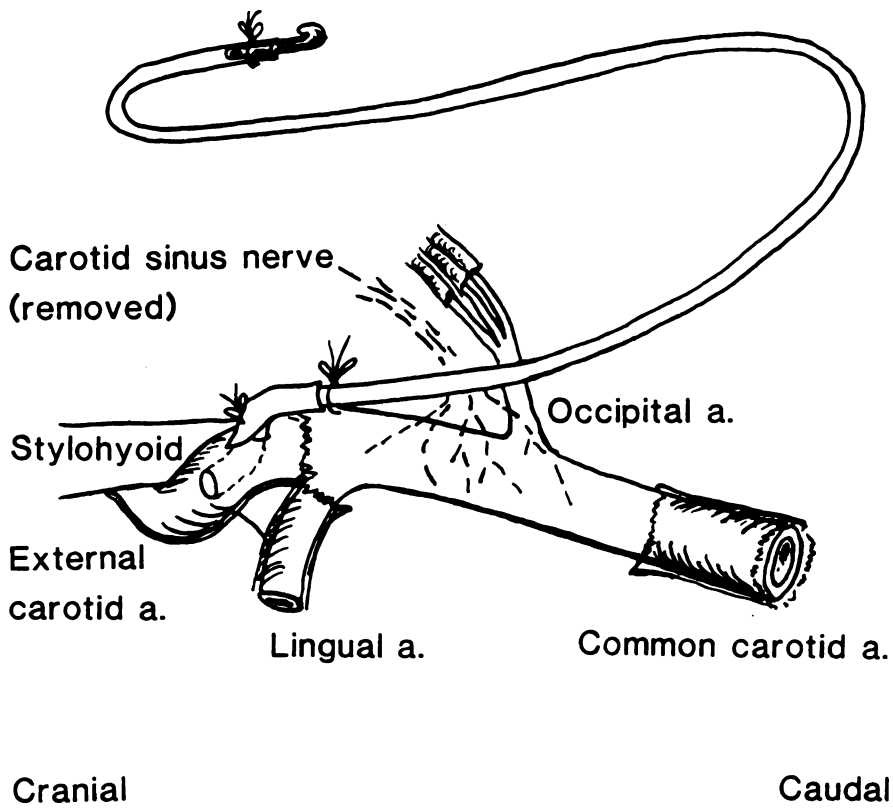


Figure 1. Anatomy of the carotid sinus region of the sheep and placement of the external carotid artery catheter.

The four catheters were tunneled subcutaneously to a prepared spot on the neck near the shoulder, where they were brought through the skin individually (in order to prevent retrograde infection) and tied to a loop of polyvinyl tubing placed through the skin. The two occluder lines were allowed to hang straight down and were brought through the skin on either side of the incision. The incision was closed, leaving a Penrose drain to prevent excessive swelling by allowing drainage of fluid. This drain was removed within 72 hours after surgery. Immediately after surgery each sheep was given 6×10^4 units/kg procaine penicillin G and then maintained on 3.6×10^4 units/kg/day procaine penicillin G for five days. The catheters were filled once daily with 1:1000 sodium heparin. The sheep were given one week to recover from surgery.

Experimental Procedures

PGE_2 Infusions

There were three routes of infusion of PGE_2, all at the rate of 10 ng/kg/min: 1) into the common carotid artery past an intact carotid sinus, 2) into the common carotid artery past a denervated carotid sinus, and 3) into the external carotid artery beyond the carotid sinus. The experimental procedure was the same for each route of PGE_2 infusion. With the sheep standing quietly in its cage, carotid blood pressure was recorded onto a Gould-Brush chart recorder using Gould Statham pressure transducers zeroed at the level of the heart. Data also were recorded onto magnetic tape for storage and playback using a Hewlett-Packard FM tape recorder. Heparinized saline was infused into each catheter at a rate of 0.3 ml/min. Control blood pressure and heart rate were obtained

in this manner, and then PGE_2 was infused, 10 ng/kg/min, at a rate of 0.3 ml/min into the appropriate artery. (PGE_2 was made up as a stock solution of 1 mg/ml in ethanol and stored at -20°C. Just prior to infusion the proper amount was diluted in normal saline). PGE_2 infusion was continued for 35 minutes. Infusions by each route were repeated four times randomly on different days. These replicates were averaged for each sheep. Statistical analysis was performed on the data per sheep rather than per replicate.

Cardiac Output Determinations

Three sheep in which carotid sinus denervation was verified (described below) were used for cardiac output determinations during control and during PGE_2 infusion into the external carotid artery or into the common carotid artery past the denervated carotid sinus. These experiments were performed to make certain that the increases in blood pressure caused by these two routes of infusion also were due to an increase in total peripheral resistance, as had been demonstrated previously by Hull and Chimoskey with PGE_2 infusions into the common carotid artery past an intact carotid sinus (1984).

The experimental procedure for the PGE_2 infusions was the same as described previously, with the addition that cardiac output was determined several times during control and during PGE_2 infusion using the indocyanine green dye dilution technique. Indocyanine green dye was injected into the jugular vein while blood was being withdrawn from one of the carotid arteries through a Gilson model DTL dye tracer. The values of cardiac output, blood pressure, heart rate, and total peripheral resistance (blood pressure divided by cardiac output) for 2-3

dye injections during control and during PGE_2 infusion were averaged to produce control and PGE_2 values per sheep per day. For the external carotid artery route of infusion, statistical analysis was performed on the values from three sheep, three days each, for a total of nine replicates. For the denervated sinus route of infusion, statistical analysis was performed on the values from three sheep, two days each, for a total of six replicates.

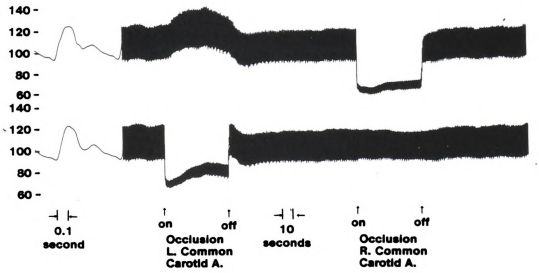


Figure 2. Verification of carotid sinus denervation by unilateral carotid artery occlusion. Figure 2 shows arterial pressure (mmHg) in right carotid artery (above) and left carotid artery (below). The right carotid sinus has been denervated. Occlusion of the common carotid artery proximal to the intact left carotid sinus causes a rise in systemic blood pressure (measured in the right carotid artery). Occlusion of the common carotid artery proximal to the denervated right carotid sinus fails to elicit a carotid sinus baroreflex.

Verification of Denervation

After all experiments had been performed, each sheep with a carotid sinus denervation was re-anesthetized with sodiudm thiamylal and maintained in a very light plane of anesthesia with halothane. While blood pressure was being recorded in both common carotid arteries, the occluder on the intact common carotid artery was inflated and the artery occluded for 60 seconds. The same procedure was repeated on the denervated side. The two sides were compared several times. Denervation was verified when occlusion on the denervated side failed to cause a rise in systemic blood pressure while occlusion on the intact side did produce a reflex rise in systemic blood pressure (Figure 2). It was imperative to keep anesthesia time short and anesthetic depth light in order consistently to produce a carotid baroreflex on the intact side. This was because changes in pressure at the carotid sinus region were small due to backflow of blood from the other carotid artery through the circle of Willis and/or from the vertebral artery through its anastomosis with the occipital artery (Baldwin and Bell 1963a, 1963b, 1963c; McQueen and Belmonte 1974). Additionally, the response of the carotid sinus to this small change probably was buffered by the aortic arch receptors and may have been inhibited by anesthesia. Despite these problems, verification of denervation was performed in the anesthetized state since blood pressure tended to be more labile in the conscious state, and transient increases in pressure due to extrinsic factors (such as noises in the hall) could not be differentiated from increases in pressure due to carotid occlusion. Denervation was verified in five of the six sheep (3 right, 2 left). This is reflected in Table 1 which contains only data from the 5 sheep in which denervation was verified.

Statistical Analysis

For all variables (blood pressure, heart rate, cardiac output, and total peripheral resistance), the paired t test was used to compare values during control to those during PGE_2 infusion. Since it had been shown previously that PGE_2 infusion past an intact carotid sinus caused all four variables to increase significantly (Hull and Chimoskey 1984), one-tailed t tables were used to find the value of p. Values of p are presented in the results; .05 or smaller was considered significant.

In four animals data from control and from PGE_2 infusion by each of the three routes were compared using a blocked Analysis of Variance (ANOVA) and Student-Newman-Keuls test for multiple comparisons to determine whether there were significant differences in the blood pressure or heart rate produced by infusion of PGE_2 past an intact carotid sinus, past a denervated sinus or beyond the carotid sinus.

RESULTS

Infusion of PGE_2 at a rate of 10 ng/kg/min into the external carotid artery of six conscious sheep resulted in an increase in blood pressure of 17 mmHg ($p<.0005$) and an increase in heart rate of 7 bpm ($p<.025$) (Table 1). During cardiac output determinations, blood pressure rose 19% from 79 to 94 mmHg ($p<.0005$), cardiac output (4.86 L/min) did not change significantly, total peripheral resistance rose 23% from 16.47 to 20.08 mmHg min/L ($p<.0005$), and heart rate rose 8% from 83 to 90 bpm ($p<.025$) (Figure 3A).

Infusion of PGE_2, 10 ng/kg/min, past the denervated carotid sinus of five of the six sheep in which denervation was verified resulted in an increase in blood pressure of 22 mmHg ($p<.0005$) and an increase in heart rate of 6 bpm ($p<.05$) (Table 1). During cardiac output determinations, blood pressure rose 25% from 80 to 100 mmHg ($p<.0005$), cardiac output (4.99 L/min) did not change significantly, total peripheral resistance increased 29% from 16.84 to 21.69 mmHg min/L ($p<.01$), and heart rate increased 6% from 76 to 80 bpm ($p<.025$) (Figure 3B).

Data from these experiments for each individual sheep are shown in Appendix B, Table B-1.

Table 1. Blood Pressure and Heart Rate Responses to Infusion of PGE_2, 10 ng/kg/min, either into the External Carotid Artery Beyond the Carotid Sinus or into the Common Carotid Artery Past a Denervated Carotid Sinus

Route of Infusion:	External Carotid Artery (6 sheep/23 replicates)			Common Carotid Artery (Carotid Sinus Denervated) (5 sheep /20 replicates)		
	Control	PGE_2	S.E.D.[a]	Control	PGE_2	S.E.D.
Blood Pressure (mmHg)	86	103**	(1.74)	81	103**	(1.96)
Heart Rate (beats/min)	77	84*	(1.93)	79	85*	(2.20)

a Standard error of the difference.
* $p<.05$ re Control ** $p<.0005$ re Control

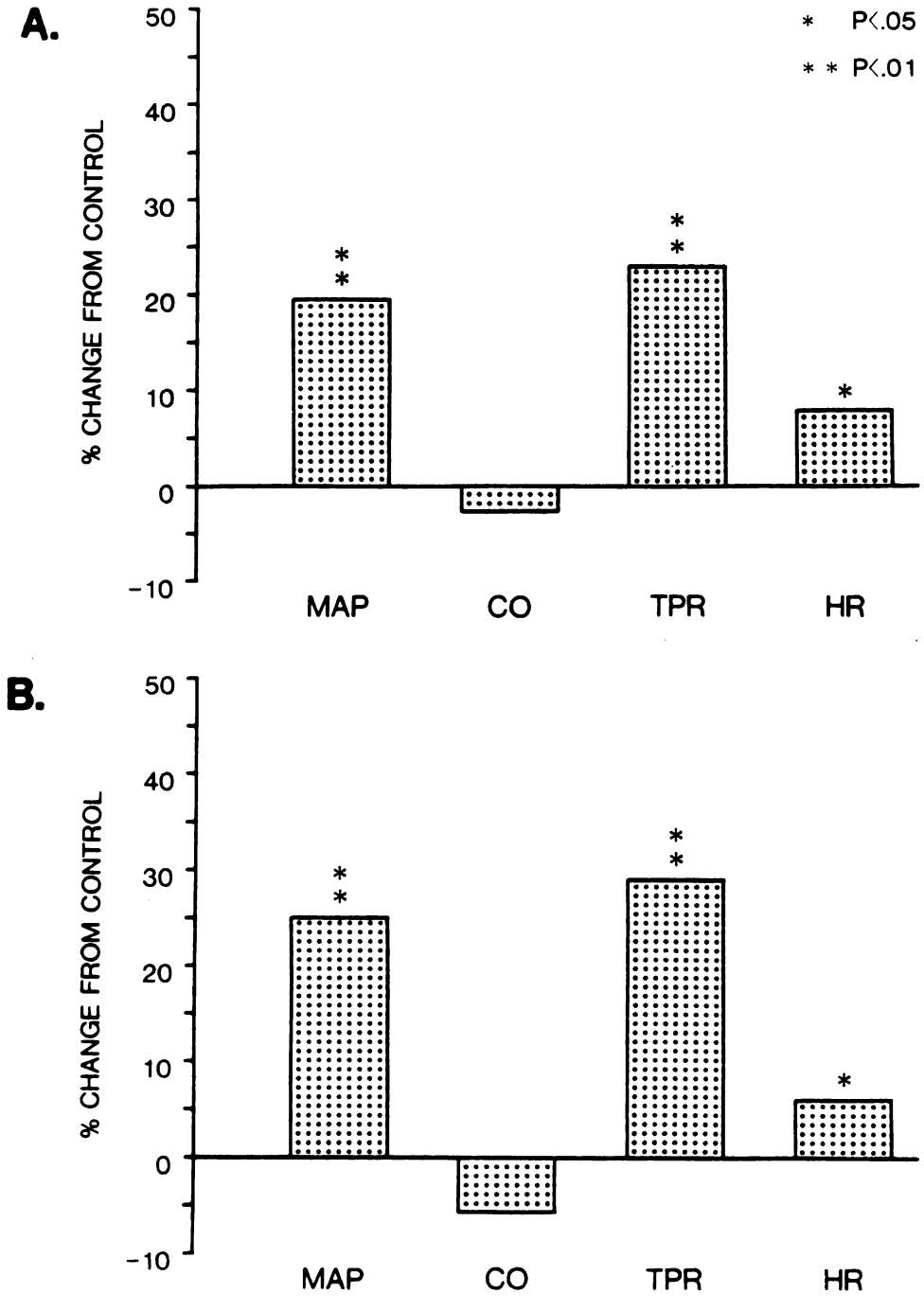


Figure 3. **A.** Hemodynamic changes during external carotid infusion of PGE_2, 10 ng/kg/min, in three conscious sheep (9 replicates). **B.** Hemodynamic changes during infusion of PGE_2, 10 ng/kg/min, past a denervated carotid sinus in three conscious sheep (6 replicates). The increase in blood pressure is due to an increase in total peripheral resistance in both cases. (Asterisks indicate which variables are significantly different from control 25 minutes after the start of PGE_2 infusion.

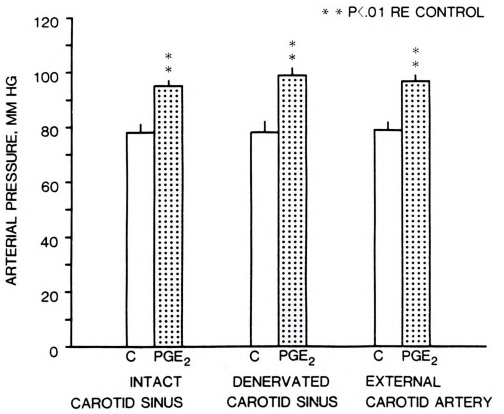


Figure 4. Blocked comparison of blood pressure during control and during PGE_2 infusion, 10 ng/kg/min, by three routes: into the common carotid artery past an intact carotid sinus, into the common carotid artery past a denervated carotid sinus, and into the external carotid artery beyond the carotid sinus (4 conscious sheep/16 replicates per route.

In four sheep in which data were obtained by all three routes of infusion of PGE_2, including infusion past an intact carotid sinus, analysis by blocked ANOVA followed by multiple comparisons using the Student-Newman-Keuls statistical test showed that while blood pressure during PGE_2 infusion differed significantly from control for each route of infusion, there were no statistically significant differences in blood pressure among control groups nor among PGE_2 groups (Figure 4). Similarly, there were no significant differences in heart rate among control or PGE_2 groups.

DISCUSSION

In cross-circulation studies in anesthetized dogs, Kaplan et al. (1969) showed that injection of PGE_1 past an intact carotid sinus causes blood pressure to fall while injection past a denervated carotid sinus causes blood pressure to rise. They concluded that direct action of PGE_1 on the carotid sinus masks or reverses a central pressor effect. In contrast to these results, McQueen and Belmonte (1974) more recently showed that PGE_2 (0.4-8.0 ug/kg) injected into the bloodstream just caudal to the carotid sinus in anesthetized cats has no direct effect on the firing of carotid sinus baroreceptor nerve fibers.

Hull and Chimoskey (1984) found that midcervical infusion of PGE_2 into the common carotid artery shifted the set point of the baroreflex without changing its sensitivity. This resetting of the baroreflex could have been an indirect effect of PGE_2 acting in the central nervous system or it could have been due to a local or direct action of PGE_2 on the carotid sinus baroreceptors. Local vasodilation in the carotid sinus region may cause a decrease in afferent baroreceptor nerve firing, resulting in an increase in sympathetic nerve activity. Direct action of PGE_2 on the carotid sinus baroreceptors could alter the sensitivity of the receptors, in which case one might expect the other, non-affected baroreceptors (ie.: aortic arch and the other carotid sinus) to dominate and to keep blood and heart rate from changing. Another possibility, however, is that direct action of PGE_2 on the carotid sinus baroreceptors could alter nerve traffic to the nucleus tractus solitarius, in which case the central nervous system would be affected indirectly. Thus, direct action of PGE_2 on the carotid sinus may cause the rise in blood pressure observed when PGE_2 is infused into the common

carotid artery of conscious sheep. However, due to the evidence that PGE_2 does indeed act in the central nervous system (by the vertebral route or IVT), it is more likely that any direct action of PGE_2 on the carotid sinus is either through augmentation or inhibition of the centrally-mediated action of PGE_2.

In this study, the question of whether the pressor response to common carotid artery infusion is influenced by a direct action of PGE_2 on the carotid sinus baroreceptors was approached in two ways. First, PGE_2 was infused into an artery to the head bypassing the carotid sinus. The external carotid artery was chosen for this purpose since the adult sheep has no internal carotid artery and since the vertebral artery of the sheep only perfuses the caudal medulla (Baldwin and Bell 1963a, 1963b, 1963c). Thus the external carotid artery is the main source of blood to the brain of the sheep and infusion of PGE_2 into this artery would be most likely to reach the same areas of the brain as those supplied by the common carotid artery. Secondly, PGE_2 was infused into the common carotid artery at a site proximal to a denervated carotid sinus, thereby ensuring that the same areas of brain that had been studied previously were perfused.

Our results agree with the work of McQueen et al. (1974). Since there were no significant differences in the changes in blood pressure or heart rate when PGE_2 was infused into an intact common carotid artery, past a denervated carotid sinus, or beyond the carotid sinus, we conclude that the carotid sinus has no direct role in the pressor response observed when PGE_2 is infused into the common carotid artery of conscious sheep. This conclusion supports the hypothesis that the cardiovascular responses observed when PGE_2 is infused into the arterial supply to the head are centrally-mediated.

IV. CHAPTER TWO

PARASYMPATHETIC NERVOUS SYSTEM

RESPONSE TO INTRACAROTID PGE_2 IN CONSCIOUS SHEEP

INTRODUCTION

Prostaglandin E_2 (PGE_2), a potent peripheral vasodilator (Moncada et al. 1980), causes blood pressure and heart rate to increase when infused into a carotid artery (10 ng/kg/min) in conscious sheep (Breuhaus and Chimoskey 1983; Hull and Chimoskey 1984). The increase in blood pressure during intracarotid (IC) PGE_2 infusion is caused by increased total peripheral resistance (Breuhaus and Chimoskey 1983; Hull and Chimoskey 1984) and can be prevented by the alpha-adrenoceptor blocking agent phentolamine in the presence of propranolol (Hull and Chimoskey 1984). Pretreatment with both propranolol and phentolamine does not abolish the increase in heart rate during IC PGE_2 infusion (Hull and Chimoskey 1984).

The increase in heart rate which remains during IC PGE_2 infusion after adrenoceptor blockade may be caused by inhibition of parasympathetic nervous tone to the heart. However, PGE_2 may increase heart rate by increasing cardiac beta-adrenoceptor activity and the dose of propranolol used in the previous study (Hull and Chimoskey 1984) was not sufficient to block it. Prostaglandin E_2 does not produce tachycardia by direct action on the heart, since 400 ng/kg/min PGE_2 infused into the

right atrium does not alter heart rate (Hull and Chimoskey 1984). This latter finding also demonstrates that, at the doses used (10–400 ng/kg/min), PGE_2 is metabolized rapidly by the lungs (Moncada et al. 1980; Hull and Chimoskey 1984) so that it does not enter the systemic circulation to cause peripheral vasodilation and reflex tachycardia.

In conscious sheep, IC PGE_2 increases the set point of the baroreflex without changing its sensitivity (Hull and Chimoskey 1984). Intracarotid PGE_2, 10 ng/kg/min, has no direct action on carotid sinus baroreceptors (Breuhaus and Chimoskey 1983). Centrally-administered PGE_2 may reset the baroreflex by activating only sympathetic centers in the brain. However, if IC PGE_2 causes both sympathetic activation and inhibition of parasympathetic tone, PGE_2 must act either at multiple sites in the brain or within the baroreflex pathway itself.

Evidence is presented below that PGE_2 has a complex action on cardiac autonomic regulation. Neither atropine alone nor a larger dose of propranolol alone can block the increase in heart rate caused by IC infusion of PGE_2 in conscious sheep. However, a combination of atropine and propranolol does block the heart rate increase. Taken together, the data presented in this chapter and the information cited above support the hypothesis that PGE_2 acts within the central baroreflex pathway both to activate the peripheral sympathetic nervous system and to inhibit the peripheral parasympathetic nervous system, resulting in increased blood pressure, heart rate, total peripheral resistance, and cardiac output (Hull and Chimoskey 1984). If either division of the autonomic nervous system is blocked peripherally, heart rate still increases during IC PGE_2 infusion by greater contribution to the heart rate response by the other unblocked division of the autonomic nervous system.

METHODS

Eight mature female sheep, ranging in weight from 50 to 80 kg, were used for the PGE_2 infusions and muscarinic receptor blockade experiments. Seven of these same sheep also were used for the beta-adrenoceptor blockade experiments and the combined blockade experiments. The sheep were housed individually in the laboratory and they were allowed to acclimate to their surroundings and laboratory personnel. This ensured that the sheep were calm during the experiments.

Instrumentation

Each sheep was anesthetized with intravenous sodium thiamylal, intubated, and maintained in a surgical anesthetic plane with halothane and oxygen. They were placed in dorsal recumbency and prepared for aseptic surgery. A midline incision was made in midcervical skin and subcutaneous tissue. Careful surgical dissection was performed to expose both carotid arteries without disturbing the vagus nerves. Non-occlusive polyvinyl catheters were implanted in each carotid artery according to the technique of Herd and Barger (1964). One external jugular vein also was catheterized in the same manner. The catheters were tunneled subcutaneously to a prepared spot on the neck, where they were brought through the skin and tied to a loop of polyvinyl tubing placed through the skin. The incision was closed and the sheep was allowed to recover from anesthesia. Immediately after surgery each sheep was given $5X10^4$ units/kg procaine penicillin G intramuscularly and 1.92 gm trimethoprim-sulfa (Tribrissin^R; Burroughs Wellcome Co.) orally. They were maintained on the trimethoprim-sulfa for an additional three days. The sheep were given ten days to two weeks to recover from

surgery. During this time they became further accustomed to handling and to the equipment to be used for experiments. The catheters were filled once daily with 1:1000 sodium heparin.

Experimental Procedures

PGE_2 Infusions

With a sheep standing quietly in its cage, mean and pulsatile arterial blood pressure were recorded from a carotid artery catheter onto a Gould-Brush chart recorder using Gould-Statham pressure transducers placed level with the heart. Data also were recorded onto magnetic tape for storage and playback using a Hewlett-Packard FM tape recorder. Heparinized saline was infused into the carotid artery (IC) at a rate of 0.3 ml/min. Control blood pressure and heart rate were obtained in this manner and then the heparinized saline was discontinued and PGE_2, 10 ng/kg/min, was infused IC at the same rate, 0.3 ml/min. (PGE_2 was made up as a stock solution of 1 mg/ml in ethanol and stored at $-20^{\circ}C$. Just prior to infusion the proper amount was diluted in physiological saline.) PGE_2 infusion was continued for 35 minutes. Infusions of vehicle (the same microliter amount of ethanol diluted in saline) also were continued for 35 minutes. Blood pressure and heart rate data were averaged over a two minute period during control and for two minutes at the beginning of each five minute interval during PGE_2 or vehicle infusion. Infusion of PGE_2, 10 ng/kg/min IC, caused blood pressure and heart rate to increase gradually, beginning approximately five minutes after the start of infusion. The increases in blood pressure and heart rate ceased 15-20 minutes later. Steady-state PGE_2 values were obtained 25 minutes into the infusions. This experiment was

performed only once on any given day and was repeated (replicates) at least twice. The average number of replicates per sheep was six. There was no tachyphylaxis to PGE_2 infusion in any sheep. The values obtained from all experiments in any one sheep were averaged to produce mean values characteristic of that sheep. Statistical analysis was performed on the data per sheep rather than per replicate.

Muscarinic Receptor Blockade

Three series of experiments were performed with atropine. Timed control experiments were performed by giving atropine by itself. In a second series of experiments atropine was given five minutes before the start of IC PGE_2 infusion. Finally, atropine was given 22 minutes after the start of IC PGE_2 infusion. Muscarinic receptor blockade was produced with atropine methyl bromide (Sigma), 1 mg/kg intravenously (IV). Atropine methyl bromide was used in these experiments because it does not cross the blood-brain barrier (Weiner 1980a). Effectiveness of the blockade was determined in the following manner. For each sheep, a control dose of acetylcholine (ACH) which would stop the heart for 2-5 seconds was found. Among all sheep this dose ranged from .01 to .02 mg/kg IV, but once the correct dose for each sheep was found, it did not vary from day to day. Five and 15 minutes after administration of atropine, a dose of ACH two times the control dose produced no change in heart rate. At 45 minutes after administration of atropine, 10 times the control dose of ACH was given. At no time did this produce a decrease in heart rate. Further evidence that 1 mg/kg atropine methyl bromide IV results in adequate muscarinic blockade was obtained in separate experiments in four of these sheep. Forty minutes after the

initial bolus of atropine, additional boluses of 0.5 mg/kg or 1 mg/kg atropine methyl bromide IV resulted in no additional increase in heart rate.

