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thesis entitled

Mechanisms of a Central Pressor Effect of Prostaglandin E_2

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Physiology

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Date May 2, 1983

MECHANISMS OF A CENTRAL PRESSOR EFFECT OF PROSTAGLANDIN E_2

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology

1983

ABSTRACT

MECHANISMS OF A CENTRAL PRESSOR EFFECT
OF PROSTAGLANDIN E_2

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The hemodynamic effects of intra-carotid Prostaglandin E_2 (PGE_2) were examined in conscious sheep, dogs and calves. The sheep and dogs were instrumented with chronically indwelling catheters in the carotid artery and jugular vein. The calves were instrumented with electromagnetic flow detectors on the main pulmonary, renal, iliac and superior mesenteric arteries. Catheters were placed in the right atrium, descending aortic arch and the carotid artery. This allowed measurement of cardiac output, iliac, renal and superior mesenteric blood flows and calculation of systemic and regional vascular resistances.

During intra-carotid PGE_2 infusion (10 ng/kg/min) arterial pressure significantly increased in all three species and heart rate significantly increased in the sheep and calves. The arterial pressure increase in the calves was principally mediated through an increase in total peripheral resistance. Resistances significantly increased in the three measured vascular beds.

Systemic venous infusions of PGE_2 (400 ng/kg/min) did not significantly alter arterial pressure or heart rate in the three species. Distal aortic PGE_2 infusion (200 ng/kg/min) into the calves

significantly decreased blood pressure and significantly increased heart rate. No changes in arterial blood gases were observed in the three species during intra-carotid PGE_2 infusion (10 ng/kg/min); thus, no chemoreflex activation occurred. The cardiac baroreflex sensitivity was unchanged during intra-carotid PGE_2 infusion (10 ng/kg/min) in all species despite significant increases in arterial pressure. Intra-carotid angiotensin II infusions (10 ng/kg/min) markedly decreased the baroreflex sensitivity in all species.

Intra-carotid PGE_2 infusions (100 ng/kg/min) did not increase arterial pressure in anesthetized calves or sheep (barbiturate/halothane, chloralose/urethane, respectively). In both species arterial pressure during anesthesia was significantly elevated above control.

Alpha, but not beta, adrenergic blockade prevented the pressor effect of intra-carotid PGE_2 infusion (10 ng/kg/min). Angiotensin converting enzyme blockade attenuated the intra-carotid PGE_2 pressor response.

Plasma renin activity doubled during intra-carotid PGE_2 infusion (10 ng/kg/min). Increasing PGE_2 infusion rates incrementally increased arterial pressure while plasma renin activity increased though not in proportion to the arterial pressure increases.

In conclusion, intra-carotid PGE_2 acts centrally to increase arterial pressure through increased sympathetic vasoconstrictor tone with a small contribution from the peripheral renin-angiotensin system.

Dedication:

To Kathy and our Families

Acknowledgments

Many institutions and people contributed to this dissertation. Some preliminary experiments were performed while I was a summer student at the Worcester Foundation for Experimental Biology. Preliminary studies continued while I was at Worcester Polytechnic Institute. I acknowledge the contributions of the faculty of these institutions.

Each member of my dissertation committee has uniquely contributed to my doctoral education. Special thanks are due to Dr. John Chimoskey for encouraging me to continue to examine the hemodynamic effects of PGE_2. In addition many faculty of Michigan State University gave considerable time to discussions of the research.

I appreciate the financial support derived from the interdepartmental cardiovascular training grant (NIH HL07404-05) and departmental general funds. I also wish to acknowledge the generous gifts of drugs from Squibb (captopril) and Ciba-Geigy (angiotensin II, phentolamine).

I also wish to thank the Michigan Heart Association for supporting this research for two years.

Many technicians, veterinary students and summer students contributed to this dissertation. I am grateful for their time, questions and good will.

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Literature Review

General Introduction to the Literature

The literature reporting arterial blood pressure effects of centrally administered E series Prostaglandins (PGE) is small enough to be throughly reviewed in the following pages. A large proportion of these reports deal with central PGE effects in conscious animals, in which a pressor response is consistently observed. A smaller, generally older literature has reported central PGE pressor effects (usually PGE_1) in anesthetized animals; however, the PGE induced hypertension is often variable within the study and occasionally is absent.

Most reports have only examined the effect of central PGE on blood pressure and heart rate. A smaller number of these studies reported a specific pressor mechanism in conscious and anesthetized animals. The most recent literature has implicated both humoral and autonomic nervous system mediation of the PGE pressor effect in conscious animals. Only one report, however, partitions the central PGE pressor effect into cardiac (cardiac output) and total peripheral resistance components.

The pressor effects of PGE_1 and PGE_2 are included in this literature. An occasional report considers the hemodynamic effects of other prostaglandins and autocoids and, where relevant, these effects are considered.

The literature is presented in three subgroups and discussed chronologically within each subgroup. Reports of central PGE effects in anesthetized animals are presented first. The second subgroup examines reports in which PGE was infused centrally, but no pressor effect was observed. The last and largest subgroup examines reports of central PGE pressor effects in conscious animals.

Anesthetized Animals; Reported Pressor Response to Central PGE

Carlson and Oro (1966) first demonstrated central PGE_1 pressor responses in the pentobarbital anesthetized dog. They observed that carotid infusion of PGE_1 (1200 ng/kg/min, 5 to 10 min) produced a pressor response in six of eight animals. The pressor effect was small (6 mmHg) and heart rate also increased (12 beats/min). They also showed that pretreatment with hexamethonium blocked the PGE_1 pressor response and concluded that the pressor effect was mediated through sympathetic vasoconstrictor activity. They proposed that the PGE_1 pressor effect may have been due to a direct effect on the carotid artery, on structures within the carotid artery, or on the central nervous system itself.

Kaplan et al. (1969) confirmed the initial observation of Carlson and Oro. These authors also used pentobarbital anesthetized dogs and designed experiments to test for central or humoral mediation of the central PGE_1 pressor effect. The experimental design used a complicated two-dog, cross circulation technique. A donor dog provided arterial blood to the head of another dog and recieved venous jugular blood. In this latter recipient dog the neural vasculature was left

intact and all vascular connections between the trunk and the head were tied off. When PGE_1 (5-10 ug) was injected into the carotid artery of the neurally intact recipient dog a pressor response (typically 40-50 mmHg) was observed in the recipient dog trunk. A depressor response was observed in the donor dog after intra-carotid PGE_1 injection in the recipient dog. The pressor response to intra-carotid PGE_1 in the recipient dog trunk was blocked by hexamethonium. The authors concluded that PGE_1 exerts a central pressor effect mediated through the sympathetic nervous system.

The effects of infusion of PGE_1 into the vertebral artery in the chlorolose anesthetized cat were reported by Gyang et al. (1973). PGE_1 was infused (1-20 ng/kg/min, 4 to 6 min) and blood pressure increased 10 to 48 mmHg. Heart rate increased concomitantly with blood pressure. Intravenous infusion of PGE_1 at the same infusion rates produced much smaller pressor effects. They concluded that PGE_1 can evoke a pressor response when carotid sinus structures are not directly perfused.

Rinchuse and Deuben (1976) demonstrated central pressor responses to intra-carotid PGE_1 in the urethane anesthetized rat. Mean arterial blood pressure increased 15 to 20