Beta-Adrenoceptor Blockade

Three series of experiments were performed with propranolol. In one series of experiments, propranolol was given by itself to serve as a time control. In a second series of experiments, propranolol was given ten minutes before the start of IC PGE_2 infusion. In a third series of experiments, propranolol was given 17 minutes after the start of PGE_2 infusion. Beta-adrenoceptor blockade was performed in seven of the same sheep used above with d,l propranolol (Sigma), 1 mg/kg + .025 mg/kg/min IV. This is twice the total amount of propranolol given by Hull and Chimoskey (1984) and is sufficient to reduce the response to a challenge bolus of isoproterenol to at least one tenth the response produced by isoproterenol during control. To test the effectiveness of the blockade, a 5 ug bolus of isoproterenol was given IV during control. At seven and 15 minutes after the initial bolus of propranolol, 25 ug isoproterenol IV had no effect on heart rate. At 45 minutes after propranolol, the heart rate responses to ten times the control dose of isoproterenol (50 ug) were compared to the control responses.

Combined Blockade

In a final series of experiments, IC PGE_2 was infused in the presence of both beta-adrenoceptor and muscarinic receptor blockade. These experiments were performed in the same seven sheep used in the beta-adrenoceptor blockade experiments. The same doses of blocking

agents and challenging agents were used. To test the ability of the combined blockade to prevent the increases in heart rate during IC PGE_2 infusion, propranolol was started at time zero and atropine was given five minutes later. Ten minutes after the initial propranolol bolus, the IC PGE_2 infusion was started. Combined blockade also was performed in the reverse order: PGE_2 was started at time zero, propranolol was given 17 minutes after the start of PGE_2 infusion, and atropine was given 22 minutes after the start of infusion.

Statistical Analysis

For each variable (blood pressure and heart rate), control and experimental values corresponding to 25 minutes of PGE_2 infusion were used. Values from replicates of experiments were averaged for each sheep and these averaged data were analyzed by blocked ANOVA. Student-Newman-Keuls test for multiple comparisons was used to determine whether there were significant differences in control values of blood pressure or heart rate among experiments or in the blood pressure or heart rate responses to PGE_2 alone, the blocking agent alone, or PGE_2 plus blocking agent. The experiments in which all three drugs were given were analyzed in two ways. First, the raw data were analyzed as described above. Second, since the control values for blood pressure and heart rate in all previous experiments were the same, and since individual responses to each experimental protocol were constant, the data also were analyzed in terms of the change in blood pressure or the change in heart rate. This allowed comparison of the changes which actually were observed when both blocking agents and PGE_2 were given together to the changes which were expected by calculating the sums of the individual

changes caused by each drug given separately. A non-parametric test, the chi-square test, was used to test for significant differences between the observed results and the expected (calculated) results (Steel and Torrie 1980). For all analyses, values of $p<.05$ were considered to indicate significant differences.

RESULTS

Control values for blood pressure and heart rate for each individual sheep did not vary from day to day, and the average control values for blood pressure and heart rate for all sheep were not significantly different from one another in any of the experiments. When the same experiments were repeated in individual sheep, blood pressure and heart rate responses to the test agents given were highly repeatable. Infusions of vehicle did not alter blood pressure or heart rate.

Muscarinic Receptor Blockade

In eight conscious sheep, atropine alone increased heart rate 26 bpm ($p<.05$) (Table 2) and 24 bpm ($p<.05$) (Figure 5) five and thirty minutes after injection, respectively. Prostaglandin E_2 infusion, 10 ng/kg/min IC, increased heart rate 14 bpm ($p<.05$) (Table 2 and Figure 5). When atropine was given after PGE_2 infusion had raised heart rate, there was a further 24 bpm increase in heart rate ($p<.05$), resulting in a total increase of 38 bpm from control ($p<.05$) (Table 2). When PGE_2 was infused after atropine, heart rate increased a total of 35 bpm ($p<.05$), which was significantly different from the increases in heart rate with atropine alone or with PGE_2 alone (Figure 5B).

Atropine alone caused small but significant increases in blood

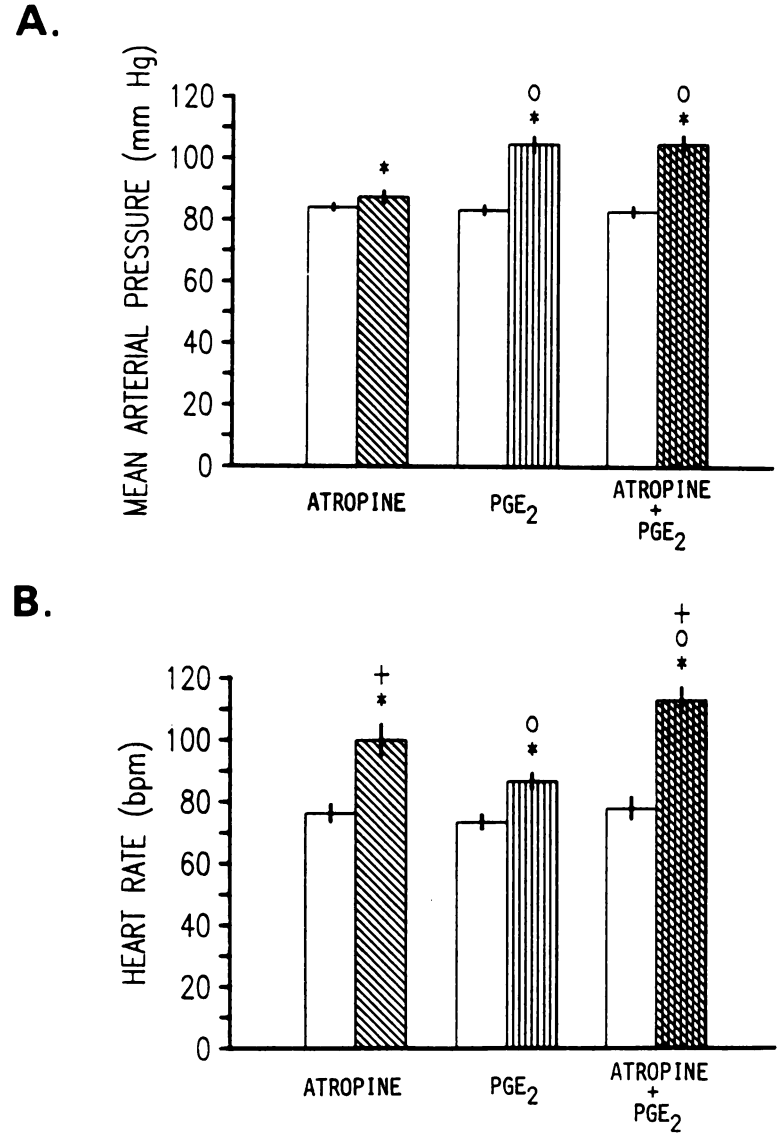


Figure 5. **Atropine + PGE_2, 8 conscious sheep.** Blood pressure and heart rate in three separate experiments (all experiments performed in all sheep) comparing the responses to 1 mg/kg atropine IV given alone, to 10 ng/kg/min PGE_2 IC given alone, and to atropine + PGE_2 given together. Open bars represent control values. Shaded bars represent test values 30 minutes after atropine alone (Atropine), 25 minutes after the start of PGE_2 infusion alone (PGE_2), and 30 minutes after atropine and 25 minutes after the start of PGE_2 infusion when the two drugs were given together (Atropine + PGE_2). **A.** Blood pressure: *=p<.05 re Control; o=p<.05 re Atropine. **B.** Heart rate: *=p<.05 re Control; o=p<.05 re Atropine; +=p<.05 re PGE_2.

pressure five and thirty minutes after injection: 8 mmHg (Table 2) and 4 mmHg (Figure 5), respectively ($p<.05$). Infusion of PGE_2 increased blood pressure 22 mmHg ($p<.05$) (Table 2 and Figure 5). Injection of atropine during steady-state PGE_2 infusion caused no further increase in blood pressure (Table 2). When the PGE_2 infusion was started five minutes after atropine had caused blood pressure to increase, there was a further significant increase in blood pressure, but the final blood pressure was no greater than the blood pressure with PGE_2 infusion alone (Figure 5A). Since the control pressures were not different, the increase in blood pressure caused by PGE_2 infusion after atropine was not as great as the increase when PGE_2 was given by itself.

Table 2. Muscarinic blockade with atropine methyl bromide (1 mg/kg IV) during IC PGE_2 infusion (10 ng/kg/min) in eight conscious sheep

Experimental Protocol	Blood Pressure (mmHg) Control	Experimental	S.E.D.[a]	Heart Rate (beats/min) Control	Experimental	S.E.D.
PGE_2 (25 min)	83	105^{*o}	(1.51)	73	87^{*o}	(2.09)
Atropine (5 min)	83	91^{*}	(2.93)	76	102^{*}	(4.18)
PGE_2 (25 min) + Atropine (5 min)	82	102^{*o}	(1.48)	78	116^{*o+}	(5.50)

a Standard error of the difference.
* significantly different from Control, $p<.05$
o significantly different from Atropine alone, $p<.05$
\+ significantly different from PGE_2 alone, $p<.05$

Data from these experiments for each individual sheep are shown in Appendix C, Tables C-1 and C-2.

Beta-Adrenoceptor Blockade

In seven conscious sheep, propranolol alone did not change heart rate (Table 3 and Figure 6). Prostaglandin E_2 infusion in these sheep increased heart rate 15 bpm ($p<.05$) (Table 3 and Figure 6). When PGE_2 had raised heart rate, subsequent administration of propranolol decreased heart rate 10 bpm ($p<.05$), but heart rate still was greater than control ($p<.05$) (Table 3). When propranolol was given before the start of PGE_2 infusion, PGE_2 still increased heart rate 7 bpm ($p<.05$), but this increase was less than the increase caused by PGE_2 infusion alone ($p<.05$) (Figure 6). The effect of propranolol on the heart rate responses to IC PGE_2 was the same regardless of whether propranolol was given before or after PGE_2.

Table 3. Beta-adrenoceptor blockade with propranolol (1 mg/kg + 0.025 mg/kg/min IV) during IC PGE_2 infusion (10 ng/kg/min) in seven conscious sheep

Experimental Protocol	Blood Pressure (mmHg)			Heart Rate (beats/min)		
	Control	Experimental	S.E.D.[a]	Control	Experimental	S.E.D.
PGE_2 (25 min)	84	105*	(1.63)	77	92*	(2.19)
Propranolol (10 min)	85	85	(0.34)	76	72	(1.65)
PGE_2 (25 min) + Propranolol (10 min)	87	109*	(2.90)	78	83*+	(1.86)

a Standard error of the difference.
* significantly different from Control and Propanolol alone, $p<.05$
\+ significantly different from PGE_2 alone, $p<.05$

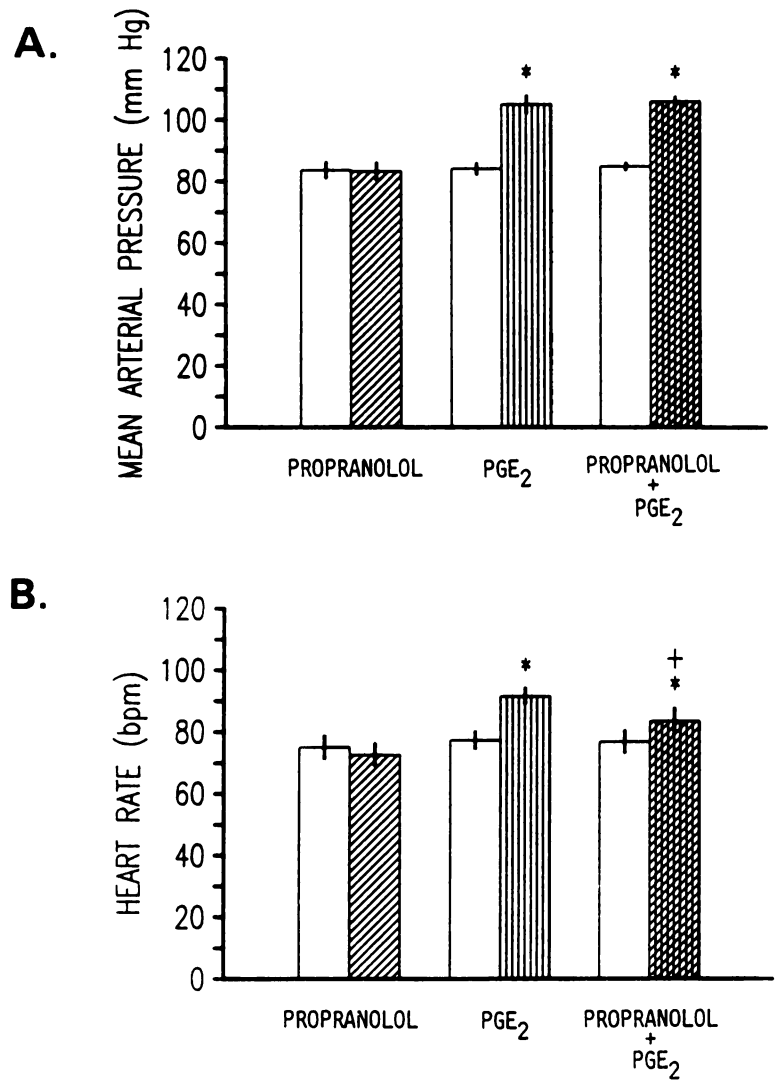


Figure 6. **Propranolol + PGE_2, 7 conscious sheep.** Blood pressure and heart rate in three separate experiments (all experiments performed in all sheep) comparing the responses to 1 mg/kg + .025 mg/kg/min propranolol IV given alone, to 10 ng/kg/min PGE_2 IC given alone, and to propranolol + PGE_2 given together. Open bars represent control values. Shaded bars represent test values 35 minutes after propranolol alone (Propranolol), 25 minutes after the start of PGE_2 infusion alone (PGE_2), and 35 minutes after propranolol and 25 minutes after the start of PGE_2 infusion when the two drugs were given together (Propranolol + PGE_2). **A.** Blood pressure: *=$p<.05$ re Control and Propranolol. B. Heart rate: *=$p<.05$ re Control and Propranolol; +=$p<.05$ re PGE_2.

In these sheep PGE_2 increased blood pressure 21 mmHg ($p<.05$). Propranolol had no effect on control blood pressure or on the blood pressure response to IC PGE_2.

Data from these experiments for each individual sheep are shown in Appendix C, Tables C-3 and C-4.

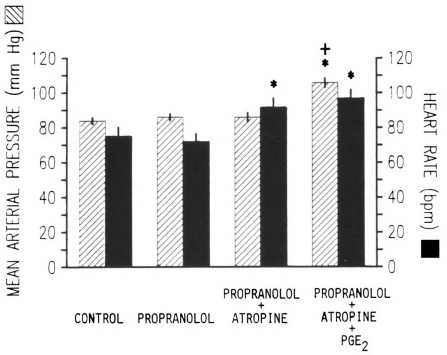


Figure 7. **Propranolol (1 mg/kg + 0.025 mg/kg/min IV) + Atropine (1 mg/kg IV) + PGE_2 (10 ng/kg/min IC), 7 conscious sheep.** Bars represent values of blood pressure and heart rate during consecutive administration of substances during one experiment. Note that administration of propranolol did not change heart rate or blood pressure. Addition of atropine five minutes later caused a significant increase in heart rate with no change in blood pressure. Subsequent infusion of IC PGE_2 caused no further increase in heart rate and raised blood pressure. * = $p<.05$ re Control and Propranolol. + = $p<.05$ re Propranolol + Atropine.

Combined Blockade

In the experiments in which both propranolol and atropine were given before PGE_2, propranolol by itself did not change heart rate, but heart rate increased when atropine was added, 17 bpm ($p<.05$, Figure 7). Subsequent infusion of IC PGE_2 caused no further increase in heart rate. When all three drugs were given together, heart rate was not significantly different from heart rate during atropine alone.

The combination of propranolol and atropine did not alter the blood pressure response to subsequent PGE_2 infusion.

Data from these experiments for individual sheep are shown in Appendix C, Tables C-5 and C-6.

When the changes in blood pressure and heart rate during combined blockade were compared by chi-square test to the sums of changes caused by each drug alone (the calculated or expected changes), neither the observed changes in blood pressure nor the observed changes in heart rate were as great as the expected changes ($p<.05$), regardless of whether the blockers were given before or during PGE_2 infusion. Figure 8 compares the observed (experimentally-derived) changes in blood pressure and heart rate to the expected changes in these variables (calculated from the sums of experimentally-derived changes) for the case in which propranolol and atropine were given during PGE_2 infusion. Note that the observed increase in heart rate when all three drugs were given (which was shown to be not significantly different from the increase in heart rate caused by atropine alone) appears to be the same as the increase in heart rate calculated from the sum of the changes in heart rate during propranolol alone and atropine alone.

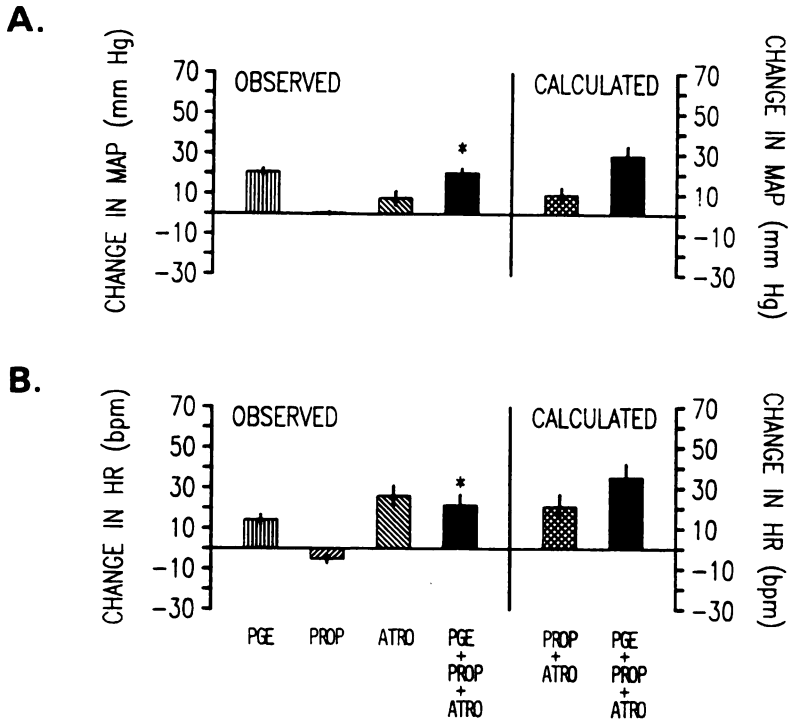


Figure 8. **PGE_2 (10 ng/kg/min IC) + Propranolol (1 mg/kg + 0.025 mg/kg/min IV) ± Atropine (1 mg/kg IV), 7 conscious sheep.** **A.** Comparison of changes in blood pressure. Note that the observed change in blood pressure at 25 minutes PGE_2 infusion + 10 minutes propranolol + 5 minutes atropine (PGE+PROP+ATRO) is significantly less than the change in blood pressure calculated (PGE+PROP+ATRO calculated) from the sum of the observed changes in blood pressure during PGE_2 alone (PGE), propranolol alone (PROP), and atropine alone (ATRO). "PROP+ATRO calculated" is the sum of PROP + ATRO. *=$p<.05$ re (PGE+PROP+ATRO calculated). **B.** Comparison of changes in heart rate. Note that the observed change in the heart rate at 25 minutes PGE_2 infusion + 10 minutes propranolol + 5 minutes atropine (PGE+PROP+ATRO observed) is significantly less than the change in heart rate calculated (PGE+PROP+ATRO calculated) from the sum of observed changes in heart rate during PGE_2 alone (PGE), propranolol alone (PROP), and atropine alone (ATRO). "PROP+ATRO calculated" is the sum of PROP + ATRO. *=$p<.05$ re PGE+PROP+ATRO calculated.

Table 4 demonstrates that isoproterenol, 5 ug, raised heart rate 65-72 beats per minute in all experimental series prior to administration of propranolol. Following propranolol, isoproterenol, 50 ug, raised heart rate only 6-10 beats per minute, whether propranolol was given alone, before PGE_2, after PGE_2, or before PGE_2 and atropine.

Table 4. Heart rate increases to test boluses of isoproterenol, 5 ug before and 50 ug after experimental agents, during four experimental protocols in seven conscious sheep

Experimental Protocol	Heart Rate Increase (beats per minute) Isoproterenol, 5 ug Before Propranolol (mean ± S.E.M.)[a]	Isoproterenol, 50 ug After Propranolol (mean ± S.E.M.)
Propranolol, alone	68 ± 2.74	9 ± 1.24
PGE_2, then Propranolol	68 ± 4.54	6 ± 1.29
Propranolol, then PGE_2	72 ± 5.38	9 ± 2.46
Propranolol, then Atropine, then PGE_2	65 ± 7.69	10 ± 1.77

a Standard error of the mean.

DISCUSSION

This laboratory has shown that the increase in heart rate observed during IC administration of PGE_2, 10 ng/kg/min to conscious sheep and calves, is not abolished with alpha- and beta-adrenoceptor blockade (Hull and Chimoskey 1984). The current study was performed to determine if inhibition of parasympathetic tone to the heart is partially responsible for the increase in heart rate. If IC PGE_2 acts only

at areas in the brain that control peripheral sympathetic nervous system activity, it might be expected that either the peripheral parasympathetic nervous system plays no role at all in the response to IC PGE_2, or that the parasympathetic nervous system actually is activated by the baroreflex to oppose the increases in blood pressure and heart rate caused by PGE_2 driving the peripheral sympathetic nervous system. Alternatively, IC PGE_2 may act at multiple sites in the brain or within the baroreflex pathway itself, both to activate the peripheral sympathetic nervous system and to inhibit resting parasympathetic tone.

In the first series of experiments presented here, muscarinic receptor blockade did not affect the heart rate response to IC PGE_2. These data, taken alone, suggest that in sheep inhibition of the parasympathetic nervous system does not contribute to the increase in heart rate observed when 10 ng/kg/min PGE_2 is given IC, nor does activation of the parasympathetic nervous system inhibit the PGE_2-induced increase in heart rate.

Inhibition of cardiac parasympathetic tone may account for a small part of the increase in blood pressure caused by IC PGE_2 infusion, since the increase in blood pressure during IC PGE_2 infusion in the presence of atropine is less than the increase in blood pressure when PGE_2 is infused alone. If inhibition of parasympathetic tone to the heart contributes to the increase in blood pressure during IC PGE_2 infusion, PGE_2 must increase cardiac output. Cardiac output was shown to increase during IC PGE_2 infusion in a previous study by this laboratory (Hull and Chimoskey 1984).

Since the atropine experiments failed to show that the increase in heart rate caused by IC PGE_2 is caused by parasympathetic inhibition, we

re-examined the effect of beta-adrenoceptor blockade on this increase. Since propranolol is a competitive antagonist (Weiner 1980b), it is possible that the inability of Hull and Chimoskey to prevent the PGE_2-induced tachycardia was caused by insufficient beta-adrenoceptor blockade. In the present study, a larger dose of propranolol significantly reduced, but still did not abolish, the increase in heart rate caused by IC PGE_2 infusion. Although small, this remaining increase in heart rate during beta-adrenoceptor blockade is significant, especially since the parasympathetic nervous system's ability to lower heart rate in response to the PGE_2-induced 20+ mmHg increase in blood pressure is unopposed. In these experiments it is unlikely that PGE_2 overcame the propranolol blockade, since the degree of blockade (as determined by heart rate responses to isoproterenol) was not different when propranolol was given alone or with PGE_2 (Table 4). Furthermore, the degree of blockade produced by propranolol was the same, regardless of whether the PGE_2-induced tachycardia was prevented or not. Thus, only part of the increase in heart rate during PGE_2 infusion is caused by beta-adrenoceptor activation alone.

Recently, a nonadrenergic-nonmuscarinic mechanism of increased heart rate following increases in afterload has been reported (Evans et al. 1984; Rigel et al. 1984). To determine whether this mechanism contributes to the change in heart rate during IC PGE_2 infusion, combined blockade with both propranolol and atropine was performed prior to IC PGE_2 infusion. In these experiments PGE_2 did not raise heart rate beyond the level obtained during propranolol and atropine.

Since combined beta-adrenergic and muscarinic blockades abolish the PGE_2-induced tachycardia, and since alpha-adrenergic blockade prevents

the PGE_2-induced increase in blood pressure (Hull and Chimoskey 1984), the increases in blood pressure and heart rate during IC PGE_2 infusion, 10 ng/kg/min in conscious sheep, are caused primarily by peripheral sympathetic activation and secondarily by parasympathetic inhibition. Furthermore, it appears that during blockade of one division of the autonomic nervous system, IC PGE_2 increases heart rate by means of the other, unblocked, division of the autonomic nervous system. Thus, the contribution of the sympathetic nervous system to the heart rate response to IC PGE_2 is increased during muscarinic receptor blockade, thereby compensating for the loss of ability to increase heart rate by withdrawing parasympathetic tone. Likewise, during beta-adrenoceptor blockade, the contribution of parasympathetic withdrawal to the PGE_2-induced tachycardia is increased, perhaps even changing from a merely permissive role to a causative role.

The interpretation described above of the data in this chapter implies that there is a set point and control system for heart rate, just as there is for blood pressure. It is known that the baroreceptors are sensitive to pulse strength as well as to mean arterial pressure (Angell James and Daly 1970); they may also be sensitive to frequency. If IC PGE_2 increases the heart rate "set point", then one might expect the central nervous system to attempt to raise heart rate by whatever means is available to it.

The central pressor and tachycardic effects of PGE_2 also have been studied in rats (Hoffman and Schmid 1979; Kondo et al. 1979; Hoffman et al. 1981; Takahashi and Bunag 1981b; Okuno et al. 1982). Our results in sheep are in agreement with the results of Hoffman and Schmid in rats (1979), who also found evidence for both sympathetic activation and

parasympathetic inhibition in response to centrally-administered PGE_2.

It has been shown previously that PGE_2, 10 ng/kg/min IC in conscious sheep, causes a shift of the baroreflex to a higher operating level without a change in sensitivity (Hull and Chimoskey 1984). Intracarotid PGE_2 does not produce its central cardiovascular effects by acting directly on the carotid sinus baroreceptors (McQueen and Belmonte 1974; Breuhaus and Chimoskey 1983). These observations (McQueen and Belmonte 1974; Breuhaus and Chimoskey 1983; Hull and Chimoskey 1984), together with the data from this study, support the hypothesis that IC PGE_2 acts within the central baroreflex pathway to cause both activation of the peripheral sympathetic nervous system and inhibition of peripheral parasympathetic nervous tone.

V. CHAPTER THREE

INTRACEREBROVENTRICULAR METHOD AND RESPONSES TO CENTRAL AND PERIPHERAL INFUSIONS OF ANGIOTENSIN II

INTRODUCTION

A central pressor effect of angiotensin II (AII), separate from its direct effect on vascular smooth muscle, first was demonstrated by Bickerton and Buckley in a cross-circulation preparation in which blood-borne AII, infused into the head of a vascularly-isolated, neurally-intact recipient, increased blood pressure in the recipient's trunk (Bickerton and Buckley 1961). Since that study, a separate brain renin-angiotensin system has been documented (Fischer-Ferraro et al., 1971; Ganten et al., 1971; Phillips et al., 1979) and many investigations of the central actions of AII have been performed (for reviews see Buckley and Ferrario 1977 and 1981; Phillips 1978 and 1980). In addition to its pressor effect, centrally-administered AII has been shown to cause antidiuresis, urinary sodium excretion, release of certain anterior pituitary hormones including ACTH and vasopressin, and drinking behavior in conscious animals. Since it is not possible or practical to use cross-circulation techniques in conscious chronically-instrumented animals, and since circulating AII is a direct vasoconstrictor, central actions of AII in conscious animals have been studied by comparing responses to intracarotid (IC) vs intravenous (IV) infusions of AII and by infusion of AII into cerebral spinal fluid.

The purpose of the present study was threefold. First, this paper describes a method of chronic catheterization of the cerebral ventricles of sheep which is more convenient and easier to maintain than conventional methods (Pappenheimer et al. 1962; Akerlund et al. 1973). Second, the presence or absence of drinking responses to intracerebroventricular (IVT) infusions of AII in sheep was used to verify proper ventricular catheter placement and patency. Drinking and pressor responses to IVT AII were compared to responses to IC or IV AII. Third, a dose of AII was determined which, when given IVT to conscious sheep, increases blood pressure 20-25 mmHg. Other hemodynamic responses, plasma sodium, osmolal and vasopressin concentrations, and packed cell volumes also were measured during IVT infusion of AII.

METHODS

Eighteen mature female sheep ranging in weight from 45 to 80 kg were used in this study. The sheep were housed in individual pens and they were allowed to acclimate to their surroundings and to laboratory personnel before surgical preparation. Because sheep are flock animals, at least two sheep were kept in each room. These conditions ensured that the sheep were calm and unstressed during the experiments.

Ventricular cannulas were placed in the first five sheep according to the technique of Pappenheimer et al (1962). Because of problems with maintenance of the catheters that developed in these sheep, the technique was modified, and the fifth sheep and thirteen additional sheep were catheterized as described below. Problems encountered with the metal ventricular cannulas will be presented in the results, but data from these sheep were not included in this presentation.

Instrumentation

Twelve sheep were anesthetized with intravenous sodium thiamylal, intubated, and maintained in a surgical plane of anesthesia with halothane and oxygen. They were placed in dorsal recumbency and their necks were prepared for aseptic surgery. Through a midline incision, both carotid arteries were exposed without disturbing the vagus nerves, and non-occlusive polyvinyl catheters made from Tygon^{R} tubing (Fischer Scientific) were implanted in each artery (Herd and Barger, 1964). One external jugular vein also was catheterized in the same manner. The catheters were tunneled subcutaneously to a prepared spot near the shoulder, brought through the skin individually, and tied to a loop of polyvinyl tubing.

In order to measure cardiac output and to calculate total peripheral resistance, the last of the twelfth sheep also had an electromagnetic flowmeter (Zepeda; Seattle WA) placed on its main pulmonary artery in a subsequent surgery (described next). Two additional sheep (making a total of fourteen sheep) were anesthetized as described above, placed on a respirator, and a left lateral thoracotomy was performed to implant an electromagnetic flowmeter on the main pulmonary artery and non-occlusive catheters in the right atrium and aortic arch. The thoracotomy was closed and the left common carotid artery was catheterized non-occlusively through a separate incision placed directly over the artery. All catheters and the flowmeter wires were tunneled subcutaneously to a prepared spot at the shoulder, as described previously.

All fourteen sheep then were placed in sternal recumbency with their heads supported on sandbags and taped in a position such that the parietal surface of the skull was horizontal (Pappenheimer et al. 1962). This positioning put the axis of the brainstem in the horizontal plane and the tip of the ventricular catheter, which was perpendicular to the horizontal axis, followed a trajectory that was inclined 25° rostrally from the coronal zero described by McKenzie and Smith (1973) (Figure 9).

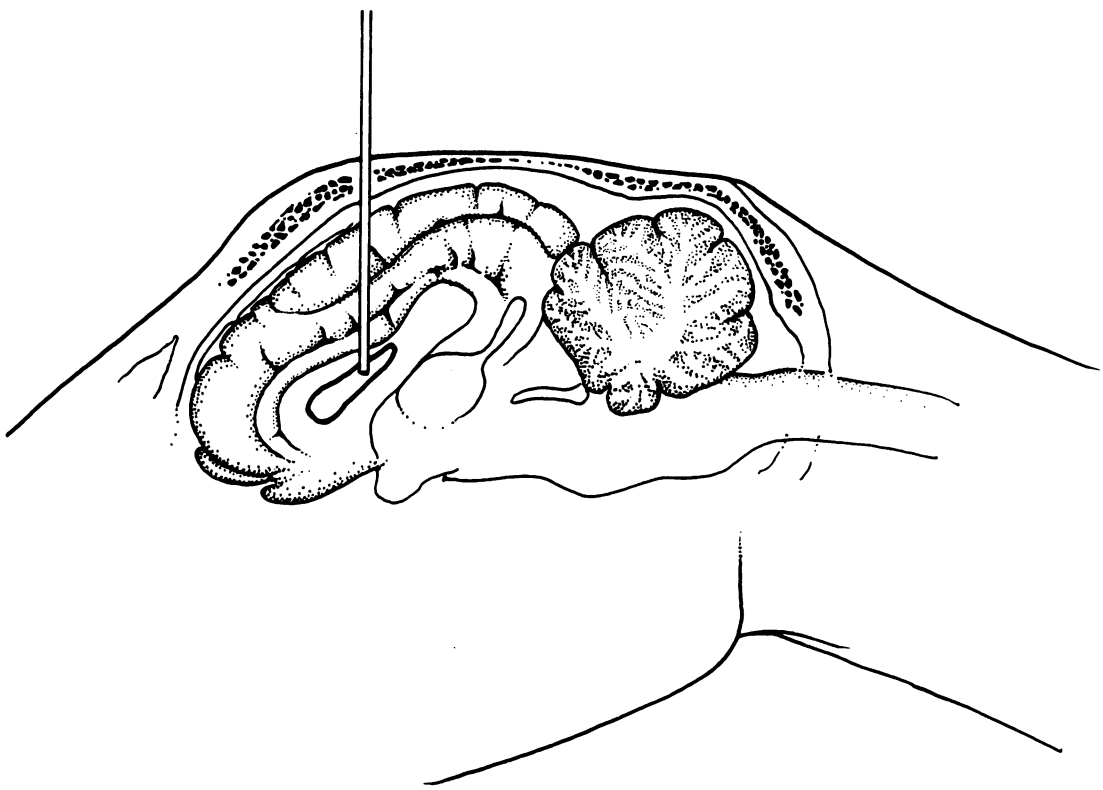


Figure 9. Position of ventricular catheter in lateral cerebral ventricle.

The surgical site was prepared for aseptic surgery and a caudad-directed curvilinear incision was made in the skin over the junction of the parietal and frontal bones (the coronal suture). The rostral flap of skin was dissected off the surface of the parietal bone and reflected forward (Figure 10A). Hemorrhage was controlled by ligation. To ensure viability at the end of the procedure, the skin flap was covered with a sponge moistened with physiologic saline. The periosteum of the skull was incised with a scalpel along the midline and reflected laterally to the side of catheter placement (7 left, 7 right).

A trephine with a 5 mm diameter was used to create a hole in the skull. Pappenheimer et al (1962) used the coronal suture as a landmark for cannula placement in the goat. According to May (1970), the coronal suture of the sheep remains distinct throughout life but is very irregular. Because the coronal suture was not always easy to find in these mature sheep without performing more extensive surgical dissection (and trauma), its position was identified as follows.

The most rostral boundary of attachment of the muscles of the neck to the skull could be identified easily. Imaginary lines were drawn from the midline of this boundary rostrally through the center of each cornual process. The trephine was placed to one side of the midline and moved forward from the muscle boundary until it was centered between the midline and the oblique imaginary line with approximately 2 mm ease on each side (Figure 10B). By following this procedure, the coronal suture was located and formed the rostral border of the trephine hole in nine sheep. In five sheep the trephine hole was placed directly over the suture.

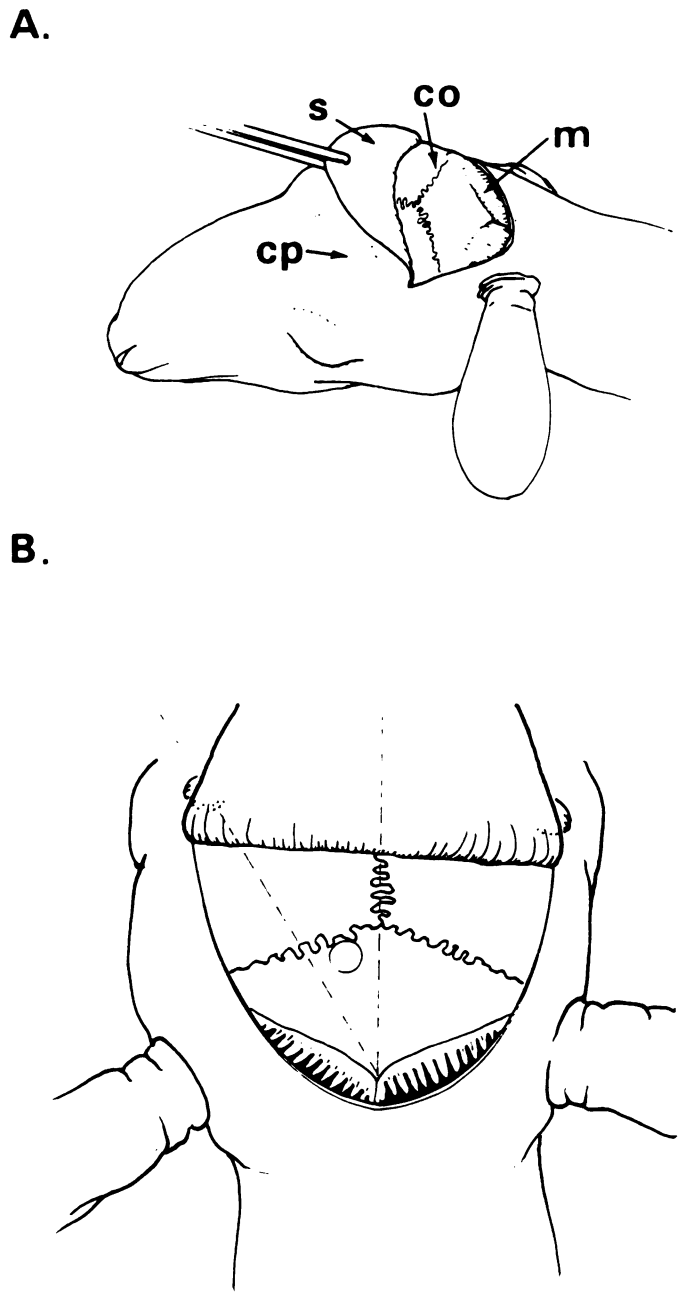
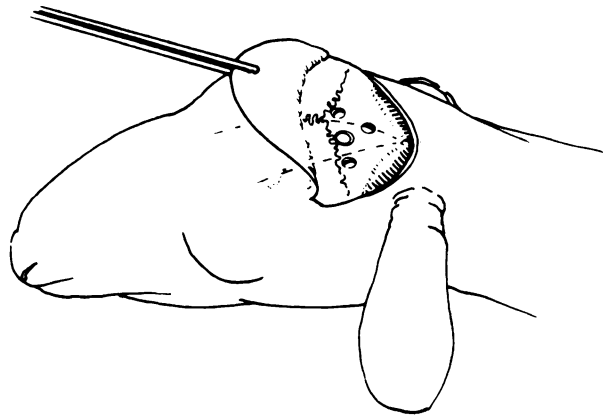


Figure 10. Placement of the ventricular catheter. **A.** s = skin, co = coronal suture, m = muscle, and cp = cornual process. **B.** Trephine hole centered between the midline and an oblique imaginary line passing through the cornual process.

C.



D.

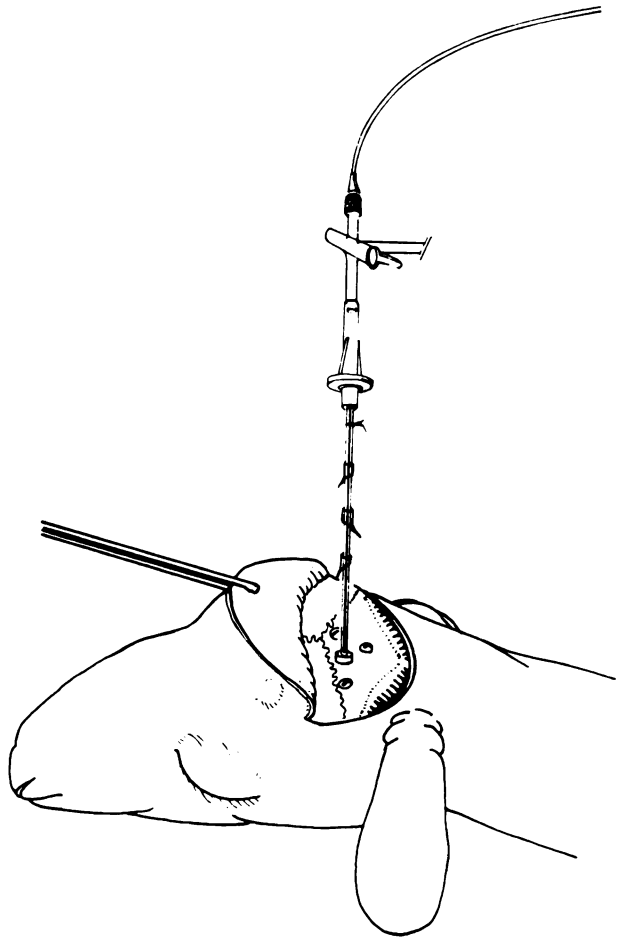


Figure 10 (cont'd.). **C.** Trephine hole made and screws placed. **D.** Catheter (with infusion line attached) ready to be advanced into brain.

Before the trephine hole was completed, three smaller holes were drilled around it and filled with stainless steel machine screws. The trephine hole was completed and the plug of bone removed (Figure 10C). If bleeding occurred, a corner of a gauze sponge was placed in the hole temporarily until the bleeding stopped.

The ventricular catheter then was prepared for placement. This catheter had been made previously from .053" I.D./.085" O.D. polyvinyl tubing cut 2 mm longer than the shaft of a 16 gauge 5-1/4 inch Angiocath^{R} stylet (The Deseret Co., Sandy, Utah). Three tabs of a larger size tubing had been placed 4 cm, 6.5 cm, and 10 cm from the bottom of the catheter, in the same way that tabs are placed on Herd-Barger catheters (Herd and Barger 1964). The Angiocath^{R} stylet was slipped into the ventricular catheter and tied at the top with a piece of silk (Figure 10D). A sterile infusion line filled with artificial cerebral spinal fluid (CSF) (Table 5) was connected to the stylet, and pressure in the stylet-ventricular catheter was measured with a Gould-Statham pressure transducer onto a Gould-Brush chart recorder.

The catheter was held by a micromanipulator and positioned over the trephine hole so that it was perpendicular to the parietal surface of the skull. A .039 ml/min infusion of artificial CSF was started through the stylet, and the catheter and stylet were advanced into the brain. Pressure was allowed to rise. The stylet and catheter then were advanced slowly until pressure dropped abruptly to ventricular pressure. The depth in millimeters from the brain surface was noted. The stylet and catheter then were advanced further until pressure again started to rise, at which point they were withdrawn until pressure dropped back to ventricular pressure, and this depth from the brain surface was noted.

The catheter was left at the deepest level possible where ventricular pressure was steady. This was important, since catheters left at the depth where pressure first dropped abruptly were more likely eventually to be sealed by the ventricular lining and become non-functional.

Table 5. Composition of Artificial Cerebral Spinal Fluid (CSF)

	mM/L
NaCl	125.25
KCl	3.10
$CaCl_2 \cdot 2H_2O$	1.20
$MgCl_2 \cdot 6H_2O$	1.00
$NaHCO_3$	24.50
$NaH_2PO_4 \cdot 2H_2O$	0.50
Dextrose*	2.53
Dissolved in distilled water gassed with 5% CO_2	
Total Na Concentration	150.30
Total Cl Concentration	138.80
pH	7.40

* CSF sterilized as individual 100 ml bottles. Dextrose was added to each 100 ml bottle as it was used.

After the correct depth of catheter placement was determined, a silk suture was tied from the lowest tab on the catheter to one of the screws. The catheter was cemented in place with dental acrylic worked into the hole in the skull and around the screws. The dental acrylic used was a cold-cure type (Hygenic Corp., Akron, Ohio) which bonded to the polyvinyl tubing but did not dissolve it. Once the dental acrylic had begun to set, the silk suture around the top of the catheter was cut carefully and the stylet was removed. The infusion line was reconnected directly to the catheter and pressure continued to be monitored to make certain that removal of the stylet had not inadvertantly disturbed the

catheter placement.

The second tab on the catheter was sutured to subcutaneous tissue to provide strain relief. The rest of the catheter was brought through the skin caudad to the incision, and the third tab on the catheter was sutured loosely to the skin. An obturator was placed in the end of the catheter. The curved flap of skin was brought back over the mound of dental acrylic and the skin incision was closed in two layers with interrupted sutures. The flap ensured that the skin incision was not directly over the point of entry of the catheter into the brain, and the curvature of the incision aided wound healing by minimizing strain on the suture line caused by the mound of dental acrylic.

Immediately after surgery each sheep was given 3×10^6 units of a combination of procaine and benzathine penicillin G (H-L-Bi-Pen™, Haver-Lockhart) intramuscularly and 1.92 gm trimethoprim-sulfa (Tribrissin®, Burroughs Wellcome Co.) orally. They were maintained on the trimethoprim-sulfa for an additional three days. Flunixin meglumine (Banamine®, Shering, Inc), a non-steroidal anti-inflammatory drug, was given IV, 1 mg/kg, for the first two days after surgery. This was done in an attempt to eliminate or decrease any adhesions or scar tissue in the ventricles resulting from placement of the ventricular catheter. The sheep were given ten days to two weeks to recover from surgery. During this time they became further accustomed to handling and to the equipment to be used for experiments. The carotid and jugular catheters were filled daily with 1:1000 sodium heparin. The ventricular catheters were left sealed except during IVT experiments.

Experimental Procedures

All experiments were performed with each sheep standing quietly in its cage. One of the carotid artery catheters was connected to a Gould-Statham pressure transducer placed at heart level, and arterial pressure was recorded continuously onto magnetic tape (Hewlett-Packard) and displayed in both pulsatile and mean form on a Gould-Brush oscillograph. Mean arterial pressure data were obtained by averaging blood pressure every 30 seconds over a two-minute period, beginning at the designated time. For example, arterial pressure data five minutes after the start of any infusion represent the average of values taken every 30 seconds, from five minutes to seven minutes after the start of the infusion. Heart rate data were obtained by counting the number of peaks in the pulsatile blood pressure trace over this same two minute period. During control periods, heparinized saline, .3 ml/min, was infused into the carotid artery catheter and, if appropriate, into the venous catheter. Subsequent peripheral drug infusions were substituted for the heparinized saline infusions and given at the same volume flow rate.

In the sheep with electromagnetic flowmeters on their main pulmonary arteries, right atrial pressure and cardiac output also were recorded. Total peripheral resistance was calculated from (arterial pressure - right atrial pressure) divided by cardiac output. Stroke volume was calculated from cardiac output divided by heart rate. Each sheep's flowmeter was calibrated in situ with the indocyanine green dye dilution technique.

During IVT infusion experiments, ventricular pressure also was monitored and recorded as described above. Care was taken when handling

the ventricular catheter to prevent infections. Special packs containing syringes, sponges, gloves, stopcocks, needle hub adaptors, an infusion line (PE 20), and a line to the transducer (PE 240) were gas sterilized with ethylene oxide. The infusion line was filled with sterile artificial CSF and connected by a stopcock to the catheter end of the transducer line. The transducer line was flushed retrograde with sterile CSF before being connected to the transducer.

The ventricular catheter was wiped with ethanol and then a clean sponge was placed under the catheter and secured by hooking it over the crownpiece of the sheep's halter. This kept the catheter tip up out of the sheep's wool. The obturater was removed and placed in a beaker of ethanol for the duration of the experiment. The free end of the ventricular catheter was immersed in an iodine solution (Betadine^{R}, The Purdue Frederick Co., Norwalk, Conn.) for one minute. The tip then was rinsed in two sequential ethanol dips, rinsed with artificial CSF, and attached to the transducer-infusion line via a sterile 16-gauge needle hub adaptor.

In those sheep in which CSF flowed freely from the catheter, the iodine and ethanol dips were performed while the obturator was still in the catheter. This was done to prevent either solution from being drawn up the catheter into the ventricle, in the event that the sheep lowered its head.

During control periods, artificial CSF was infused IVT at a volume flow rate of .039 ml/min. This infusion rate did not alter ventricular pressure and was tolerated easily by the sheep. Subsequent IVT AII infusions were performed by diluting the appropriate amount of AII in artificial CSF and giving it at the same volume flow rate as during

control. Intracerebroventricular experiments were repeated a minimum of four days apart and often seven days apart. Experiments in which AII was given IV or IC were performed in between IVT experiments.

Angiotensin II (Sigma) was prepared as a stock solution of 10 mg/ml in artificial CSF and stored refrigerated. Just prior to use the proper amount was diluted in artificial CSF for IVT experiments or in saline for IC or IV infusions. In pilot experiments, IVT infusions of AII at 10-50 ng/kg/min all caused drinking. The dose of IVT AII used in this study (50 ng/kg/min) was selected because it caused blood pressure to increase approximately 20 mmHg. Vehicle control experiments also were performed. During some experiments, blood was drawn to measure packed cell volume and plasma vasopressin, osmolal, and sodium concentrations. Angiotensin II also was given at a rate of 10 ng/kg/min IC or IV to eight sheep and at 50 ng/kg/min IV to six of these same sheep. The sheep were not allowed to drink during any of the AII infusions, since drinking behavior increases the magnitude of the pressor response (Hoffman et al. 1977; Fitzsimons and Kucharczyk; 1978). Presence or absence of drinking behavior was assessed at the end of the infusions.

In experiments in which blood samples were taken, blood was withdrawn during control and 35 minutes after the start of AII infusion from a carotid artery catheter through a length of polyethylene tubing which was long enough to extend out of the cage, and which held three milliliters of fluid. This allowed the investigator to take blood samples without standing up or approaching (and possibly disturbing) the sheep. Blood was withdrawn into iced syringes. The first ten ml of blood withdrawn were discarded, and then, using a new syringe, the sample was drawn and transferred into iced EDTA tubes. After the blood

sample was taken, the volume of blood withdrawn was replaced with physiologic saline. In these experiments, packed cell volume was determined in microhematocrit tubes after the experiment was over. The rest of the blood was centrifuged immediately at 4^{o} F for five minutes at 2700 rpm. Plasma sodium concentration was determined by flame photometry (Instrumentation Laboratory, Model #943) and plasma osmolality was determined by the freezing point depression technique (Micro-Osmette; Fischer Scientific). Plasma also was stored at -80^{o}C until analyzed for vasopressin concentration, described below.

Vasopressin Assay

Plasma vasopressin concentrations were determined by Dr. Roger Grekin at the University of Michigan, Ann Arbor. The assay has been described previously (Pierce et al., 1984). Briefly, vasopressin was extracted from 2 ml plasma samples with octadecylsilane cartridges (Sep-pak C_{18}; Waters Associates, Milford, MA) and assayed in triplicate. Synthetic arginine vasopressin (Sigma, 350 IU/ng) was used for the standard curve. There was no detectable cross-reactivity with oxytocin, vasotocin, or ACTH, and no interference from unknown plasma factors. The assay could detect as little as 1.25 pg/ml vasopressin.

Statistical Analysis

Where possible, experiments were repeated in each sheep, and data for each sheep represent the average of several experiments of each type. In the results section, "n" identifies the number of sheep used in the experiment, and "r" represents the total number of replicates of the experiment that were performed in those sheep. Statistics were

performed on the data per sheep (n-1 degrees of freedom) and not on the data per replicate.

Mean arterial pressure, heart rate, right atrial pressure, cardiac output, total peripheral resistance, stroke volume, packed cell volume, plasma sodium, and plasma osmolality were compared by the paired t-test or by blocked analysis of variance (ANOVA), as was appropriate. Multiple comparisons were made by Student-Newman-Keuls test. Some plasma vasopressin concentrations were less than the sensitivity of the assay (1.25 pg/ml). These samples were assigned values of 0.63 pg/ml (halfway between 0 or 1.25 pg/ml) and nonparametric statistics (Signed Ranks Test) were used to determine whether IVT AII infusion altered plasma vasopressin concentration. Values of $p<.05$ were considered significant.

Verification of Catheter Placement

All ventricular catheters were injected or infused postmortem with crystal violet or methylene blue dye. The brains were removed from the skulls and cut in half midsagitally. The point of entry and the position of the tip of each catheter was recorded.

RESULTS

Problems encountered with stainless steel ventricular guide cannulae included neurologic deficits, inflammation/infection, and poor durability. Two sheep were sacrificed less than one month after cannula placement when they developed severe head tilts (with the ear on the side of cannula placement held higher than the opposite ear) and loss of equilibrium. In one of these sheep the deficit was preceded by a fever,

suggesting that the source of inflammation was infection. In the second sheep, no fever was detected. Antibiotic and non-steroidal anti-inflammatory therapy returned body temperature to normal in the first sheep, but the neurologic deficits progressed in both sheep. The third sheep was found dead the day after surgery. Although it appeared that the cause of death was enterotoxemia, complications from placement of the ventricular cannula cannot be ruled out. The cannula was malpositioned in the fourth sheep, and the fifth sheep had a seizure one week after catheter placement and sheared off the hub of the cannula.

The problems described above did not occur in the sheep which received polyvinyl ventricular catheters (see Appendix D for individual sheep history). All polyvinyl ventricular catheters entered the posterior part of the lateral ventricle 2 to 18 mm rostral to the splenium of the corpus callosum except one (discussed under "angiotensin II infusions"). One sheep would not eat after surgery. A second developed septicemia, apparently from its carotid artery catheter. These two sheep were sacrificed immediately, before sufficient data could be obtained to include them in the results. In the remaining twelve sheep, the ventricular catheters remained functional an average of 6 ± 1 month, ranging from two months to greater than one year. During this time the sheep remained healthy and peripheral arterial or venous infusions could be performed daily. Intracerebroventricular experiments usually were performed weekly, although it was possible to perform IVT experiments as often as every five days for extended periods of time with no problems encountered. It also was possible to repeat an IVT experiment within two days, but this could not be done habitually without causing slight changes in control blood pressure and increasing

the risk of infection. The relatively low control values of blood pressure and heart rate and the stability of these values from day to day for each sheep indicate that the presence of the ventricular catheters and the experiments performed were neither painful nor stressful for these animals and that the sheep were handled humanely.

Angiotensin II Infusions

Thirteen of the fourteen sheep which received polyvinyl cerebroventricular catheters drank water immediately when they were released after experiments in which AII was given IVT. The sheep were released 15–30 minutes after the AII infusion had been turned off. One sheep drank only rarely after IVT AII, and when she did drink it was only a few sips and usually was delayed 5–10 minutes after being released. At postmortem this sheep's catheter stopped 3 mm above the ventricle. Data from IVT experiments in this sheep have not been included.

Intracerebroventricular infusions of AII, 50 ng/kg/min in eleven conscious sheep (42 replicates), caused a gradual rise in arterial pressure. Thirty minutes after the start of infusion, blood pressure had increased from 86 $\pm$ 1.6 mmHg to 109 $\pm$ 2.5 mmHg ($p<.01$, Figure 11). Blood pressure began to fall as early as 15 minutes after the end of infusion, but did not return to control levels until 30–60 minutes afterwards. Heart rate (78 $\pm$ 3 bpm) did not change. In the three sheep with electromagnetic flowmeters (six replicates), right atrial pressure increased from 1.1 $\pm$ .8 mmHg to 3.7 $\pm$ 1.1 mmHg ($p<.05$) cardiac output (6.5 $\pm$ .2 L/min) did not change, total peripheral resistance increased from 13.14 $\pm$ .86 to 16.34 $\pm$ .70 mmHg min/L ($p<.01$), and stroke volume increased from 86 $\pm$ 2 to 88 $\pm$ 1 ml ($p<.05$).

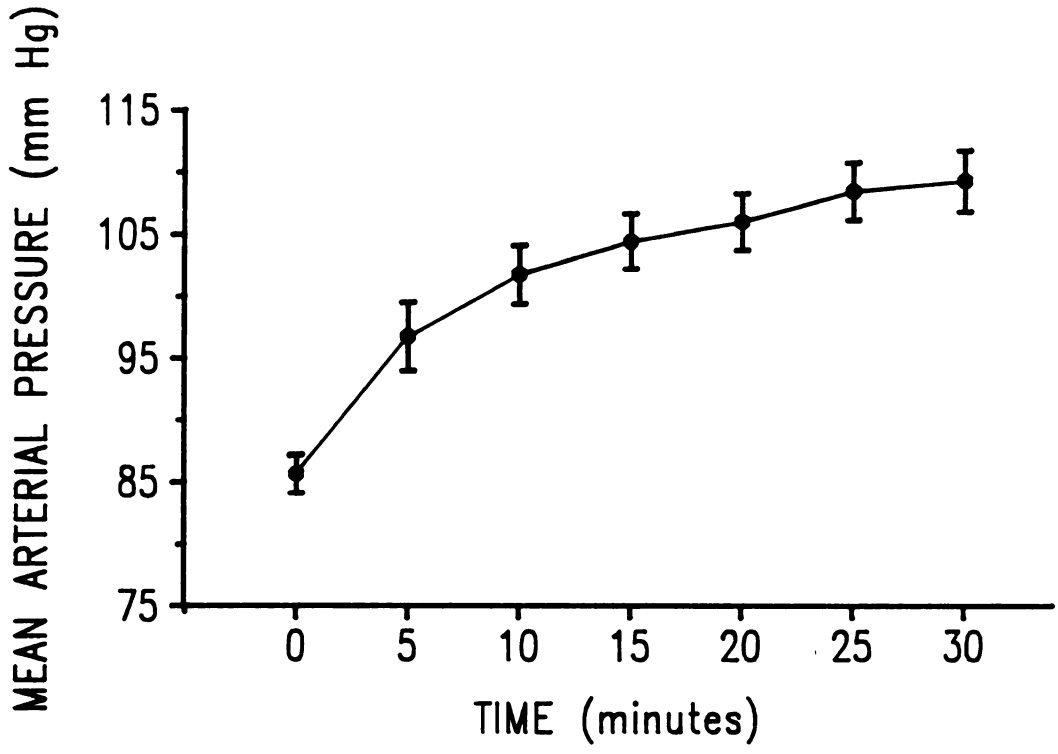


Figure 11. Time course of blood pressure response to IVT AII, 50 ng/kg/min, in eleven conscious sheep. All points differ from control and each other ($p<.05$) except 15 and 20 minutes, which do not differ from each other, and 25 and 30 minutes, which do not differ from each other.

Values of packed cell volume, plasma sodium concentration, plasma osmolality, and plasma vasopressin concentration before and 35 minutes after the start of IVT AII infusion are shown in Table 6. Only plasma vasopressin (which increased) changed significantly ($p<.01$). Although statistics were performed on all the AVP data (see Methods), the value of vasopressin reported during IVT AII infusion is the average value in the five sheep in which it was greater than the sensitivity of the assay. Although plasma osmolality decreased in seven of the nine sheep, the change was not significant ($p=.07$).

Intracerebroventricular infusions of vehicle (CSF) for the same duration as IVT AII had no effect on any variable reported above.

Table 6. Effect of Intracerebroventricular (IVT) Infusion of Angiotensin II (AII), 50 ng/kg/min, on Packed Cell Volume, Plasma Sodium Concentration, Plasma Osmolality, and Plasma Vasopressin Concentration in Conscious Sheep

	n	Control (mean + SEM)		IVT AII (mean + SEM)
Packed Cell Volume (%)	9	27 ± 1		27 ± 1
Plasma [Na^+] (mEq/L)	9	143.3 ± .9		143.0 ± .9
Plasma Osmolality (mOsm/L)	9	309 ± 3		306 ± 3
Plasma Vasopressin (pg/ml)	8	< 1.25*	($p<.05$)	5.90 ± 1.62

* All control values were less than the sensitivity of the assay.

Table 7 summarizes the arterial pressure, heart rate, and drinking responses of eight conscious sheep to IC and IV AII, 10 ng/kg/min, and of six sheep to IV AII, 50 ng/kg/min. The hemodynamic data shown were taken during control and 25 minutes after the start of infusion. With peripheral AII infusions, blood pressure rose quickly (within five minutes), leveled off for the duration of the infusion, and fell rapidly after the infusion was turned off. (Blood pressure began to decrease within minutes after the end of infusion and had returned to control by 15-30 minutes afterwards.) Drinking responses were recorded after the sheep were released, approximately ten minutes after the end of the AII infusions. The "(IC-IV) AII, 10 ng/kg/min" values for changes in arterial pressure and changes in heart rate were calculated to estimate the central component of the responses to peripherally-infused AII at 10 ng/kg/min (Fink et al. 1980). No statistics were performed on these values.

Table 7. Blood Pressure, Heart Rate, and Drinking Responses to Peripheral Infusions of Angiotensin II (AII)

Angiotensin II Route	Dose (ng/kg/min)	Blood Pressure (BP, mmHg) Control mean±SEM	Change in BP	Heart rate (HR, beats/min) Control mean±SEM	Change in HR	Drinking Behavior After Release #yes	#no
IC AII (n=8,r=11)	10	86 ± 1	+30*	77 ± 4	- 4	3	5
IV AII (n=8,r=10)	10	85 ± 1	+25*	85 ± 2	-13*	2	6
(IC-IV) AII (n=8)	10		+ 5		+ 9		
IV AII (n=6,r=6)	50	86 ± 2	+47*	80 ± 5	-10*	1	5

* $p<.01$ re Control

Blood pressure and heart rate data for individual sheep from all experiments presented in this chapter can be found in Appendix E.

DISCUSSION

The use of flexible polyvinyl tubing catheters instead of rigid stainless steel cannulae for catheterization of the lateral cerebral ventricles of sheep has certain advantages. The catheters appear to cause less inflammation, since longer implantation times are possible without development of neurologic abnormalities. This may be due to the difference in material, but it may reflect a difference in the ease of adequately cleaning the free end of the catheter or cannula before infusions. The free ends of the polyvinyl catheters can be lifted up

out of the wool and dipped in disinfectant solutions, whereas this is not possible with the rigid hubs of the steel cannulae. Additionally, the further these hubs stick out from the skull (making them easier to clean) the more susceptible they are to being broken off (sheep #11).

One disadvantage of the polyvinyl catheters is that patency is not easily re-established if they become plugged. In these sheep, patency was maintened longer when the tips of the catheters were left against the tissue at the bottom of the ventricle rather than just barely into the ventricle, since this prevented the ventricular lining from sealing over the catheter. However, leaving the catheter tip against tissue prevents CSF from flowing freely back up the catheter. This makes it necessary to assess proper catheter placement by some other method, and excludes the use of this technique for CSF sampling. The technique can be used for infusions, however, since infusion fluid will flow back up around the catheter into the ventricular space (Hoffman and Phillips, 1976b). Regular weekly infusions into the catheters probably helps to maintain catheter patency.

Drinking behavior in response to IVT infusion of AII can be used in conscious sheep as a premortem indication that the ventricular catheter is patent and is indeed in a cerebral ventricle, despite failure of CSF to flow from the catheter. Phillips (1978) reported that IVT AII is 1000X more potent than IV AII in its ability to cause drinking. In the present study, only one of six sheep drank after 50 ng/kg/min AII IV, whereas 13 of 14 sheep drank after 50 ng/kg/min AII IVT. The only sheep that did not drink in response to IVT AII had a catheter that ended several millimeters above the lateral ventricle. When sheep were sacrificed either because they had lost a drinking response to IVT AII

that they had had earlier or because ventricular infusion pressure was becoming elevated, postmortem examination revealed that their catheters were becoming or had become plugged.

Previous studies with peripherally or centrally-administered AII in sheep and goats have focused on AII's effects on drinking behavior and the hormones of body fluid balance (Andersson and Westbye 1970; Andersson et al. 1972; Andersson 1977; Blair-West et al. 1980; Nicholls et al. 1983). The peripheral rate of AII infusion used in the present study produces circulating levels of AII well within the physiologic range of AII in sheep (Blair-West et al. 1980). The IVT rate of AII infusion was higher than that used in other studies, but was necessary to produce the desired change in blood pressure. This is in agreement with work in conscious rats in which IV AII also produced greater pressor responses than IVT AII (Hoffman and Phillips 1976b).

The results of AII infusions reported here in conscious sheep are comparable to those reported by Bonjour and Malvin (1970) in conscious dogs, and by Mouw et al. (1971) in pentobarbital-anesthetized dogs. In the latter study IV and IC infusions of AII, 10-17 ng/kg/min, produced similar changes in blood pressure to those reported here, and smaller doses of IVT AII (.01-5 ng/kg/min) increased plasma vasopressin concentration without changing blood pressure, plasma sodium concentration, plasma osmolality, or packed cell volume. The present study provides new evidence that the IVT AII-induced increase in blood pressure in conscious sheep is caused by an increase in total peripheral resistance. Cardiac output and heart rate do not change, and right atrial pressure and stroke volume are increased slightly.

VI. CHAPTER FOUR

COMPARISONS OF INTRACAROTID VS. INTRACEREBROVENTRICULAR INFUSIONS OF PGE_2

INTRODUCTION

Prostaglandins of the E series (PGEs) are potent vasodilators when injected peripherally (Moncada et al. 1980), but as early as 1969 Kaplan et al. demonstrated a centrally-mediated vasopressor effect of PGE_1 in anesthetized dogs. Further studies of the central pressor effects of PGE_s have been performed because of their potential role in central arterial pressure regulation (Skarnes et al. 1981; Takahashi and Bunag 1981b). Prostaglandin E_2 is synthesized by brain neural and vascular tissue (Hagen et al. 1979; Abdel-Halim et al. 1980;) and does not circulate, since it is metabolized rapidly by the lungs (Ferreira and Vane 1970; Piper et al. 1970; Moncada et al. 1980;). Thus PGE_2 could act locally in the brain to increase blood pressure without leaking into the periphery to decrease blood pressure. Since the studies of Kaplan et al. (1969), PGE_s have been shown to increase blood pressure when infused into the cerebral blood supply or into cerebral spinal fluid of a variety of species. (Hoffman and Schmid 1979; Kondo et al. 1979; Hoffman et al. 1981; Skarnes et al. 1981; Takahashi and Bunag 1981b; Feurstein et al. 1982; Okuno et al. 1982; Breuhaus and Chimoskey 1983; Hull and Chimoskey 1984).

The present study was performed (1) to determine if PGE_2 increases arterial pressure when infused into the lateral cerebral ventricles (IVT) of conscious sheep and, if so, then (2) to compare dose response curves of IC vs IVT PGE_2, (3) to note any hemodynamic or behavioral differences between the responses to IC and IVT PGE_2, and (4) to measure plasma catecholamines, vasopressin, sodium, and osmolality and packed cell volume before and during IC and IVT PGE_2 infusion in conscious sheep. Evidence is presented that PGE_2 is much less effective at raising arterial pressure when given IVT than when given IC. Despite this, certain plasma changes and behavioral effects, which are not observed when PGE_2 is given IC, occur when PGE_2 is given IVT. Prostaglandin E_2 administered into cerebral spinal fluid may not act at the same sites in the brain as does blood-borne PGE_2.

METHODS

Fifteen mature female sheep ranging in weight from 45 to 80 kg were used in this study. The sheep were housed in individual pens and they were allowed to acclimate to their surroundings and to laboratory personnel before instrumentation. Because sheep are flock animals, at least two sheep were kept in each room. These conditions ensured that the sheep were calm and unstressed during the experiments.

Instrumentation

The methods used to place vascular and cerebral ventricular catheters in sheep were described in Chapter 3.

Experimental Procedures

All experiments were performed with each sheep standing quietly in its cage. One of the carotid artery catheters was connected to a Gould-Statham pressure transducer placed at heart level, and arterial pressure was recorded continuously onto magnetic tape (Hewlett-Packard) and displayed in both pulsatile and mean form on a Gould-Brush oscillograph. During control periods, heparinized saline, .3 ml/min, was infused into the carotid artery catheter and, if appropriate, into the venous catheter. Subsequent peripheral drug infusions were substituted for the heparinized saline infusions and given at the same volume flow rate.

During IVT infusion experiments, ventricular pressure also was monitored and recorded as described above. Care was taken when handling the ventricular catheters to prevent infections. The ventricular catheter tips were disinfected as described in Chapter 3. During control periods, sterile artificial CSF was infused IVT at a volume flow rate of .039 ml/min. This infusion rate did not alter ventricular pressure and was tolerated easily by the sheep. Subsequent IVT PGE_2 or vehicle infusions were performed by diluting the appropriate amount of PGE_2 in artificial CSF and giving it at the same volume flow rate as during control. Intracerebroventricular experiments were repeated a minimum of four days apart and often seven days apart. There was no set order in which experiments were performed, although very high doses of IVT PGE_2 never were given before the individual sheep's sensitivity to IVT PGE_2 was tested using lower doses of PGE_2. Also, because prostaglandins are known mediators of inflammation, doses of PGE_2 greater than 100 ng/kg/min were not repeated in consecutive IVT

experiments. Experiments in which PGE_2 was given IC or IV were performed on days when IVT experiments were not performed.

In experiments in which blood samples were taken, blood was withdrawn during control and 35 minutes after the start of PGE_2 or vehicle infusion from a carotid artery catheter through a length of polyethylene tubing which was long enough to extend out of the cage and which held three milliliters of fluid. This allowed the investigator to take blood samples without standing up or approaching (and possibly disturbing) the sheep. Blood was withdrawn into iced syringes. The first ten ml of blood withdrawn were discarded, and then, using a new syringe, the sample was drawn and transferred into iced EDTA tubes. After the blood sample was taken, the volume of blood withdrawn was replaced with physiologic saline. In these experiments, packed cell volume (PCV) was determined in microhematocrit tubes after the experiment was over. The rest of the blood was centrifuged immediately at 4° F for five minutes at 2700 rpm. Plasma sodium concentration was determined by flame photometry (Instrumentation Laboratory, Model #943) and osmolality was determined by freezing point depression technique (Micro-Osmette). Plasma also was stored at -80°C until it was analyzed for catecholamines and vasopressin, described below.

Intracarotid PGE_2 Infusions: Intracarotid PGE_2 infusions were performed as reported previously (Breuhaus and Chimoskey 1983). For IC infusions, PGE_2 (Upjohn) was made up as a stock solution of 1 mg/ml in ethanol and stored at -20° C. Just prior to use, the proper amount was diluted in physiologic saline at room temperature. Infusions of vehicle were performed by adding the same microliter amount of ethanol (without

PGE_2) as was used for PGE_2 infusions at 10 ng/kg/min. Twelve sheep were used to generate IC PGE_2 dose response curves. All sheep received IC PGE_2 at a rate of 10 ng/kg/min. Seven of these sheep received an infusion of vehicle (0 ng/kg/min), eight received 5 and 30 ng/kg/min, six received 60 ng/kg/min and eight received 100 ng/kg/min. Blood samples for PCV, plasma osmolality, and plasma concentrations of sodium, vasopressin, and circulating catecholamines were collected during exeriments in which PGE_2 was given IC at 10 ng/kg/min and at 100 ng/kg/min. Body temperature was recorded in seven sheep before and during IC infusion of PGE_2, 10 ng/kg/min.

Intracerebroventricular PGE2 Infusions: Twelve sheep received IVT PGE_2 in doses ranging from 0 ng/kg/min (vehicle) to 1000 ng/kg/min. Infusions of vehicle were performed by adding the same microliter amount of ethanol (without PGE_2) as was used for infusions of PGE_2 at 300 ng/kg/min. Seven sheep received infusions of vehicle; nine received 10, 30, and 100 ng/kg/min; all twelve received 300 ng/kg/min; four received 750 ng/kg/min; and three received 1000 ng/kg/min IVT PGE_2. Only sheep that showed little or no change in blood pressure at 300 ng/kg/min IVT PGE_2 received higher doses. For IVT infusions, PGE_2 was made up in stock solutions of 10 mg/ml ethanol and 100 mg/ml ethanol to minimize the amount of ethanol infused into the ventricles, especially at the higher doses of PGE_2. The stock solutions were stored at -20° C, and just prior to use the proper amount of PGE_2 was diluted in artificial CSF at room temperature. An attempt was made to find an IVT PGE_2 infusion rate which caused a similar increase in blood pressure as did 10 ng/kg/min IC PGE_2 in each sheep. This was not always possible.

Blood samples for PCV, plasma sodium, plasma osmolality, vasopressin, and circulating catecholamines were taken during infusion of PGE_2 at 300 ng/kg/min IVT. Body temperature was recorded in seven sheep before and during IVT PGE_2 infusions.

Intravenous PGE_2 Infusions: Intravenous (IV) PGE_2, 10 ng/kg/min, was infused in six sheep. This was done to determine whether the serous nasal discharge observed during IC and IVT PGE_2 was caused by a central action of PGE_2 on the lungs, and will be explained in the discussion.

Adrenergic Blockade of IVT PGE2: In four sheep, the ability of adrenoceptor blockade to prevent the IVT PGE_2-induced increase in blood pressure was tested. Alpha-adrenoceptor blockade was obtained with phentolamine, 1 mg/kg IV given five minutes before the start of IVT PGE_2 infusion. Because phentolamine alone causes pronounced tachycardia in conscious sheep (Hull and Chimoskey 1984), the beta-adrenoceptor blocker propranolol, 1 mg/kg IV, was given ten minutes before administration of phentolamine. Adequacy of phentolamine blockade (tested with phenylephrine) and of propranolol blockade (tested with isoproterenol) will be described in the results. Separate experiments were performed in the same four sheep to determine the hemodynamic effects of adrenoceptor blockade alone.

Catecholamine Assay

Plasma was acidified by the addition of 2N perchloric acid containing 100 mg/100 ml EGTA (1 part acid to 9 parts plasma). Plasma concentrations of norepinephrine and epinephrine were measured by a

modification of a radioenzymatic assay described by Umezu and Moore (1979). The assay was run on three separate occasions. Sensitivity of the assay was 125 pg/ml on two occasions and 250 pg/ml on one occasion.

Vasopressin Assay

Plasma vasopressin concentrations were determined for us by Dr. Roger Grekin at the University of Michigan, Ann Arbor. The assay has been described previously (Pierce et al. 1984). Briefly, vasopressin was extracted from 2 ml plasma samples with octadecylsilane cartridges (Sep-pak C_{18}; Waters Associates, Milford, MA) and assayed in triplicate. Synthetic arginine vasopressin (Sigma, 350 IU/ng) was used for the standard curve. There was no detectable cross-reactivity with oxytocin, vasotocin, or ACTH, and no interference from unknown plasma factors. The assay could detect as little as 1.25 pg/ml vasopressin.

Statistical Analysis

Where possible, experiments were repeated in each sheep, and data for each sheep represent the average of several experiments of each type. Statistical analyses were performed on the data per sheep and not on the data per replicate.

Mean arterial pressure, heart rate, PCV, plasma sodium concentration, plasma osmolality, plasma catecholamines, and body temperature data were compared by the paired t-test or by blocked analysis of variance (ANOVA), as was appropriate. Multiple comparisons were made by Student-Newman-Keuls test. One-way ANOVA was used to compare blood pressure and heart rate responses to different doses of PGE_2, since not every animal received every dose of PGE_2. Some plasma

samples had less vasopressin than was detectable by the assay (1.25 pg/ml). These samples were assigned values of 0.63 pg/ml (halfway between 0 and 1.25 pg/ml) and a nonparametric test, the Signed Ranks test, was used to determine whether PGE_2 altered plasma vasopressin concentration. Values of $p<.05$ were considered significant. Results are expressed as means $\pm$ standard errors of the mean (SEM), or for paired samples, means and the standard error of the difference (SED).

Verification of Ventricular Catheter Placement

Proper positioning of the ventricular catheters and free access of infusions to CSF were tested before death by observations of the presence or absence of drinking responses to infusions of angiotensin II, 50 ng/kg/min IVT (Chapter 3; Hoffman and Phillips 1976b). If, after a period of time, a sheep lost its drinking response to IVT angiotensin, no data were used from IVT experiments performed after the last IVT angiotensin experiment in which the sheep drank.

All ventricular catheters were injected or infused postmortem with crystal violet or methylene blue dye. The brains were removed from the skulls and cut in half midsagitally. The point of entry and the position of the tip of each catheter in the lateral cerebral ventricle was verified.

RESULTS

Of the fifteen sheep used in the study, fourteen were prepared surgically with polyvinyl ventricular catheters as described above. One sheep did not drink in response to IVT angiotensin II and, at postmortem examination, the tip of its ventricular catheter was several millimeters

above the lateral ventricle. Data from IVT experiments in this sheep were not included.

Intracarotid PGE_2 Infusions

Figure 12 shows blood pressure and heart rate responses to increasing doses of IC PGE_2 in twelve conscious sheep. (Not every sheep received every dose of PGE_2, as indicated in "Methods".) The dose-response curve for IC PGE_2 is limited; doses greater than 30 ng/kg/min cause no greater steady-state increases in blood pressure than the increases caused by 30 ng/kg/min, although blood pressure does rise more quickly at higher doses.

Intracarotid PGE_2 caused a small increase in body temperature which was significant ($p<.01$, Table 8) because it increased in every sheep. Packed cell volume and plasma sodium, osmolality, vasopressin, epinephrine, and norepinephrine did not change during IC PGE_2, 10 ng/kg/min. Intracarotid PGE_2, 100 ng/kg/min, did increase PCV ($p<.01$, Table 8).

Intracarotid PGE_2 infusions produced a serous nasal discharge and sneezing at all doses in all animals. At higher doses (60 and 100 ng/kg/min IC), PGE_2 caused slight shivering in some sheep. Often the sheep urinated and defecated as IC PGE_2 began to increase blood pressure and heart rate. However, the sheep showed no signs of discomfort, alarm, or excitement during IC PGE_2 infusion and often chewed their cuds. They showed no tendency to drink after PGE_2, but usually went straight to their feed buckets when released after an experiment.

Intracarotid infusions of vehicle had no effect on any reported above.

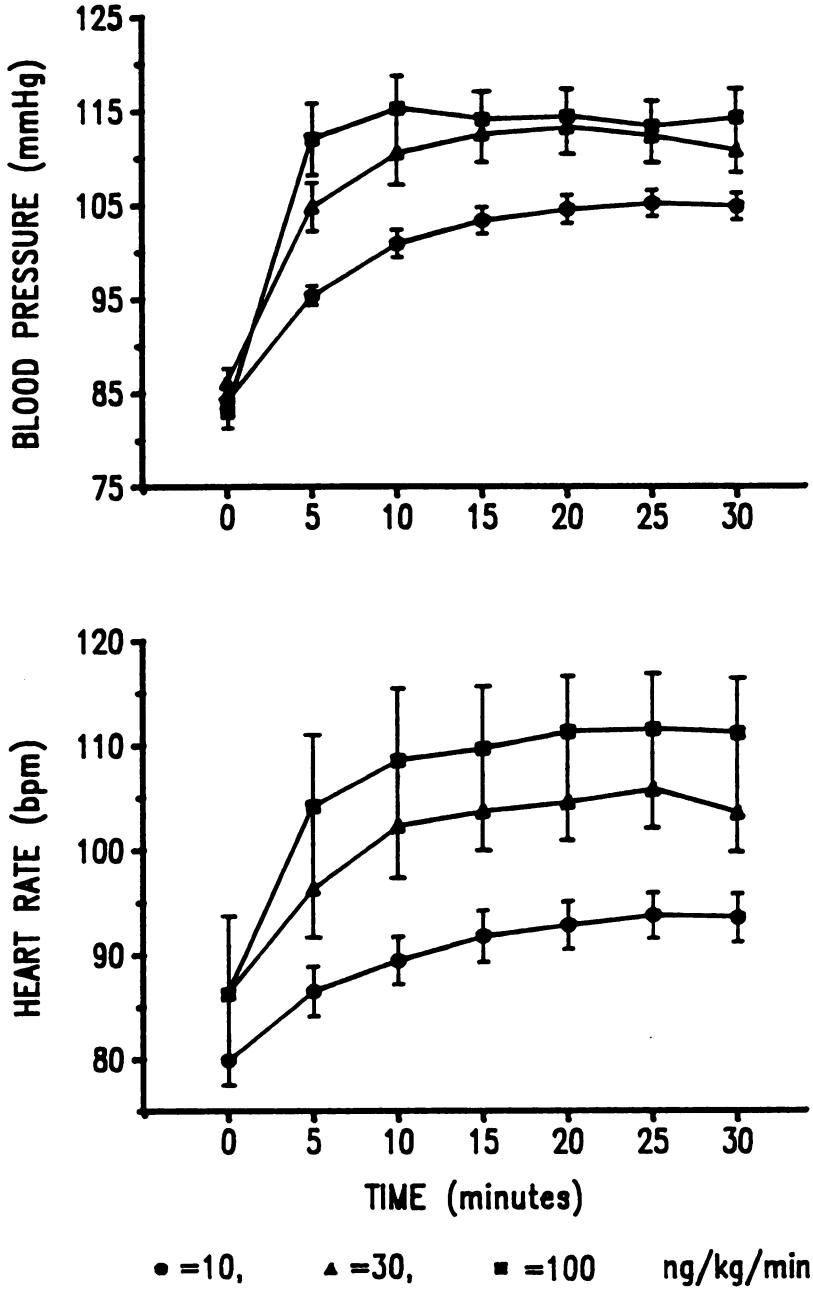


Figure 12. Intracarotid PGE_2 at three doses. **A.** Time course of blood pressure responses. **B.** Time course of heart rate responses. Blood pressure and heart rate are significantly greater than control at all time points for all doses. At 10 ng/kg/min, PGE_2 reaches steady state 15 minutes after the start of infusion. At 30 ng/kg/min, PGE_2 reaches steady state at 10 minutes. At 100 ng/kg/min, PGE_2 reaches steady state at 5 minutes. (Control values for blood pressure and heart rate are not significantly different from each other).

Table 8. Hemodynamic, Hematologic, and Humoral Responses to Centrally-Administered PGE_2

Route, Dose:	IC, 10 ng/kg/min			IC, 100 ng/kg/min			IVT, 300 ng/kg/min		
	Control	PGE_2	(SED)[a]	Control	PGE_2	(SED)	Control	PGE_2	(SED)
Blood Pressure (mmHg)	84	105**	(1.4)	85	114**	(2.4)	85	94**	(2.3)
Heart Rate (bpm)	80	94**	(1.5)	86	111**	(3.6)	75	78	(2.2)
Body Temperature (^{o}F)	101.0	101.7**	(.07)	---	---	---	100.7	100.9	(.26)
Packed Cell Volume (%)	25.3	25.9	(.44)	26.3	29.4**	(.73)	26.8	30.1**	(.91)
Plasma Sodium (mEq/L)	140.0	138.5	(.89)	142.1	141.8	(.27)	143.5	141.7**	(.47)
Plasma Osmolality (mOsm/L)	302	303	(2.5)	309	306[b]	(1.5)	308	306*	(1.0)
Vasopressin (pg/ml)	1.17	1.27	(.16)	1.40	2.38	(.75)	1.26	3.15*	(.93)
Epinephrine (pg/ml)	158	166	(31)	358	350	(17)	332	343	(37)
Norepinephrine (pg/ml)	322	275	(73)	652	655	(61)	518	646	(99)

a Standard error of the difference.
b p=.07

* p<.05 re Control
** p<.01 re Control

Intracerebroventricular PGE2 Infusions

The blood pressure responses of eleven sheep to doses of IVT PGE_2 from 10 to 300 ng/kg/min are shown in Figure 13. Blood pressure did not increase in four of the eleven sheep, and larger doses of PGE_2 in these sheep (to 1000 ng/kg/min) also were ineffective. Greater doses of IVT PGE_2 were not used because of the occurrence of side effects or toxicity described below. The increases in arterial pressure in response to IVT PGE_2 were not as great as the increases in blood pressure in response to IC PGE_2 (One-way ANOVA, $p<.05$, Figure 14).

Heart rate did not change at any dose of IVT PGE_2. In seven of these sheep in which body temperature was measured during IVT PGE_2 infusions, there were no significant changes in body temperature at any dose 35 minutes after the start of infusion. Intracerebroventricular PGE_2 significantly increased PCV ($p<.01$), decreased plasma sodium concentration ($p<.01$), decreased plasma osmolality ($p<.05$), and increased plasma vasopressin concentration ($p<.05$). Plasma concentrations of circulating catecholamines did not change (Table 8).

Intracerebroventricular PGE_2 caused shivering in all sheep at some dose. In general, shivering began at doses of PGE_2 that did not increase blood pressure as much as did 10 ng/kg/min IC PGE_2 (an IC dose at which no shivering occurs). In fact, one sheep began to shiver and showed other side effects of IVT PGE_2 at 30 ng/kg/min, yet never had much of a hemodynamic response to IVT PGE_2, even at 1000 ng/kg/min.

A serous nasal discharge and sneezing also occurred during IVT PGE_2 infusions in all sheep, regardless of whether or not they responded hemodynamically. Other effects of IVT PGE_2 which were not observed during IC PGE_2 infusion were: pupillary constriction in eight of eleven

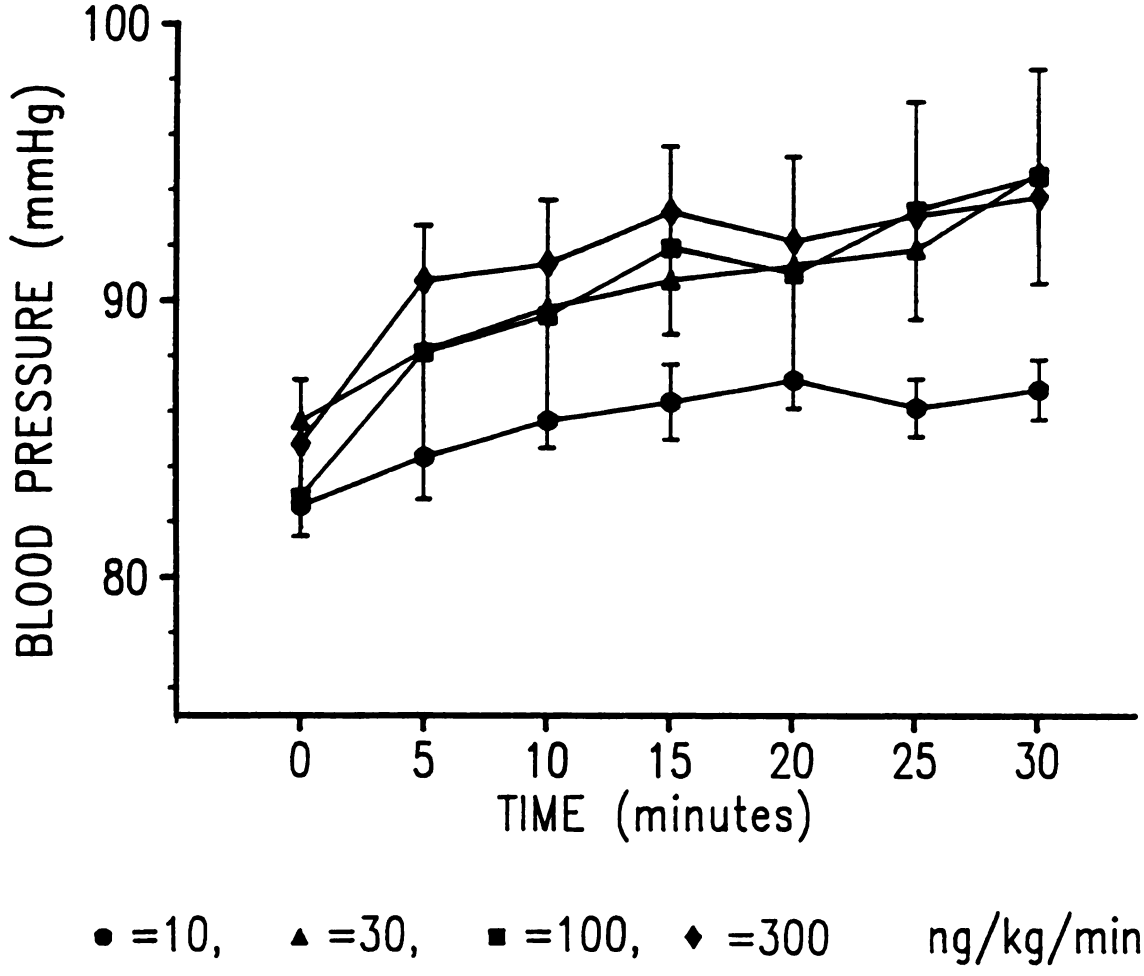


Figure 13. Time course of blood pressure responses to IVT PGE_2 at four doses in eleven conscious sheep. For all doses, all time points are significantly greater than control but are not different from each other. Steady state was reached 5 minutes after the start of infusion. (Control values for all doses are not significantly different from each other).

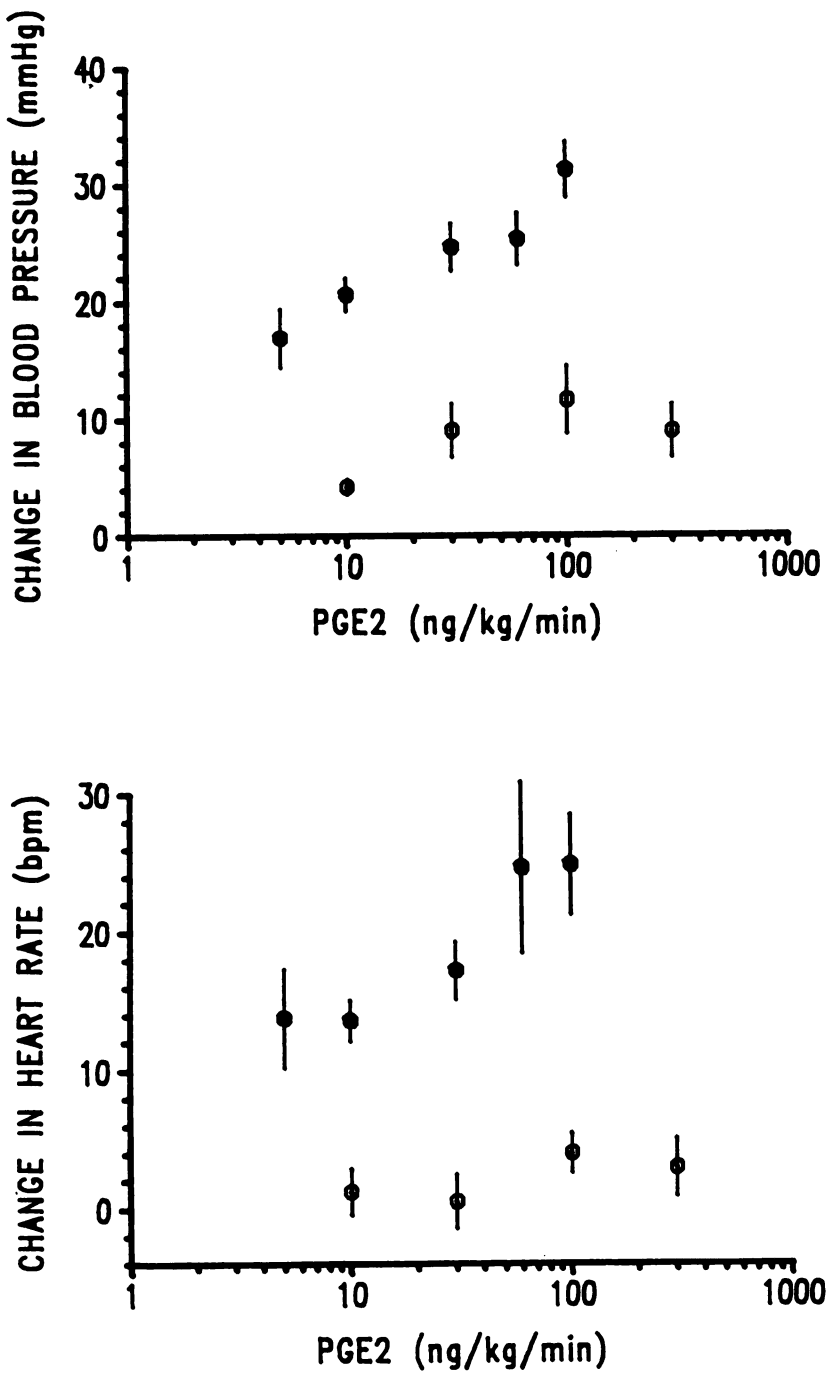


Figure 14. Comparison of log dose response data for intracarotid PGE_2 (solid circles) and IVT PGE_2 (open circles). Intracarotid PGE_2 produces significantly greater pressor and tachycardic responses than IVT PGE_2 at all doses. (IVT PGE_2 does not change heart rate). **A.** Changes in blood pressure. **B.** Changes in heart rate.

sheep, a dry hacking cough near the end of the 35 minute infusion period or after the PGE_2 infusion had been turned off in all sheep (but not during every experiment), and vocalization in seven out of eleven sheep. The frequency of vocalization and the length of time of infusion before the onset of vocalization appeared to be dose-related. Those sheep that vocalized showed no signs of pain or discomfort. Vocalization did not result in further increases in blood pressure in those sheep that had a pressor response to IVT PGE_2, and it did not produce increases in blood pressure or heart rate in sheep that otherwise had no pressor response to IVT PGE_2.

Intracerebroventricular infusions of vehicle had no effect on any variable reported above.

Blood pressure and heart rate data for individual sheep from these experiments can be found in Appendix F.

Intravenous PGE2 Infusions

During IV PGE_2 infusion, 10 ng/kg/min in six conscious sheep, there were no changes in blood pressure or heart rate. No nasal discharge occurred. There was no visible effect of IV PGE_2 at this dose.

Adrenergic Blockade of IVT PGE2

Adequacy of propranolol blockade was tested with isoproterenol. Before propranolol, 5 ug isoproterenol increased heart rate 67 $\pm$ 7 bpm. Sixty minutes after administration of propranolol, 25 ug isoproterenol increased heart rate 12 $\pm$ 6 bpm. Adequacy of phentolamine blockade was tested with phenylephrine. Before phentolamine, 100 ug phenylephrine increased blood pressure 25 $\pm$ 4 mmHg and decreased heart rate 20 $\pm$ 5

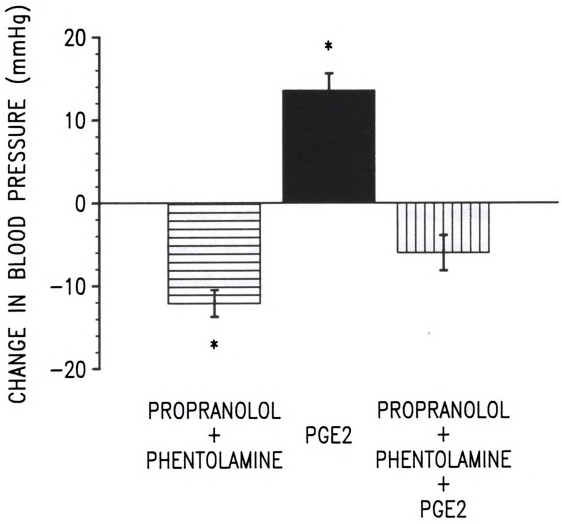


Figure 15. Changes in blood pressure during three experimental protocols in the same four sheep. The first bar shows the change in blood pressure 45 minutes after the administration of propranolol and 35 minutes after the administration of phentolamine. The second bar shows the pressor response of these sheep to IVT PGE_2, 300 ng/kg/min, 30 minutes after the start of infusion. The last bar shows that when propranolol and pentolamine are given prior to PGE_2 infusion, there is no change in blood pressure 30 minutes after the start of PGE_2 infusion. Asterisks indicate that blood pressure is significantly different from control, $p<.05$.

bpm. Sixty minutes after phentolamine, 1000 ug phenylephrine increased blood pressure 26 ± 5 mmHg and decreased heart rate 27 ± 5 bpm.

The combination of propranolol and phentolamine had no effect on heart rate, but decreased blood pressure (Figure 15). Propranolol and phentolamine prevented the PGE_2-induced increase in blood pressure (Figure 15).

DISCUSSION

This laboratory (Breuhaus and Chimoskey 1983, 1984a, 1984b; Hull and Chimoskey 1984) has shown that intracarotid (IC) infusions of PGE_2, 10 ng/kg/min, increase arterial pressure and heart rate in conscious sheep, dogs and calves. The increase in arterial pressure is caused by an increase in total peripheral resistance mediated by increased alpha-adrenergic vasomotor tone, although there also is a slight, but significant, increase in cardiac output. Blood flow in certain regional vascular beds, measured in calves, is decreased; renal, cranial mesenteric, and iliac resistances are increased. The increase in heart rate is caused by both cardiac beta-adrenergic activation and by inhibition of resting cardiac parasympathetic tone. The baroreflex was studied over a limited range and found to be reset without a change in sensitivity. The resetting of the baroreflex probably was central in origin, since the effect of IC PGE_2 on blood pressure is not mediated by direct action at the carotid sinus baroreceptors (Breuhaus and Chimoskey 1983) and since IC PGE_2 has no direct action on carotid sinus nerve firing (McQueen and Belmonte 1974).

In the present study, IVT PGE_2 was less potent than IC PGE_2 in terms of its hemodynamic effects. Intracarotid PGE_2 increased blood pressure

and heart rate in all sheep, whereas IVT PGE_2 had no effect on blood pressure or heart rate in four sheep and increased heart rate in only four of the seven sheep in which it did have a pressor effect. One possible explanation for this is that PGE_2 is removed rapidly from CSF by the choroid plexus and transported to venous blood (Bito 1972, 1975a, 1975b). Once there, PGE_2 would circulate to the lungs and be metabolized (Ferreira et al. 1967; Piper et al. 1970; Moncada et al. 1980). This laboratory has shown previously that 400 ng/kg/min PGE_2 causes no change in blood pressure or heart rate when infused into the right atria of conscious calves (Hull and Chimoskey 1984).

Despite its less potent hemodynamic effects, IVT PGE_2 produced some changes that are not observed during IC PGE_2 infusion, including behavioral changes and changes in plasma sodium concentration, plasma osmolality, and plasma vasopressin concentration. In this study, the largest increases in plasma vasopressin concentration were observed in sheep that had no pressor response to IVT PGE_2. This observation and the fact that adrenergic blockade completely prevented the PGE_2-induced pressor response, confirm that vasopressin release does not contribute to the pressor response to PGE_2 in conscious sheep. Increased plasma levels of vasopressin may account for the decreases in plasma osmolality and plasma sodium concentration, however, since vasopressin causes antidiuresis and natriuresis (Chan and Sawyer 1962). Urinary water and sodium excretion were not measured in this study, but were found to be increased by IVT PGE_2 in conscious goats (Andersson and Leksell 1975; Leksell 1976). Since there was no evidence of loss of plasma volume during IVT PGE_2 infusion (plasma osmolality was decreased instead of increased), the increase in packed cell volume probably was due to

splenic contraction rather than due to hemoconcentration.

Prostaglandin E_2 was infused IV to determine whether it would cause a serous nasal discharge, an effect seen during IC and IVT PGE_2 infusions. This was done to determine whether the nasal discharge was due to facial vasodilation, was a central effect of PGE_2, or whether it might be caused by action of PGE_2 returning in the venous system to the lungs to increase pulmonary secretions. Since IV PGE_2 did not produce a nasal discharge (thus eliminating the last possibility) and since IVT PGE_2 did produce a serous nasal discharge (thus eliminating the first possibility) it appears that this effect of IC and IVT PGE_2 is centrally-mediated.

We conclude that PGE_2 given IVT may not reach the same sites in the brain as IC PGE_2, or it may reach the same sites in different concentrations. Since PGE_2 is synthesized by brain vascular tissue (Hagen et al. 1979; Abdel-Halim et al. 1980), it is likely that endogenous PGE_2 gains entry to cardiovascular centers in the brain from the blood route rather than from CSF. This does not exclude the possibility that PGE_2 could participate in cardiovascular regulation through synthesis by neural tissue in discrete areas of the brain.

VII. CHAPTER FIVE

CENTRAL ANGIOTENSIN II AND PGE_2 ACT INDEPENDENTLY TO INCREASE BLOOD PRESSURE IN CONSCIOUS SHEEP

INTRODUCTION

It is well-known that prostaglandin synthesis and renin release are interrelated in the kidney. Prostaglandins cause renin release from the kidney (Larsson et al. 1974; Spokas et al. 1982; Freeman et al. 1984) and chronic infusions of PGE_2 into the renal arteries of conscious dogs increase plasma renin activity (Hockel and Cowley 1979). Conversely, angiotensin II (AII) stimulates prostaglandin release from several tissues (McGiff et al. 1970; Gimbrone and Alexander 1975; Terragno 1981; Nasjletti and Malik 1982).

Central effects of PGE_2 and AII are similar: both raise arterial pressure by increasing sympathetic nerve activity and causing vasopressin release (Hoffman and Phillips 1976; Takahashi and Bunag 1981b; Unger et al. 1981), both cause antidiuresis and natriuresis, and the actions of both are potentiated by hypertonic saline (Andersson and Leksell 1975; Leksell 1976; Kapsha and Severs 1981). It has been shown that chronic intrarenal infusions of PGE_2 in conscious dogs produce mild hypertension which can be reversed by angiotensin receptor or converting-enzyme blockade (Hockel and Cowley 1980). It has been suggested that intracerebroventricular (IVT) AII causes vasopressin

release by stimulating PGE_2 synthesis in the brain (Share 1979).

This study was performed to determine whether PGE_2 and AII act in series in the brain to increase blood pressure. Specifically, we tested whether central AII-receptor blockade prevents the pressor effect of centrally-administered PGE_2 or whether prostaglandin synthesis blockade prevents the pressor response to centrally-administered AII.

METHODS

Instrumentation

Nine mature female sheep (50-80 kg) underwent sterile surgery for placement of vascular catheters and cerebral ventricular catheters as described in Chapter 3.

Experimental Procedures

All experiments were performed with each sheep standing quietly in its cage. One of the carotid artery catheters was connected to a Gould-Statham pressure transducer placed at heart level, and arterial pressure was recorded continuously onto magnetic tape (Hewlett-Packard) and displayed in both pulsatile and mean form on a Gould-Brush oscillograph.

Intracarotid PGE2 Infusions: Intracarotid PGE_2 infusions were performed as reported previously (Breuhaus and Chimoskey 1983). During control periods, heparinized saline, .3 ml/min, was infused into the carotid artery catheter. Prostaglandin E_2 (Upjohn) was made up as a stock solution of 1 mg/ml in ethanol and stored at $-20^{\circ}C$. Just prior to

use, enough PGE_2 for a 35 minute infusion was diluted in physiologic saline at room temperature and substituted for the heparinized saline.

Intracerebroventricular Infusions: During IVT infusion experiments, ventricular pressure also was recorded. Care was taken when handling the ventricular catheters to prevent infections. The ventricular catheter tips were disinfected and attached to a sterile transducer/infusion line via a sterile 16-gauge needle hub adaptor. During control periods, sterile artificial CSF was infused IVT at a volume flow rate of .039 ml/min. This infusion rate did not alter ventricular pressure and was tolerated easily by the sheep. Subsequent IVT drug infusions were performed by diluting the proper amount of drug in artificial CSF and giving it at the same volume flow rate as during control. (For IVT experiments, stock solutions of drugs were made up as follows: PGE_2, 100 mg/ml in ethanol stored at $-20^{\circ}C$; AII (Sigma), 10 mg/ml in artificial CSF stored at $4^{\circ}C$; and [Sar^1Thr^8] AII (Sarthran, Sigma), 20 mg/ml in artificial CSF stored at $4^{\circ}C$.)

AII-Receptor Blockade of Central PGE2: The first series of experiments tested whether central administration of PGE_2 in conscious sheep exerts its pressor effect through activation of the brain renin-angiotensin system. Each sheep's hemodynamic response to intracarotid (IC) PGE_2, 10 ng/kg/min, and to IVT PGE_2, 300 ng/kg/min, infused for 35 minutes (Chapter 4; Chimoskey and Breuhaus 1985) was

first characterized. Next, a dose of AII was found which, when given IVT for 35 minutes, increased blood pressure as much as PGE_2. This dose was found to be 50 ng/kg/min (Chapter 3). A dose of sarthran was then found which, when also given IVT, prevented the IVT AII pressor response. In pilot studies this dose was found to be 1000 ng/kg/min. Sarthran was started 15 minutes before and continued during the IVT AII infusion. Finally, the ability of 1000 ng/kg/min IVT sarthran to prevent the pressor responses to 10 ng/kg/min IC PGE_2 or to 300 ng/kg/min IVT PGE_2 was tested. Again, the sarthran infusion was started 15 minutes before and continued during the PGE_2 infusions. Sarthran was given by itself for 50 minutes to determine whether it had any effect on resting blood pressure. Intracarotid and IVT vehicle infusions also were performed.

Prostaglandin Synthesis Blockade of Central AII: The second series of experiments tested whether prostaglandin synthesis blockade prevents the pressor response to IVT AII in conscious sheep. Prostaglandin synthesis blockade was obtained with two different types of cyclo-oxygenase inhibitors and two different protocols. In the first protocol, six sheep received indomethacin, 4 mg/kg, dissolved in sesame oil and given subcutaneously two hours before IVT AII infusion. This dose and method of administration of indomethacin have been shown to prevent PGE_2 synthesis and the central pressor and hyperthermic

responses to endotoxin in conscious sheep. The blockade is effective within one hour of administration of indomethacin and lasts at least four hours (Skarnes et al. 1981). In this study IVT AII was given well within the known effective period of indomethacin (IVT AII was infused from 120 to 155 minutes after injection of indomethacin). Additionally, the efficacy of indomethacin blockade was tested in five sheep by determining whether it would prevent the pressor response to IC arachidonic acid (see below).

In the second protocol, five sheep were given flunixin meglumine, 3 mg/kg intravenously, thirty minutes before IVT AII infusion. This dose is 3X the recommended clinical dose, which is supposed to prevent prostaglandin synthesis for 24 hours. Efficacy of prostaglandin synthesis blockade was tested in five sheep in the following way. A dose of arachidonic acid was found which increased blood pressure. On different days, either indomethacin was given subcutaneously two hours before IC arachidonic acid infusion or flunixin meglumine was given one half hour before IC arachidonic acid infusion.

Statistical Analysis

Blood pressure and heart rate data were compared by the paired t test or by blocked analysis of variance (ANOVA), as was appropriate. Multiple comparisons were performed with Student-Newman-Keuls test. Values of $p<.05$ are considered significant. Results are expressed as means $\pm$ standard error of the mean (SEM).

Verification of Ventricular Catheter Placement

Proper positioning of the ventricular catheters and free access of infusions to CSF were tested before death by observations of the presence or absence of drinking responses to infusions of angiotensin II, 50 ng/kg/min IVT (Chapter 3; Hoffman and Phillips 1976b). If, after a period of time, a sheep lost its drinking response to IVT angiotensin, no data were used from IVT experiments performed after the last IVT angiotensin experiment in which the sheep did drink.

All ventricular catheters were injected or infused postmortem with crystal violet or methylene blue dye. The brains were removed from the skulls and cut in half midsagitally. The point of entry and the position of the tip of each catheter in the lateral cerebral ventricle was verified.

RESULTS

One sheep did not drink after IVT AII and at postmortem this sheep's ventricular catheter had become sealed. Data from this sheep were not included. All other sheep drank after IVT AII and their ventricular catheters were positioned properly in their lateral cerebral ventricles.

AII-Receptor Blockade of Central PGE2: Intracerebroventricular AII, 50 ng/kg/min, caused a gradual rise in blood pressure which reached steady state 15 minutes after the start of infusion (Figure 16). At 30 minutes, blood pressure had increased from 87 ± 1.4 to 110 ± 2.7 mmHg ($p<.01$). Heart rate did not change. Intracerebroventricular sarthran,

1000 ng/kg/min, had no effect on blood pressure or heart rate, but prevented the IVT AII pressor response (Figure 16) and the IVT AII drinking response.

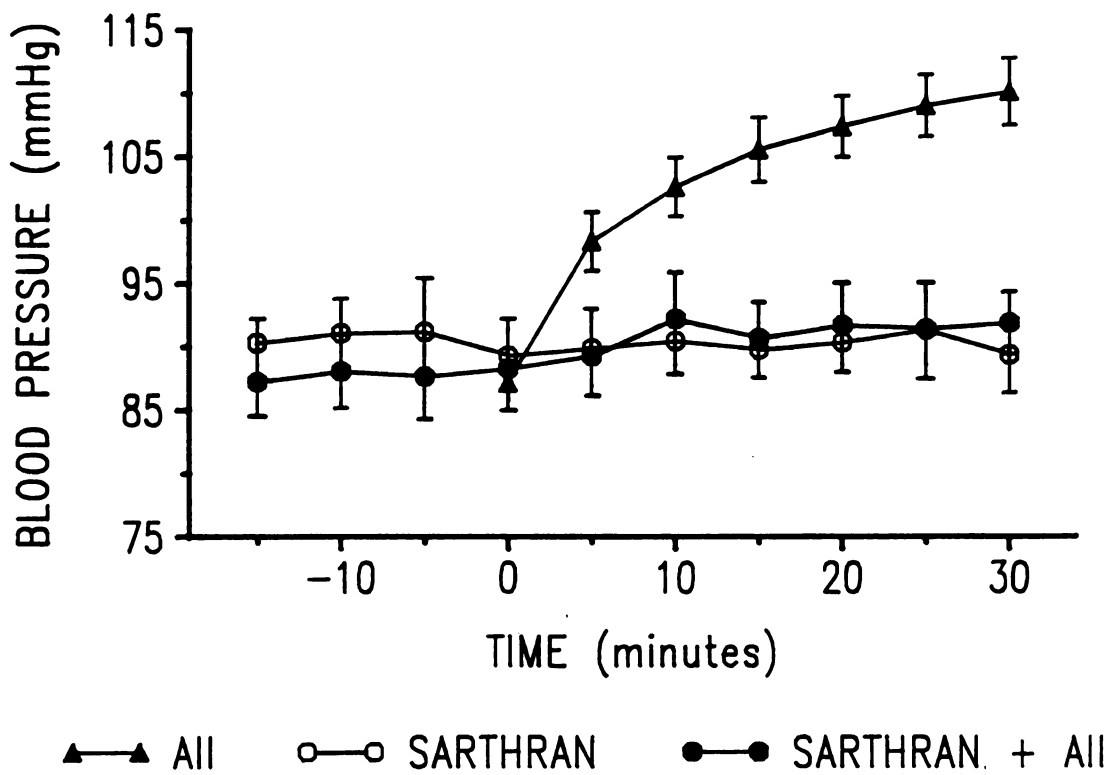


Figure 16. Blood pressure responses to IVT AII, 50 ng/kg/min from 0 to 30 minutes; to IVT sarthran, 1000 ng/kg/min from -15 to 30 minutes; and to the combination of IVT sarthran started at -15 minutes and IVT AII started at 0 minutes. During IVT AII infusion alone, blood pressure is greater than control ($p<.05$) after time 0, and reaches steady state at 20 minutes (ie. pressures at times 20-30 minutes are not different from each other). IVT sarthran and IVT sarthran + IVT AII do not alter blood pressure and are not different from each other.

In addition to a gradual increase in blood pressure (Figure 17), IC PGE_2, 10 ng/kg/min, caused an increase in heart rate. Steady state was reached at 15 minutes. At 30 minutes, blood pressure and heart rate had increased from 85 $\pm$ 1.6 to 106 $\pm$ 2.0 mmHg ($p<.01$) and from 80 $\pm$ 3.0 to 93 $\pm$ 2.8 bpm ($p<.01$). Intracarotid infusion of vehicle (the same microliter amount of ethanol diluted in saline) had no effect on blood pressure or heart rate. Sarthran, 1000 ng/kg/min IVT, did not alter the pressor (Figure 17) or tachycardic responses to IC PGE_2.

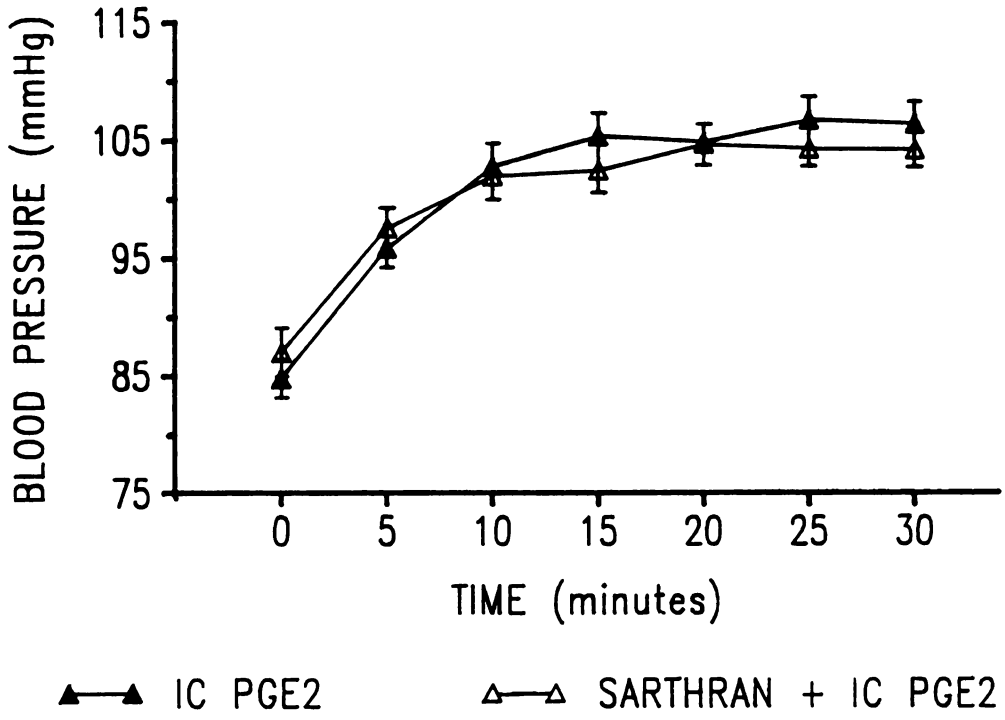


Figure 17. Blood pressure responses to IC PGE_2, 10 ng/kg/min from 0 to 30 minutes; and to the combination of IVT sarthran, 1000 ng/kg/min started at -15 minutes, and IC PGE_2 started at 0 minutes. There is no difference in the blood pressure responses to IC PGE_2 with or without sarthran pretreatment. For IC PGE_2 infusion alone, blood pressure is greater than control ($p<.05$) from 5-30 minutes after the start of infusion, and reaches steady state at 15 minutes (ie. pressures at times 15-30 minutes are not different from each other). For sarthran + IC PGE_2, blood pressure is greater than control ($p<.05$) from 5-30 minutes after the start of infusion, and reaches steady state at 10 minutes.

Intracerebroventricular PGE_2, 300 ng/kg/min, increased blood pressure from 86 $\pm$ 2.0 to 100 $\pm$ 3.9 mmHg ($p<.01$) 30 minutes after the start of infusion (Figure 18). Heart rate did not change. Intracerebroventricular infusion of vehicle (the same microliter amount of ethanol diluted in artificial CSF) had no effect on blood pressure or heart rate. Sarthran, 1000 ng/kg/min IVT, did not alter the pressor response to IVT PGE_2 (Figure 18).

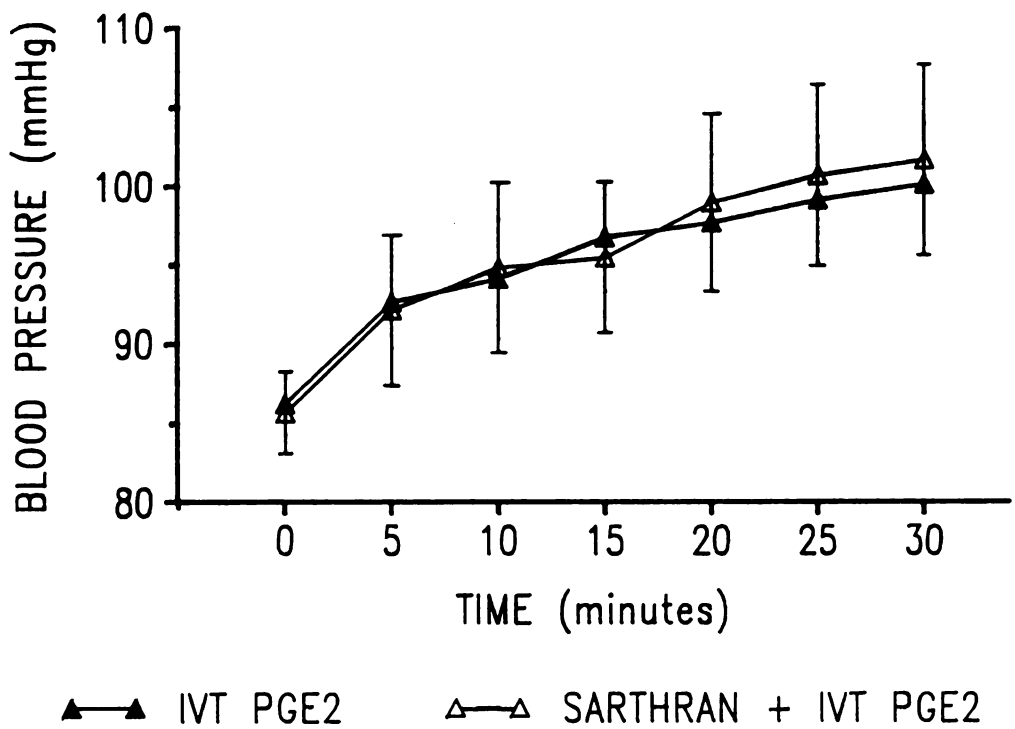


Figure 18. Blood pressure responses to IVT PGE_2, 300 ng/kg/min from 0 to 30 minutes, and to the combination of IVT sarthran, 1000 ng/kg/min started at -15 minutes, and IVT PGE_2 started at 0 minutes. There is no difference in the blood pressure responses to IVT PGE_2 with or without sarthran pretreatment. For IVT PGE_2 alone, blood pressure is greater than control at all time points ($p<.05$) and reaches steady state 15 minutes after the start of infusion (ie. pressures from 15-30 minutes are not differint from each other). For sarthran + IVT PGE_2, blood pressure is greater than control ($p<.05$) at 10-30 minutes.

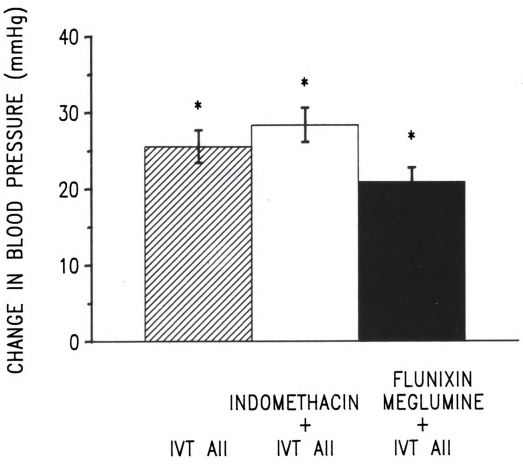


Figure 19. Comparison of changes in blood pressure caused by IVT AII alone, 50 ng/kg/min; caused by IVT AII after pretreatment with indomethacin, 4 mg/kg subcutaneously; and caused by IVT AII after pretreatment with flunixin meglumine, 3 mg/kg intravenously. All increases in blood pressure differ significantly from control (*=p<.05), but are not different from each other.

Prostaglandin Synthesis Blockade of Central AII: The results of prostaglandin synthesis blockade with either indomethacin or flunixin meglumine were the same; neither blocker prevented the IVT AII pressor responses (Figure 19), and both appeared to potentiate the IVT AII drinking responses (subjective observation).

In five sheep IC infusions of arachidonic acid, 4 mg/min for 20 minutes, increased blood pressure 15 mmHg ($p<.01$). Intracarotid infusion of the vehicle for arachidonic acid (0.1 M $NaHCO_3$ in 10% ethanol) did not alter blood pressure. After indomethacin or flunixin meglumine, IC arachidonic acid also did not alter blood pressure.

Blood pressure and heart rate data for individual sheep from experiments presented in this chapter can be found in Appendix G.

DISCUSSION

Potentiation of the central AII pressor response with centrally-administered PGE has been described (Gyang et al. 1973; Leksell 1976). Although the hypothesis that central AII acts through brain PGE_2 synthesis is attractive and is supported by circumstantial evidence (Share 1979), the present study does not support it, since indomethacin and flunixin meglumine did not prevent the IVT AII pressor response. These results in conscious sheep are in agreement with those of Phillips who found that meclofenamate increased the drinking response to IVT AII in conscious rats, but had no effect on the pressor response to IVT AII (Phillips 1980).

This is the only study in which central AII-receptor blockade has been performed with central administration of PGE_2. The results indicate that PGE_2 does not exert its central effects solely by stimulation of the brain renin-angiotensin system.

In conclusion, this study provides evidence that central AII and PGE_2 do not act in series (i.e. production or administration of one does not exert its effects solely through synthesis or release of the other). It is possible that central administration of one substance increases

blood pressure partially through synthesis or release of the other and partially through other mechanism(s), and that during prostaglandin synthesis blockade or AII-receptor blockade the increase in blood pressure is maintained by greater contribution from these other mechanism(s). Whether these other mechanism(s) exist and what they may be is unknown, but this type of compensatory switching of mechanisms was observed in Chapter 2. This study also does not exclude the possibility that AII and PGE_2 have similar central actions because they share common central pathways. If this is true, however, it is likely that PGE_2 and AII activate these pathways independently of each other.

VIII. SUMMARY AND CONCLUSIONS

In summary, although PGE_2 is a potent vasodilator in the periphery, (Moncada et al. 1980), central administration of PGE_2 causes blood pressure to rise. In sheep, the increase in blood pressure is mediated totally by peripheral adrenergic activation (Chapter 4; Hull and Chimoskey 1984).

Intracarotid (IC) administration of PGE_2 also causes heart rate to increase due to a combination of cardiac beta-adrenergic activation and cardiac parasympathetic inhibition (Chapter 2). Intracarotid PGE_2 has no effect on plasma renin activity or partial pressure of CO_2 (Hull and Chimoskey 1984), or on plasma osmolality or plasma concentrations of sodium, epinephrine, norepinephrine, or vasopressin (Chapter 4). At higher doses, IC PGE_2 increases packed cell volume (PCV), probably by splenic contraction.

In conscious sheep, IC PGE_2 is more potent than intracerebroventricular (IVT) PGE_2 (Chapter 4). This difference cannot be explained by direct action of IC PGE_2 on carotid sinus baroreceptors (Chapter 1). It may be caused by PGE_2 reaching different sites in the brain by the two routes or by PGE_2 reaching the same sites in different concentrations, especially since IVT PGE_2 produces certain changes such as pupillary constriction, vocalization, and coughing which are not observed during IC PGE_2 administration.

Intracerebroventricular PGE_2 increases PCV and decreases plasma osmolality and sodium concentration in conscious sheep. The decreases in plasma sodium concentration and plasma osmolality may be the result of increased urinary sodium excretion and decreased free water clearance (Andersson and Leksell 1975) caused by increased vasopressin secretion. Although IVT PGE_2 does increase plasma vasopressin concentration in sheep, this increase is not partially responsible for PGE_2's pressor effect in sheep (as it is in rats: Takahashi and Bunag 1981b, Okuno et al. 1982), since adrenergic blockade alone prevents the PGE_2 pressor response. Also unlike IVT PGE_2's effects in conscious rats (Hoffman and Schmid 1979; Feurstein et al. 1982; Okuno et al. 1982), IVT PGE_2 does not increase plasma catecholamines or heart rate in conscious sheep.

Intracerebroventricular administration of angiotensin II (AII) has cardiovascular effects similar to those of IVT PGE_2. Interactions between the renin-angiotensin system and PGE_2 have been described (McGiff et al. 1970; Larsson et al. 1974; Gimbrone and Alexander 1975; Terragno 1981; Nasjletti and Malik 1982; Spokas et al. 1982; Freeman et al. 1984). The original hypothesis of Chapter 5 was that central PGE_2 increases blood pressure by stimulating release or synthesis of central AII. Instead, the experiments presented in Chapter 5 show that PGE_2 and AII can act independently of each other in the brain; AII receptor blockade has no effect on the pressor responses to IC or IVT PGE_2, and prostaglandin synthesis blockers have no effect on the pressor response to IVT AII.

Thus, the central mechanism of the PGE_2 pressor response remains to be elucidated. More recent studies support an interaction of PGE_2 with central catecholaminergic pathways (Breuhaus and Chimoskey 1984a). In

these studies in conscious sheep, the central alpha$_2$ agonist clonidine both prevented and reversed the central pressor and tachycardic effects of PGE$_2$. Since PGE$_2$ inhibits catecholamine release in a variety of tissues including brain (Bergström et al. 1973; Frame and Hedqvist 1975; Malik 1978; Hillier and Templeton 1980), central administration of PGE$_2$ may increase blood pressure by inhibiting catecholamine release in areas of the brain where catecholamines are tonically vasodepressor, such as the A-1 region of the ventrolateral medulla or the A-2 or C-2 regions of the dorsomedial medulla (Brody et al. 1984).

In conclusion, PGE$_2$ may play an important role in arterial pressure regulation. Prostaglandin E$_2$ is synthesized by brain tissue (Wolfe and Coceani 1969; Wolfe et al. 1976), brain blood vessels (Hagen et al. 1979; Abdel-Halim et al. 1980), and endothelial cells (Gimbrone and Alexander 1975). Because PGE$_2$ is pressor when administered centrally, increased central PGE$_2$ production would result in increased blood pressure. The effect of increased central PGE$_2$ production would be self-regulating, however, since PGE$_2$ is a potent vasodilator in the periphery. Any PGE$_2$ released from the brain into cerebral venous blood would be removed rapidly from the blood with passage through the lungs (Ferreira and Vane 1967; Piper et al. 1970; Moncada et al. 1980). As long as the rate of PGE$_2$ release did not exceed the ability of the lungs to remove it in a single pass, blood pressure would remain elevated. Further central PGE$_2$ production, however, would result in leakage of PGE$_2$ into the periphery. The resultant direct vasodilation would counter PGE$_2$'s central pressor effect.

There is evidence that endogenous prostaglandins play a role in central arterial pressure regulation. The rise in blood pressure

associated with IVT administration of bradykinin can be inhibited by inhibition of prostaglandin synthesis with indomethacin and enhanced by administration of the prostaglandin precursor arachidonic acid (Takahashi and Bunag 1981a). Similarly, the increases in blood pressure caused by endotoxin (Skarnes et al. 1981) or IVT carbachol (Phillips 1980) are prevented by prior treatment with prostaglandin synthesis inhibitors. Prostaglandin E_2 may well be the prostaglandin involved in these pathways since central administration of PGF_{2alpha} has little (Hoffman et al. 1981) or no (Kondo et al. 1979) effect on blood pressure and IVT PGI_2 causes blood pressure to decrease (Kondo et al. 1979).

Finally, if central PGE_2 plays a role in normal arterial pressure regulation, it is possible that any metabolic change resulting in an increase in PGE_2 in brain tissue could lead to hypertension. The possibility of a role for PGE_2 in the genesis of hypertension is supported by evidence that increases in blood pressure and sympathetic nerve activity due to IVT injections of PGE_2 are greater in spontaneously hypertensive rats (Takahashi and Bunag 1981b) and certain strains of genetically hypertensive rats demonstrate a decreased ability to catabolize prostaglandins before they become hypertensive (Pace-Asciak 1976; Limas and Limas 1977).

APPENDICES

APPENDIX A

IDENTIFICATION OF MAJOR CONTRIBUTORS ("THE GIRLS")

Sheep ID #	Name	Contributions
1	Betty Bot	Chapter 1: carotid sinus
2	Zorro	Chapter 1: carotid sinus
3	Possum	Chapter 1: carotid sinus, Intravertebral pilots, IVT pilots
4	Minnie Pearl	Chapter 1: carotid sinus
5	Peachy	Chapter 1: carotid sinus, Intravertebral pilots, IVT pilots
6	Ethyl	Chapter 1: carotid sinus, Intravertebral pilots, IVT pilots
7	Gertrude	Intravertebral pilots
8	Lucy	Intravertebral pilots
9	Bertha	Intravertebral pilots
10	Agnes	IVT pilots
11	Dana	Chapter 2: parasympathetic; Chapter 3: IVT AII; Chapter 4: IVT PGE2, catecholamines, vasopressin; Chapter 5: IVT sarthran; Clonidine studies; Naloxone studies
12	Roz	Chapter 2: parasympathetic; IVT pilots
13	Violet	Chapter 2: parasympathetic; Chapter 3: IVT AII; Chapter 4: IVT PGE2, catecholamines, vasopressin; Chapter 5: IVT sarthran; Clonidine studies

14	Daisy	Chapter 2: parasympathetic; Chapter 3: IVT AII; Chapter 4: IVT PGE2, catecholamines, vasopressin; Chapter 5: IVT sarthran
15	Smokey	IVT pilots; IC PGE2 catecholamines and vasopressin
16	Silver	Chapter 2: parasympathetic; Chapter 3: IVT AII; Chapter 4: IVT PGE2, catecholamines and vasopressin
17	Susie	IVT pilots; IC PGE2 catecholamines and vasopressin
18	Pinky	Chapter 2: parasympathetic; Chapter 3: IVT AII; Chapter 4: IVT PGE2, catecholamines, vasopressin; Chapter 5: IVT sarthran; Carbachol pilots; Clonidine, naloxone, and stress studies
19	Blondie	Chapters 2, 3, 4, and 5; Clonidine studies
20	Emma	Chapters 2, 3, 4, and 5; Clonidine studies
21	Charlotte	Chapters 3 and 4; Clonidine, naloxone, and stress studies; Hypertonic saline and carbachol pilots
22	Trudy	Chapters 3, 4, and 5; Clonidine, naloxone, and stress, and hypertonic saline studies
23	Helga	Chapters 3, 4, and 5; Clonidine, naloxone, stress, hypertonic saline, yohimbine, and ANP studies
24	Sadie	Chapters 3 and 4; Clonidine, naloxone, stress, hypertonic saline, yohimbine, and ANP studies
25	Maude	Chapters 3 and 4; Clonidine, naloxone, stress, hypertonic saline, yohimbine, and ANP studies

APPENDIX B

Table B-1. Blood Pressure and Heart Rate Before and After Infusion of PGE_2, 10 ng/kg/min, into the Common Carotid Artery Past an Intact Carotid Sinus or Past a Denervated Carotid Sinus, or into the External Carotid Artery

Route of Infusion:	Common Carotid Artery (Intact Carotid Sinus)			Common Carotid Artery (Denervated Carotid Sinus)			External Carotid Artery		
	Control	PGE_2	(reps)[a]	Control	PGE_2	(reps)	Control	PGE_2	(reps)
Blood Pressure (mmHg)									
1 Betty Bot	97	107	(3)	---	---	---	99	121	(3)
2 Zorro	---	---	---	93	119	(3)	100	112	(3)
3 Possum	80	95	(5)	85	101	(4)	82	97	(5)
4 Minnie Pearl	83	101	(4)	82	101	(4)	83	102	(4)
5 Peachy	70	90	(4)	68	92	(4)	71	93	(4)
6 Ethyl	81	95	(4)	79	103	(4)	81	94	(4)
Mean	82.2	97.6**	(20)	81.1	103.1**	(19)	86.0	103.3**	(23)
SD	9.7	6.5		9.2	9.9		11.3	11.4	
SEM	4.3	2.9		4.1	4.4		4.6	4.6	
Heart Rate (beats/min)									
1 Betty Bot	74	96	(3)	---	---	---	82	91	(3)
2 Zorro	---	---	---	104	112	(3)	101	112	(3)
3 Possum	53	59	(5)	61	63	(4)	58	59	(5)
4 Minnie Pearl	74	93	(4)	75	86	(4)	72	79	(4)
5 Peachy	70	81	(4)	71	79	(4)	69	78	(4)
6 Ethyl	85	88	(4)	85	84	(4)	84	84	(4)
Mean	71.2	83.4*	(20)	79.1	84.9*	(19)	77.4	83.6*	(23)
SD	11.7	14.8		16.2	17.7		14.8	17.6	
SEM	5.2	6.6		7.3	7.9		6.1	7.2	

a replicates

* $p<.05$ re Control (C) ** $p<.01$ re Control

APPENDIX C

Table C-1. Blood Pressure and Heart Rate Data from Individual Sheep from Three Separate Experiments in which Intravenous Atropine Methyl Bromide Was Given Alone (1 mg/kg), Intracarotid PGE_2 Was Given Alone (10 ng/kg/min), and Atropine Was Given During PGE_2 Infusion

	Blood Pressure (mmHg)						Heart Rate (beats/min)					
Sheep ID #	C	Atropine (5 min)	C	PGE_2 (25 min)	C	PGE_2 + Atropine	C	Atropine (5 min)	C	PGE_2 (25 min)	C	PGE_2 + Atropine
11	83	84	81	101	84	99	76	81	76	86	86	102
12	83	85	88	105	83	102	82	101	68	77	82	114
13	83	86	82	103	81	99	74	96	79	82	79	100
14	83	86	82	108	81	108	72	91	68	88	74	115
16	79	78	77	94	71	89	94	119	82	94	81	137
18	82	93	82	106	81	100	77	108	78	91	83	114
19	87	105	87	107	90	116	69	107	73	95	73	129
20	88	109	88	117	86	105	69	112	65	80	67	118
mean	83	91*a	83	105*o	82	102*o	76	102*	73	87*o	78	116*o+
SEM	0.97	3.83	1.38	2.36	1.92	2.77	2.90	4.29	2.15	2.33	2.23	4.42
number of replicates	34		50		14		34		50		14	

a Statistical analysis by blocked ANOVA. Multiple comparisons by Student-Newman-Keuls. Alpha = .05.
* $p<.05$ re Control (C)
o $p<.05$ re Atropine alone
\+ $p<.05$ re PGE_2 alone

Table C-2. Blood Pressure and Heart Rate Data from Individual Sheep from Three Separate Experiments in which Atropine Methyl Bromide Was Given Alone (1 mg/kg), Intracarotid PGE_2 Was Given Alone (10 ng/kg/min), and Atropine Was Given Before the Start of PGE_2 Infusion

	Blood Pressure (mmHg)						Heart Rate (beats/min)					
Sheep ID #	C	Atropine (30 min)	C	PGE_2 (25 min)	C	PGE_2 + Atropine	C	Atropine (30 min)	C	PGE_2 (25 min)	C	PGE_2 + Atropine
11	83	82	81	101	83	105	76	80	76	86	77	98
12	83	85	88	105	84	103	80	90	68	77	87	120
13	85	88	82	103	82	102	77	98	79	82	78	117
14	83	89	82	108	81	106	70	90	68	88	71	101
16	82	80	77	94	77	91	91	122	82	94	96	116
18	82	89	82	106	83	109	78	99	78	91	76	106
19	85	95	87	107	91	114	68	114	73	95	71	117
20	90	93	88	117	86	112	71	106	65	80	68	130
mean	84	88*a	83	105*o	83	105*o	76	100*+	73	87*o	78	113*o+
SEM	0.96	1.80	1.38	2.36	1.43	2.53	2.58	4.89	2.15	2.33	3.33	3.78
number of replicates	16		50		15		16		50		15	

a Statistical analysis as in Table C-1.
* $p<.05$ re Control (C)
o $p<.05$ re Atropine alone
\+ $p<.05$ re PGE_2 alone

Table C-3. Blood Pressure and Heart Rate Data from Individual Sheep from Three Separate Experiments in which Intravenous Propranolol Was Given Alone (1 mg/kg + .025 mg/kg/min), Intracarotid PGE_2 Was Given Alone (10 ng/kg/min), and Propranolol Was Given During PGE_2 Infusion

			Blood Pressure (mmHg)						Heart Rate (beats/minute)					
Sheep ID #	Test Propranolol bef/aft	Block[a] PGE2 + Propranolol bef/aft	C	Propranolol (10 min)	C	PGE_2 (25 min)	C	PGE_2 + Propranolol	C	Propranolol (10 min)	C	PGE_2 (25 min)	C	PGE_2 + Propranolol
11	+66/+8	+82/+6	84	85	85	102	87	109	82	78	84	99	81	88
13	+56/+9	+52/+6	84	84	82	102	85	100	81	79	84	88	89	85
14	+77/+8	+82/+4	92	93	90	109	93	107	69	56	76	95	66	68
16	+63/+16	+56/+11	77	78	77	94	81	104	85	77	82	94	80	86
18	+71/+7	+70/+7	85	84	82	106	84	102	82	77	78	91	82	88
19	+75/+6	+74/+0	85	85	87	107	88	122	71	71	73	95	78	86
20	+66/+10	+62/+8	85	85	88	117	89	119	64	63	65	80	71	79
mean	+68/+9	+68/+6	85	85	84	105*[b]	87	109*	76	72	77	92*	78	83*+
SEM			1.55	1.71	1.67	2.75	1.67	3.29	3.06	3.31	2.62	2.51	2.79	2.73
number of replicates				20		36		10		20		36		10

a Adequacy of propranolol blockade expressed as the change in heart rate in response to 5 ug isoproterenol IV during control (before=bef) compared to the change in heart rate in response to 50 ug isoproterenol IV 45 minutes after the initial bolus of propranolol (after=aft).

b Statistical analysis as in Table C-1.

* $p<.05$ re Control (C) and Propranolol alone

\+ $p<.05$ re PGE_2 alone

Table C-4. Blood Pressure and Heart Rate Data from Individual Sheep from Three Separate Experiments in which Intravenous Propranolol Was Given Alone (1 mg/kg + .025 mg/kg/min), Intracarotid PGE_2 Was Given Alone (10 ng/kg/min), and Propranolol Was Given Before the Start of PGE_2 Infusion

			Blood Pressure (mmHg)						Heart Rate (beats/minute)					
Sheep ID #	Test Propranolol bef/aft	Block[a] PGE2 + Propranolol bef/aft	C	Propranolol (35 min)	C	PGE_2 (25 min)	C	PGE_2 + Propranolol	C	Propranolol (35 min)	C	PGE_2 (25 min)	C	PGE_2 + Propranolol
11	+60/+4	+72/+12	84	86	85	102	85	106	76	77	84	99	88	92
13	+78/+10	+95/+4	82	83	82	102	85	99	79	76	84	88	82	84
14	+64/+8	+90/+8	93	91	90	109	91	108	75	63	76	95	62	64
16	+50/+10	+76/+22	72	69	77	94	83	103	83	77	82	94	86	91
18	+59/+4	+82/+10	84	82	82	106	85	108	86	84	78	91	78	95
19	+79/+8	+66/+2	85	84	87	107	84	111	70	74	73	95	73	78
20	+57/+12	+75/+8	88	88	88	117	83	109	58	59	65	80	71	83
mean	+64/+8	+72/+9	84	83	84	105*[b]	85	106*	75	73	77	92*	77	84*+
SEM			2.43	2.62	1.67	2.75	0.98	1.54	3.54	3.35	2.62	2.35	3.47	3.96
number of replicates			11		36		10		11		36		10	

a Adequacy of propranolol blockade expressed as the change in heart rate in response to 5 ug isoproterenol IV during control (before=bef) compared to the change in heart rate response to 50 ug isoproterenol IV 45 minutes after the initial bolus of propranolol (after=aft).

b Statisical analysis as in Table C-1.

* $p<.05$ re Control (C) and Propranolol alone

\+ $p<.05$ re PGE_2 alone

Table C-5. Blood Pressure and Heart Rate Data from Individual Sheep in which Intravenous Propranolol (1 mg/kg/min + .025 mg/kg/min) and Atropine (1 mg/kg) Were Given During Intracarotid Infusion of PGE_2, 10 ng/kg/min

			Blood Pressure (mmHg)				Heart Rate (beats/minute)			
Sheep ID #	Test[a] Block Before	After	Control	PGE_2 (15 min)	PGE_2 + Propranolol	PGE_2 Propranolol Atropine	Control	PGE_2 (15 min)	PGE_2 + Propranolol	PGE_2 Propranolol Atropine
11	+118	+ 8	89	111	105	109	80	108	80	90
13	+ 46	+12	80	95	90	97	78	80	73	99
14	+ 94	+14	90	105	103	105	78	81	76	89
16	+ 33	+23	77	94	95	95	91	100	90	112
18	+ 72	+ 8	81	96	102	111	85	91	88	102
19	+ 64	+10	86	100	94	103	76	82	77	98
20	+ 69	+20	86	112	103	113	63	68	72	112
mean	+ 71	+14	84	102*	99*	105*	79	87	79	100*
SEM			1.83	2.87	2.22	2.58	3.26	5.11	2.67	3.51

a Adequacy of propranolol blockade expressed as the change in heart rate in response to 5 ug isoproterenol IV during control (Before) compared to the change in heart rate in response to 50 ug isoproterenol IV 45 minutes after the initial bolus of propranolol (After).

b Statistical analysis as in Table C-1.

* $p<.05$ re Control (C)

Table C-6. Blood Pressure and Heart Rate Data from Individual Sheep in which Intravenous Propranolol (1 mg/kg + .025 mg/kg/min) and Atropine (1mg/kg) Were Given Before the Start of Intracarotid PGE_2 Infusion, 10 ng/kg/min

Sheep ID #	Test[a] Block Before	Test[a] Block After	Blood Pressure (mmHg) Con-trol	Propra- (5 min)	Propranolol + Atropine	Propranolol Atropine PGE_2	Heart Rate (beats/minute) Con-trol	Propra- (5 min)	Propranolol + Atropine	Propranolol Atropine PGE_2
11	+48	+ 6	90	90	89	113	79	73	79	87
13	+54	+ 6	85	85	85	95	70	68	86	94
14	+84	+14	88	95	93	105	61	53	73	76
16	+38	+18	73	78	71	97	102	92	113	111
18	+76	+ 6	82	81	85	112	72	74	97	104
19	+94	+12	85	89	90	113	77	77	103	109
20	+60	+10	84	83	87	104	65	67	90	96
mean	+65	+10	84	86	86	106*[b]	75	72	92*	97*
SEM			2.06	2.09	2.66	2.89	5.06	4.45	5.25	4.73

a Adequacy of propranolol blockade expressed as the change in heart rate in response to 5 ug isoproterenol IV during control (Before) compared to the change in heart rate in response to 50 ug isoproterenol IV 45 minutes after the initial bolus of propranolol (After).

b Statistical analysis as in Table C-1.

* $p<.05$ re Control (C)

APPENDIX D

Table D-1. Verification of Ventricular Catheter Placement

Sheep I.D.#	Did CSF Ever Flow From Catheter? How Long?	Drink After IVT All?	Length of Time Catheter Functional	Reason for Euthanasia Catheter-Related? Loss of Function	Neurologic Signs	Postmortem Catheter Position
3	yes	yes	1 month	no	yes	
5	yes[a]	---[b]	2 weeks	no	yes	R 2 mm
10	yes[a]	---	0 days	no	?	-------
11	yes[a]	---	1 week	no	?	L 2 mm
12	yes[a]	---	0 days	yes	no	-------
11	no	yes	10 months	no	yes	R 3 mm
13	no	yes	7 months	no	no	R 9 mm
14	yes - 1 week	yes	5 months	no	no	L 9 mm
15	no	yes	1 month	no	?	L 10 mm
16	no	yes	2 months	yes	no	R 3 mm[c]
17	no	yes	2.5 weeks	no	no	L 2 mm
18	yes - 1 week	no	1 month	no	yes	L 11 mm
19	yes - 2 months	yes	6 months	yes	no	R 10 mm[d]
20	no	yes	5 months	yes	no	L 10 mm[d]
21	yes - 2 months	yes	6 months	yes	no	L 12 mm[c]
22	yes - 7 months	yes	7 months	no	yes	L 18 mm
23	no	yes	12+ months	---	---	R ***
24	no	yes	3+ months	---	---	R ***
25	no	yes	4 months	yes	no	R ***

a - CSF obtained at the time of surgery only and not afterwards
b - A "___" means the experiment was not done or the position not checked
c - choroid plexus was beginning to seal off catheter tip
d - ventricular lining was sealing over the catheter tip.
*** - sheep still alive, for further explanation of Table D-1, see text.

APPENDIX D

VERIFICATION OF CATHETER PLACEMENT AND PROBLEMS ENCOUNTERED

The problems which we encountered in developing this preparation are presented here in the hope that this will prevent others who wish to use this technique from experiencing similar difficulties. Table D-1 summarizes the success or failure of catheter placement for each sheep used in this study. All catheters entered the posterior part of the lateral ventricle. The numbers given under "Postmortem Catheter Position" indicate the distance in millimeters that the catheter was found rostral to the splenium of the corpus collosum. The letter "L" indicates that the catheter was to the left of midline; "R" means it was to the right. "Length of Time Catheter Functional" indicates the time from surgery until either 1) elective euthanasia was performed (if no problems developed with the catheter), 2) there was a change in catheter function (loss of drinking response to IVT AII or loss of ability to infuse into the catheter indicated by elevated ventricular pressure), or 3) the animal demonstrated some neurologic abnormality (such as a head tilt). The table also indicates which sheep were sacrificed because of problems with their ventricular catheters, and whether these were related to development of neurologic signs or to loss of catheter patency.

Sheep #'s 3, 5, 10, and 12 received stainless steel guide cannulas according to the technique of Pappenheimer et al. (1962). Pilot data

only were obtained from sheep #3 before she developed a head tilt and gradually lost control of her equilibrium. Sheep #5 had an extensor paralysis in her right hind limb immediately after surgery which improved over the next few days. She began to develop a head tilt after the first IVT experiment. Several days later she had a fever, her head tilt was worse, and problems with her right hind limb returned. Euthanasia with an overdose of pentobarbital was performed in both of these sheep. In the sheep that developed head tilts, the tilt was such that the ear on the side of the catheter placement was higher than the other ear. Sheep #10 was found dead in her cage the morning after surgery. We are uncertain whether this death was related to her ventricular cannula or caused by a gastrointestinal upset. The evening before she had recovered well from anesthesia, had no neural deficits, and was eating and drinking. She had had an unusually big appetite immediately after surgery and, in retrospect, she may have upset the bacterial balance in her rumen and developed enterotoxemia, but no postmortem was performed. Sheep #12's cannula apparently was malpositioned since control IVT infusions could not be performed without causing discomfort and raising blood pressure. Therefore, no IVT experiments were performed in this sheep. Sheep #11 sheared off the hub of her ventricular guide cannula one week after surgery. No IVT experiments had been performed. She then was the first sheep to receive a polyvinyl catheter using the technique described in Chapter 3. This catheter was placed in the lateral cerebral ventricle opposite to the stainless steel cannula. At postmortem, both cannula and catheter had entered their respective ventricles at approximately the same position. No data from sheep using the stainless steel cannulas were presented.

Of the fourteen sheep that received polyvinyl ventricular catheters, data from two of these sheep (#'s 15 and 17) were not presented. Sheep #15 developed diarrhea and would not eat after surgery. The diarrhea cleared up, but she never regained an appetite. She appeared normal and was healthy in every other way, but she could not be enticed to eat. Different feeds, appetite stimulants, IV dextrose infusions, feeding a slurry made from pellets by stomach tube, and transfaunation (transferring fresh rumen contents from a normal donor to the sheep by stomach tube) were tried. Remarkably, she maintained her weight for approximately one month. Euthanasia was performed when she continued not to eat and her health began to deteriorate. At postmortem it was found that the catheter had entered the posterior part of the left lateral ventricle 10 mm in front of the splenium of the corpus callosum. However, it had passed through the ventricle, and the tip rested in the menigeal space between the cerebellum and the dorsum of the brainstem, just over the rostral colliculi. During IVT infusions, fluid apparently had leaked backwards along the catheter to the lateral ventricle, since this sheep drank in response to IVT AII, 30 ng/kg/min. The State of Michigan Animal Health Diagnostic Laboratory performed a complete gross postmortem examination on this sheep but could not determine a cause for the lack of appetite.

Sheep #17 developed a uveitis in her right eye and a septicemia. These responded initially to local and systemic antibiotic (chloramphenicol) therapy, but the uveitis returned after discontinuing treatment. This problem more likely was related to embolization from this sheep's right carotid artery catheter (which was flapped and did not withdraw) than to a problem associated with her ventricular catheter

(which was on her left side). This sheep did drink in response to IVT AII, but four weeks elapsed from the time of her last IVT experiment until euthanasia. At postmortem it was found that the ventricular catheter originally had just entered the posterior left lateral ventricle 2 mm in fromt of the splenium of the corpus callosum, but the catheter was not very deep and the ventricular lining had sealed over the tip of the catheter, leaving only a dimple to indicate that the catheter had ever been through the lining. No dye had entered the ventricles.

Sheep #11 developed disequilibrium and a head tilt approximately one year after surgery and had to be sacrificed. Her catheter was still patent at postmortem examination.

The ends of the ventricular catheters of sheep #'s 13 and 14 were dangling freely in the ventricular space. The choroid plexus had wrapped itself around the tips, but dye had flowed freely throughout the ventricles.

Euthanasia was performed on sheep #16 because an elevated ventricular pressure during IVT experiments suggested that the ventricular catheter was getting plugged. This was confirmed at postmortem; there was only a small spot of dye coming into the ventricle at the end of the catheter. The ventricular lining was in the process of sealing over the catheter.

Cerebrospinal fluid flowed freely from sheep #18's ventricular catheter the first time she had an IVT experiment. However, later that week the catheter pin was discovered to be missing. After that, CSF no longer flowed freely from the catheter. This sheep did not drink after her first IVT AII experiment, although she sniffed at the water. During

that experiment ventricular pressure was approximately 14 mmHg, so certainly the catheter was not plugged at that time. Ventricular pressure gradually increased during subsequent IVT experiments, suggesting that the catheter either was sealing off or choroid plexus was beginning to plug the tip. This sheep continued to have an inconsistent drinking response to IVT AII. When she did drink it usually was delayed. At postmortem it was found that the tip of the catheter was several millimeters above the dorsal lining of the ventricle. The lack of drinking response to IVT AII in this sheep suggested that infusions were not reaching the third cerebral ventricle. Alternatively, it may be that centrally- administered AII did not elicit a drinking response in this sheep, since she did not drink in response to IC AII either. However, the very definite pressor response to IVT PGE_2 obtained repeatedly in this sheep had to be a central effect. This sheep did, however, require higher doses of PGE_2 IVT than did other sheep that responded to IVT PGE_2, and she had relatively few side effects associated with the infusions (although she did have one—the dry hacking cough). It is possible, therefore, that PGE_2 and AII gained access to the cerebral ventricles in this sheep by diffusion through the thin layer of cerebrum that lay between the ventricles and the catheter tip. It may also be true that these data demonstrate that the central site of action of PGE_2 is different from the central site where AII elicits its drinking response. In other sheep whose catheters had sealed off, a definite change in function was observed, i.e., loss of drinking behavior in response to IVT AII. Since this sheep did not drink in response to IVT AII even when it was likely that her catheter was still patent, it was impossible to use this as a guide to know when

her catheter had sealed off. She never lost her ability to respond to IVT PGE_2. Because we cannot be certain that AII and/or PGE_2 reached this sheep's CSF, we did not include her data in any of the results presented in the body of the thesis.

Sheep #19's ventricular catheter was showing signs of becoming sealed when she ruptured a carotid artery at the site of catheterization, and had to be put to sleep.

As indicated in Table D-1, sheep #20's ventricular catheter became non-functional after five months. This was apparent antemortem when the sheep stopped drinking in response to IVT AII and began to object to ventricular infusions by shaking her head. This sheep died after surgery to replace a jugular catheter. At postmortem she was extremely bloated, and gas and a dark red serous fluid exuded from the cut surface of her cervical muscles. A presumptive diagnosis of Clostridial enterotoxemia was made. Also at postmortem it was found that the dorsal ventricular lining indeed had sealed over the end of the ventricular catheter, but, again, a scar was present in the lining indicating that at one time the catheter had penetrated into the ventricle. No IVT data were used from this sheep after she stopped drinking in response to IVT AII.

Sheep #21 was sacrificed when her catheter showed signs of becoming plugged.

Sheep #22 suddenly became unable to stand and showed signs of increased intracranial pressure seven months after catheter placement. At postmortem her catheter was still patent.

At the time of this writing, sheep #'s 23, 24, and 25 are still alive and healthy. All three drink in response to IVT AII.

APPENDIX E

Table E-1. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracerebroventricular Angiotensin II, 50 ng/kg/min

Sheep	(rep)[a]	C[b]	Blood Pressure (mmHg) 5	10	15	20	25	30 min	C	Heart Rate (beats/min) 5	10	15	20	25	30 min
11	5	88	102	107	110	113	113	118	91	82	82	82	83	81	79
13	3	88	95	99	101	105	106	105	78	76	72	75	74	76	76
14	1	91	92	97	99	102	102	104	76	69	72	67	70	69	67
16	1	75	78	89	94	94	98	97	91	85	85	81	80	80	82
18	3	83	91	99	102	105	108	113	69	66	62	60	59	58	60
19	3	90	105	109	112	111	115	116	68	65	66	65	66	66	67
20	5	87	97	101	106	108	111	114	66	64	62	59	59	60	60
21	3	81	89	95	100	100	101	103	94	94	95	96	98	99	99
22	3	87	107	110	115	116	117	115	87	92	93	95	102	104	96
23	9	80	91	95	97	98	100	100	77	77	79	79	79	78	79
24	2	91	111	116	116	118	123	123	79	74	81	75	74	75	72
25	4	85	98	101	101	104	108	109	74	70	69	68	67	71	66
mean[c]		86	97*	102*	105*	106*	109*	109*	80	77	78	77	77	78	77
SD		5.2	9.3	8.0	7.5	7.7	7.8	8.3	9.5	10.3	10.7	11.8	13.1	13.2	12.3
SEM		1.6	2.8	2.4	2.3	2.3	2.4	2.5	2.9	3.1	3.2	3.6	4.0	4.0	3.7

a (rep) = Number of replicates per sheep

b C = Control

c Mean SD, and SEM do not include sheep #18

* p<.05 re Control. Blood pressure (BP) at 15 minutes = BP at 20 minutes. BP at 25 minutes = BP at 30 minutes.

Table E-2. Hemodynamic Responses of Individual Sheep to Intracerebroventricular Angiotensin II, 50 ng/kg/min, in Sheep with Electromagnetic Flowmeters on Their Main Pulmonary Arteries

Sheep ID#	Blood Pressure (mmHg)		Right Heart Rate (bpm)		Atrial Pressure (mmHg)		Total Cardiac Output (L/min)		Peripheral Resistance (mmHg·min/L)		Stroke Volume (ml)	
	Control	AII[a]	Control	AII	Control	AII	Control	AII	Control	AII	Control	AII
23	82	101	82	81	0.5	2.0	6.87	6.55	11.80	15.04	84	86
24	93	121	70	74	0.2	3.3	6.27	6.74	14.74	17.44	90	91
25	84	104	75	68	2.7	5.8	6.28	5.91	12.89	16.54	84	87
Mean	86	108*	76	74	1.1	3.7*	6.47	6.40	13.14	16.34*	86	88*
SD	5.8	11.0	5.8	6.5	1.4	1.9	0.34	0.44	1.48	1.21	3.2	2.7
SEM	3.4	6.3	3.3	3.8	0.8	1.1	0.20	0.25	0.86	0.70	1.9	1.5

a AII = Angiotensin II data 30 minutes after the start of infusion
* $p<.05$ re Control

Table E-3. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracarotid Angiotensin II, 10 ng/kg/min

Sheep	Blood Pressure (mmHg) Control	5 min	15 min	25min	Heart Rate (beats/min) Control	5 min	15 min	25 min
11	90	117	122	122	65	60	64	68
13	81	98	103	105	82	77	74	73
14	83	103	109	108	66	61	65	69
16	82	92	98	97	83	76	72	70
18	85	105	111	113	98	88	84	85
19	90	112	115	117	86	72	73	76
20	90	136	138	134	61	65	60	61
24	82	125	132	131	79	88	95	86
mean	86	111*	116*	116*	77	73	73	73
SEM	1.4	5.1	4.9	4.5	4.4	3.9	4.1	3.0

* $p < .05$ re Control

Table E-4. Blood Pressure and Heart Rate Responses of Individual Sheep to Intravenous Angiotensin II, 10 ng/kg/min

Sheep	Blood Pressure (mmHg) Control	5 min	15 min	25 min	Heart Rate (beats/min) Control	5 min	15 min	25 min
11	89	105	111	116	93	82	77	81
13	85	105	112	113	78	72	70	72
14	92	94	108	109	82	73	71	70
16	82	92	93	98	83	75	76	73
18	84	101	104	105	76	67	68	64
19	81	101	104	105	92	68	73	74
20	83	103	107	112	76	67	68	64
24	84	110	120	122	88	74	65	68
mean	85	102*	108*+	110*+	85	75*	71*	72*
SEM	1.3	2.1	2.7	2.7	2.3	2.5	1.4	1.8

* $p<.05$ re Control
\+ $p<.05$ re 5 minutes

Table E-5. Blood Pressure and Heart Rate Responses of Individual Sheep to Intravenous Angiotensin II, 50 ng/kg/min

Sheep	Blood Pressure (mmHg) Control	5 min	15 min	25 min	Heart Rate (beats/min) Control	5 min	15 min	25 min
11	94	136	142	143	88	70	77	86
13	82	124	130	131	86	75	79	81
16	80	101	104	100	86	68	74	74
18	82	128	136	138	85	59	62	65
19	84	126	131	129	82	66	66	67
20	91	153	154	155	55	46	45	49
mean	86	128*	133*	133*	80	64*	67*	70*+
SD	5.7	17.0	16.6	18.7	12.6	10.3	12.7	13.2
SEM	2.3	6.9	6.8	7.6	5.1	4.2	5.2	5.4

* $p<.05$ re Control

\+ $p<.05$ re 5 min

APPENDIX F

Table F-1. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracarotid PGE_2, 0 ng/kg/min (Vehicle)

Sheep	(rep)[a]	Blood Pressure (mmHg) C[b]	5	10	15	20	25	30 min	Heart Rate (beats/min) C	5	10	15	20	25	30 min
11	1	87	89	91	87	89	88	89	66	73	69	67	69	68	68
13	1	86	90	85	88	90	90	86	94	91	95	94	95	93	96
14	1	82	88	85	87	84	83	85	94	91	94	98	95	93	93
18	1	85	85	92	83	85	86	88	77	77	76	75	80	75	74
19	1	90	93	90	90	92	88	88	79	82	86	79	81	85	82
20	1	87	88	87	87	86	86	84	52	50	52	52	52	52	52
21	1	81	82	82	83	82	81	82	98	100	93	94	92	96	96
mean		86	88	88	86	87	86	86	80	81	81	80	81	80	80
SEM		1.2	1.3	1.5	0.9	1.3	1.2	0.9	6.4	6.2	6.1	6.4	6.0	6.1	6.3

a (rep) = Number of replicates per sheep
b C = Control

Table F-2. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracarotid PGE_2, 5 ng/kg/min

Sheep	(rep)[a]	Blood Pressure (mmHg) C[b]	5	10	15	20	25	30 min	Heart Rate (beats/min) C	5	10	15	20	25	30 min
9	1	89	89	90	93	94	95	96	79	81	81	79	85	82	84
11	1	93	103	109	110	112	110	115	98	111	118	119	119	124	124
13	1	82	86	91	93	94	98	95	98	98	97	97	96	98	97
14	1	93	96	100	102	104	103	105	99	97	105	105	103	108	108
18	1	85	94	98	99	102	112	109	69	81	89	83	91	96	95
19	2	84	96	106	109	112	112	113	73	75	80	78	79	80	83
20	1	86	93	100	101	102	105	104	79	84	89	92	96	99	102
21	1	81	84	89	90	91	96	93	76	77	83	86	87	79	88
mean		87	93	98	100	101	104	104*	84	88	93	92	94	96	98*
SEM		1.6	2.1	2.6	2.5	2.8	2.5	2.9	4.4	4.5	4.7	5.0	4.4	5.5	4.9

a (rep) = Number of replicates per sheep
b C = Control
* $p<.01$ re Control (Pair t test: only the 30 min data were compared to Control)

Table F-3. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracarotid PGE_2, 10 ng/kg/min

Sheep	(rep)[a]	Blood Pressure (mmHg) C[b]	5	10	15	20	25	30 min	Heart Rate (beats/min) C	5	10	15	20	25	30 min
9	5	90	95	101	102	103	104	104	76	80	83	85	84	85	83
11	9	91	100	104	106	107	107	108	92	99	103	104	105	106	106
13	8	82	91	96	98	100	102	102	84	87	87	87	88	87	86
14	5	86	95	101	106	106	107	106	81	84	88	90	93	95	95
18	8	82	98	101	104	105	107	107	76	87	88	89	91	91	90
19	5	87	97	108	109	108	107	105	73	81	83	88	93	95	94
20	5	88	101	110	114	117	117	117	68	77	81	81	84	84	86
21	9	83	91	96	97	98	98	97	82	86	91	95	97	96	96
22	6	84	95	102	104	105	104	104	85	88	92	99	95	98	97
23	10	77	91	98	101	103	103	103	77	81	85	88	88	90	89
24	5	80	92	92	96	97	99	99	93	106	107	110	110	109	110
25	6	81	98	102	104	105	107	106	70	83	86	85	87	90	91
mean		84	95*	101*	103*	105*	105*	105*	80	87*	89*	92*	93*	94*	94*
SEM		1.2	1.0	1.5	1.5	1.5	1.5	1.5	2.3	2.4	2.3	2.5	2.3	2.2	2.4

a (rep) = Number of replicates per sheep
b C = Control
* $p<.05$ re Control. Steady state for blood pressure and heart rate reached at 15 minutes.

Table F-4. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracarotid PGE_2, 30 ng/kg/min

Sheep	(rep)[a]	Blood Pressure (mmHg) C[b]	5	10	15	20	25	30 min	Heart Rate (beats/min) C	5	10	15	20	25	30 min
9	1	90	99	103	105	110	109	107	81	86	91	93	93	94	92
11	1	92	113	120	123	120	121	118	106	111	118	117	119	123	121
13	1	85	105	105	105	106	104	104	107	119	124	119	116	117	114
14	1	88	105	109	110	110	108	107	86	98	100	106	109	108	107
18	1	80	103	108	114	116	114	108	78	91	110	100	101	100	95
19	1	87	112	121	122	122	119	119	78	90	90	97	98	100	99
20	1	87	111	121	120	121	121	119	78	92	97	103	105	106	101
21	1	81	91	98	102	101	101	103	76	83	88	94	95	98	99
mean		86	105*	111*	113*	113*	112*	111*	86	96*	102*	104*	105*	106*	104*
SEM		1.5	2.6	3.2	2.9	2.7	2.7	2.3	4.6	4.4	4.8	3.5	3.4	3.5	3.5

a (rep) = Number of replicates per sheep
b C = Control
* $p<.05$ re Control. Steady state for blood pressure and heart rate reached at 10 minutes.

Table F-5. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracarotid PGE_2, 60 ng/kg/min

Sheep	(rep)[a]	C[b]	Blood Pressure (mmHg) 5	10	15	20	25	30 min	C	Heart Rate (beats/min) 5	10	15	20	25	30 min
9	1	89	108	109	110	111	111	109	62	74	7	74	77	77	79
11	1	94	117	119	121	118	118	115	105	119	121	122	130	125	130
18	1	87	121	123	124	122	118	117	62	90	96	104	108	102	116
20	1	90	120	125	125	126	120	121	103	112	114	114	114	114	114
21	1	82	96	100	101	101	101	103	74	81	90	89	95	93	96
22	1	82	108	110	109	108	110	112	91	89	98	102	109	106	110
mean		88	112	114	115	114	113	113*	83	94	99	101	106	103	108*
SEM		2.0	3.9	3.8	4.0	3.9	3.0	2.7	8.0	7.2	6.8	7.1	7.3	6.8	7.2

a (rep) = Number of replicates per sheep
b C = Control
* $p<.05$ re Control (Pair t test: only the 30 min data were compared to Control)

Table F-6. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracarotid PGE_2, 100 ng/kg/min

Sheep	(rep)[a]	C[b]	Blood Pressure (mmHg) 5	10	15	20	25	30 min	C	Heart Rate (beats/min) 5	10	15	20	25	30 min
11	1	92	118	118	115	120	118	117	109	130	135	135	135	136	133
18	1	82	120	122	117	119	117	118	55	81	82	89	92	93	92
20	1	89	130	130	128	126	125	130	94	112	114	113	114	115	115
21	1	82	95	100	104	101	102	103	82	88	97	98	103	100	98
22	1	81	104	107	107	110	108	108	100	108	113	113	111	111	116
23	1	75	112	117	110	113	107	112	77	97	100	102	103	101	99
24	1	83	105	111	111	109	109	110	110	130	134	130	131	130	129
25	1	81	111	118	121	116	119	115	63	87	93	97	101	106	107
mean		83	112*	115*	114*	114*	113*	114*	86	104*	109*	109*	111*	112*	111*
SEM		1.8	3.9	3.3	2.8	2.8	2.8	2.8	7.3	6.8	6.8	5.8	5.3	5.3	5.2

a (rep) = Number of replicates per sheep
b C = Control
* $p<.05$ re Control. Steady state for blood pressure reached at 5 minutes; for heart rate at 10 min.

Table F-7. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracerebroventricular PGE_2, 0 ng/kg/min (Vehicle)

Sheep	(rep)[a]	Blood Pressure (mmHg) C[b]	5	10	15	20	25	30 min	Heart Rate (beats/min) C	5	10	15	20	25	30 min
11	1	94	93	98	97	95	97	97	93	92	90	90	94	93	92
13	1	87	87	86	86	86	86	89	104	102	102	101	100	101	104
14	1	89	94	91	93	94	94	95	94	97	98	101	102	101	98
16	1	75	75	75	76	76	75	77	82	79	79	81	81	81	84
18	1	84	85	85	84	83	84	83	83	90	88	77	83	86	86
19	1	85	90	90	90	88	86	89	81	81	80	76	78	78	83
20	1	96	102	99	100	99	99	94	70	67	62	65	65	67	67
mean		87	90	89	90	89	89	89	87	87	86	84	86	87	88
SEM		2.6	3.2	3.2	3.1	3.0	3.1	2.7	4.2	4.5	5.1	5.1	5.0	4.7	4.5

a (rep) = Number of replicates per sheep
b C = Control

Table F-8. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracerebroventricular PGE_2, 10 ng/kg/min

Sheep	(rep)[a]	C[b]	Blood Pressure (mmHg) 5	10	15	20	25	30 min	C	Heart Rate (beats/min) 5	10	15	20	25	30 min
11	1	87	89	86	89	90	89	92	90	91	90	88	90	94	92
13	1	81	85	85	84	86	83	85	102	108	112	110	112	109	111
14	1	80	83	86	84	88	84	83	88	90	88	89	89	92	92
18	1	86	87	88	91	89	91	92	50	49	49	50	50	50	47
19	1	82	81	85	84	91	87	88	77	79	76	78	76	76	80
20	1	86	86	89	91	86	87	88	53	52	53	55	52	52	51
21	1	84	89	87	90	87	90	86	97	99	99	98	99	100	99
22	1	82	86	88	89	88	88	89	97	94	96	97	96	97	96
23	1	78	76	80	80	82	82	83	105	100	97	94	96	100	98
mean[c]		83	84	86*	86*	87*	86*	87*	89	89	89	89	89	90	90
SEM		1.1	1.5	1.0	1.4	1.0	1.1	1.1	6.0	6.1	6.3	5.8	6.4	6.4	6.3

a (rep) = Number of replicates per sheep
b C = Control
c Mean, SD, and SEM do not include sheep #18
* $p<.05$ re Control and 5 min, but not different from each other

Table F-9. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracerebroventricular PGE_2, 30 ng/kg/min

Sheep	(rep)[a]	Blood Pressure (mmHg) C[b]	5	10	15	20	25	30 min	Heart Rate (beats/min) C	5	10	15	20	25	30 min
11	1	83	85	90	89	87	86	88	70	70	74	68	72	70	70
13	1	94	92	91	95	96	95	98	107	103	100	99	101	100	99
14	2	82	84	87	86	87	85	87	75	73	74	74	74	72	74
18	1	76	79	80	78	82	78	79	65	63	61	62	62	62	60
19	1	88	90	94	96	97	97	99	73	70	68	70	71	74	69
20	1	89	94	94	98	97	103	106	66	65	64	63	66	66	66
21	1	83	84	83	84	84	84	85	70	67	70	76	71	69	72
22	1	85	91	93	92	94	96	94	91	88	91	94	102	106	98
23	1	82	85	87	86	89	89	101	69	74	71	72	71	71	78
mean[c]		86	88	90*	91*	91*	92*	95*+	78	76	77	77	79	79	78
SEM		1.5	1.4	1.4	1.8	1.9	2.4	2.7	5.0	4.6	4.4	4.5	5.1	5.4	4.6

a (rep) = Number of replicates per sheep
b C = Control
c Mean, SD, and SEM do not include sheep #18
* $p<.05$ re Control and 5 min, but not different from each other
\+ $p<.05$ re Control and all blood pressure at all other time points

Table F-10. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracerebroventricular PGE_2, 100 ng/kg/min

Sheep	(rep)[a]	Blood Pressure (mmHg) C[b]	5	10	15	20	25	30 min	Heart Rate (beats/min) C	5	10	15	20	25	30 min
11	1	87	94	97	98	104	104	107	88	91	90	91	94	98	94
13	1	80	80	82	81	82	82	82	93	94	96	92	93	92	90
14	1	84	89	95	100	93	95	96	65	67	66	69	69	72	75
18	1	79	80	82	82	82	81	82	79	75	72	72	77	77	73
19	1	85	92	91	94	93	98	101	80	82	79	81	81	78	83
20	1	85	97	96	99	100	103	101	77	80	80	82	80	80	79
21	1	80	81	84	81	80	81	82	74	76	79	79	78	77	77
22	2	83	95	96	99	101	105	105	79	81	89	86	90	89	87
23	1	78	77	76	83	75	79	82	59	61	59	57	59	55	62
mean[c]		83	88*	89*	92*	91*	93*	95*	77	79	80	80	80	80	81
SEM		1.1	2.7	2.9	3.1	3.8	3.9	3.8	3.9	3.9	4.4	4.1	4.3	4.7	3.6

a (rep) = Number of replicates per sheep
b C = Control
c Mean, SD, and SEM do not include sheep #18
* p.05 re Control. Steady state reached at 15 minutes.

Table F-11. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracerebroventricular PGE_2, 300 ng/kg/min

Sheep	(rep)[a]	C[b]	Blood Pressure (mmHg) 5	10	15	20	25	30 min	C	Heart Rate (beats/min) 5	10	15	20	25	30 min
11	3	91	99	102	104	106	105	104	80	83	87	87	91	89	90
13	2	85	87	89	87	85	86	85	78	77	77	74	75	74	74
14	2	85	87	89	91	92	92	93	73	70	71	69	70	72	76
16	1	80	82	88	88	85	88	88	76	73	78	79	76	74	76
18	1	84	86	87	86	84	85	85	92	89	86	89	91	90	95
19	3	88	96	101	103	105	108	110	75	75	76	74	75	77	81
20	3	90	100	104	104	107	108	107	65	68	70	69	70	70	80
21	1	80	85	83	85	80	79	79	85	80	85	86	88	84	84
22	2	86	93	93	98	98	101	102	86	85	83	89	91	90	96
23	4	77	82	81	85	83	84	85	79	80	78	79	78	80	82
24	1	88	92	89	87	87	86	90	65	78	78	82	71	71	65
25	1	85	96	86	95	85	88	87	64	65	62	62	60	57	55
mean[c]		85	91*	91*	93*	92*	93*	94*	75	76	77	77	77	76	78
SEM		1.3	2.0	2.3	2.4	3.1	3.1	3.1	2.3	1.9	2.2	2.6	2.9	2.9	3.4

a (rep) = Number of replicates per sheep
b C = Control
c Mean, SD, and SEM do not include sheep #18
* $p<.05$ re Control, but not different from each other. Steady state reached at 5 minutes.

Table F-12. Blood Pressure and Heart Rate Responses to Intracerebroventricular PGE_2, 750 ng/kg/min, in Sheep that Did not Respond to Lower Doses

Sheep	(rep)[a]	Blood Pressure (mmHg) C[b]	5	10	15	20	25	30 min	Heart Rate (beats/min) C	5	10	15	20	25	30 min
13	1	86	86	85	87	87	91	93	98	103	101	100	97	102	91
14	1	95	97	97	95	96	100	97	93	96	93	88	85	98	88
18	2	80	86	86	85	88	89	90	73	71	71	70	69	69	71
21	1	84	92	89	87	87	89	90	81	82	80	82	79	78	77

a (rep) = Number of replicates per sheep
b C = Control

Table F-13. Blood Pressure and Heart Rate Responses to Intracerebroventricular PGE_2, 1000 ng/kg/min, in Sheep that Did not Respond to Lower Doses

Sheep	(rep)[a]	C[b]	Blood Pressure (mmHg) 5	10	15	20	25	30 min	C	Heart Rate (beats/min) 5	10	15	20	25	30 min
13	2	87	88	89	87	97	89	89	85	82	78	77	79	74	72
18	4	82	93	97	97	101	101	100	68	70	71	68	71	73	72
21	1	79	80	82	80	79	81	83	82	83	86	87	81	82	80

a (rep) = Number of replicates per sheep
b C = Control

APPENDIX G

Table G-1. Blood Pressure Responses of Individual Sheep to Intracerebroventricular Sarthran, 1000 ng/kg/min

	Blood Pressure (mmHg)									
Sheep	C	-10	-5	0	5	10	15	20	25	30 min
11	96	101	94	96	96	94	93	93	91	93
13	88	87	88	87	86	92	85	86	85	86
14	92	92	92	90	92	92	92	89	94	93
19	91	85	81	79	78	80	80	83	81	80
20	94	101	114	102	102	100	97	101	111	102
22	90	89	88	88	89	90	92	93	92	91
23	81	83	81	83	86	85	88	86	86	80
mean	90	91	91	89	90	90	90	90	91	89
SEM	1.9	2.8	4.2	2.9	3.0	2.5	2.2	2.4	3.8	3.0

Table G-2. Heart Rate Responses of Individual Sheep to Intracerebroventricular Sarthran, 1000 ng/kg/min

	Heart Rate (beats/min)									
Sheep	C	-10	-5	0	5	10	15	20	25	30 min
11	106	107	107	108	110	111	110	112	109	110
13	85	85	88	91	93	95	92	94	105	102
14	82	79	78	80	78	70	75	76	74	71
19	63	67	65	62	57	63	61	60	60	60
20	71	70	69	64	70	63	64	67	71	67
22	86	85	85	83	77	78	77	71	70	70
23	65	68	68	68	67	70	66	68	68	79
mean	80	80	80	79	79	79	78	78	80	80
SEM	5.6	5.3	5.6	6.2	6.7	6.8	6.7	6.9	7.3	7.1

Table G-3. Blood Pressure Responses of Individual Sheep to Intracerebroventricular (IVT) Sarthran, 1000 ng/kg/min, + IVT Angiotensin II, 50 ng/kg/min

Blood Pressure (mmHg)

Sheep	C[a]	-10	-5[b]	0[c]	5	10	15	20	25	30 min
11	91	96	94	100	100	106	102	104	100	101
13	90	88	85	86	88	94	90	90	86	89
14	92	91	95	91	91	96	94	98	92	92
19	88	89	89	89	89	91	90	90	90	92
20	93	95	96	95	95	99	94	94	103	100
22	83	85	84	85	86	85	86	89	87	87
23	73	73	71	73	75	76	78	77	81	82
mean	87	88	88	88	89	92*	91	92*	91	92*
SEM	2.6	2.9	3.3	3.2	3.1	3.7	2.8	3.2	2.9	2.5

a Sarthran infusion started at -15 minutes
b AII start to diffuse into ventricle
c AII infusion on at full strength

* $p<.05$ re Control

Table G-4. Heart Rate Responses of Individual Sheep to Intracerebroventricular (IVT) Sarthran, 1000 ng/kg/min, + IVT Angiotensin II, 50 ng/kg/min

	Heart Rate (beats/min)									
Sheep	0[a]	-10	-5[b]	0[c]	5	10	15	20	25	30 min
11	96	89	93	90	91	94	90	97	95	90
13	68	65	67	67	68	68	70	69	70	67
14	66	65	66	66	65	64	70	67	68	66
19	70	74	74	74	73	74	72	76	74	71
20	80	85	80	77	78	82	79	79	84	81
22	94	99	94	92	91	89	87	87	88	88
23	77	73	74	71	72	73	74	71	72	71
mean	79	79	78	77	77	78	77	78	79	76
SEM	4.6	4.9	4.3	4.0	4.0	4.2	3.1	4.1	3.9	3.8

a Sarthran infusion started at -15 minutes
b AII start to diffuse into ventricle
c AII infusion on at full strength

Table G-5. Blood Pressure Responses of Individual Sheep to Intracerebroventricular Sarthran, 1000 ng/kg/min, + Intracarotid PGE_2, 10 ng/kg/min

Blood Pressure (mmHg)

Sheep	C[a]	-10	-5	0[b]	5	10	15	20	25	30 min
11	90	90	89	87	94	101	100	100	102	101
13	87	89	90	91	97	100	102	105	103	99
14	93	101	104	101	105	106	105	108	108	109
19	87	93	90	86	96	99	102	104	102	106
20	89	96	97	97	102	111	112	113	110	107
22	87	91	89	89	96	100	98	103	104	105
23	76	78	79	83	94	96	98	100	100	102
mean	87	91*	91*	91*	98*	102+	102+	105+	104+	104+
SEM	2.1	2.7	2.9	2.4	1.6	1.9	1.8	1.7	1.3	1.4

a Sarthran infusion started at -15 minutes
b PGE_2 infusion started

* $p<.05$ re Control
\+ $p<.05$ re Control and Sarthran

Table G-6. Heart Rate Responses of Individual Sheep to Intracerebroventricular Sarthran, 1000 ng/kg/min, + Intracarotid PGE_2, 10 ng/kg/min

	Heart Rate (beats/min)									
Sheep	C[a]	-10	-5	0[b]	5	10	15	20	25	30 min
11	95	84	89	92	99	105	108	110	116	114
13	88	83	88	88	95	95	96	97	96	98
14	87	83	84	81	81	78	80	70	72	68
19	66	70	67	68	74	78	82	84	85	86
20	58	54	52	55	60	61	61	60	61	60
22	79	81	76	72	74	72	73	69	65	67
23	66	70	71	71	76	79	82	85	90	88
mean	77	75	75	75	80	81	83	82	84	83
SEM	5.2	4.2	5.0	4.8	5.1	5.5	5.8	6.6	7.3	7.3

a Sarthran infusion started at -15 minutes
b PGE_2 infusion started

Table G-7. Blood Pressure Responses of Individual Sheep to Intracerebroventricular Sarthran, 1000 ng/kg/min, + IVT PGE_2, 300 ng/kg/min

	Blood Pressure (mmHg)									
Sheep	C[a]	-10	-5[b]	0[c]	5	10	15	20	25	30 min
11	89	95	97	100	104	110	110	110	114	113
13	83	85	90	90	87	85	84	84	85	84
19	85	86	89	90	92	93	94	103	107	107
20	94	99	102	106	108	112	109	118	117	121
22	88	89	85	87	83	87	90	94	98	98
23	75	80	79	81	79	81	86	85	84	87
mean	86	89	90	92	92	95*	96*	99*	101*	102*
SEM	2.6	2.8	3.3	3.7	4.7	5.4	4.7	5.6	5.7	6.0

a Sarthran infusion started at -15 minutes
b PGE_2 start to diffuse into ventricle
c PGE_2 infusion on full strength

* $p < .05$ re Control and Sarthran

Table G-8. Heart Rate Responses of Individual Sheep to Intracerebroventricular (IVT) Sarthran, 1000 ng/kg/min, + IVT PGE_2, 300 ng/kg/min

	Heart Rate (beats/min)									
Sheep	0[a]	-10	-5[b]	0[c]	5	10	15	20	25	30 min
11	67	68	68	67	74	78	75	79	84	84
13	77	76	75	76	77	79	75	77	78	77
19	67	65	66	67	65	65	68	67	66	65
20	72	76	72	71	72	74	72	74	73	71
22	79	87	88	96	87	85	82	79	85	83
23	80	77	81	77	80	79	81	79	83	86
mean	74	75	75	76	76	77	76	76	78	78
SEM	2.3	3.2	3.4	4.4	3.1	2.7	2.2	1.9	3.1	3.4

a Sarthran infusion started at -15 minutes
b PGE_2 start to diffuse into ventricle
c PGE_2 infusion on full strength

Table G-9. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracerebroventricular Angiotensin II, 50 ng/kg/min, Two Hours After Subcutaneous Indomethacin, 4 mg/kg

Sheep	Blood Pressure (mmHg)							Heart Rate (beats/min)						
	C[a]	5	10	15	20	25	30 min	C	5	10	15	20	25	30 min
11	96	112	116	118	119	123	123	87	80	81	79	79	81	86
13	91	97	99	103	109	110	115	70	71	64	68	72	73	79
19	87	97	103	109	108	112	113	60	65	58	64	62	59	56
20	86	101	110	111	114	120	123	77	77	74	76	79	83	80
22	86	105	109	111	111	113	114	72	76	77	82	81	80	82
mean	89	102*	107*+	110*+	112*+	116*+	118*+	73	74	71	74	75	75	77
SEM	2.0	2.9	3.0	2.3	2.0	2.6	2.2	4.4	2.6	4.3	3.4	3.5	4.4	5.3

a C =Control
* p<.05 re Control
\+ p<.05 re 5 min

Table G-10. Blood Pressure and Heart Rate Responses of Individual Sheep to Intracerebroventricular Angiotensin II, 50 ng/kg/min, One Half Hour After Intravenous Flunixin Meglumine, 3 mg/kg

Sheep	Blood Pressure (mmHg)							Heart Rate (beats/min)						
	C[a]	5	10	15	20	25	30 min	C	5	10	15	20	25	30 min
11	94	105	105	112	110	114	119	93	85	83	84	82	82	83
21	83	95	97	100	103	103	104	87	78	80	81	82	82	85
22	90	109	109	112	109	110	112	85	89	85	84	82	84	84
23	80	78	77	81	81	85	96	61	64	65	65	62	63	68
mean	87	97*	97*	101*	101*	103*	108*	82	79	78	79	77	78	80
SEM	3.4	6.9	7.2	7.3	6.8	6.4	5.2	7.0	5.5	4.5	4.6	5.0	4.9	4.0

a C= Control
* $p<.05$ re Control. Steady state reached at 15 minutes.

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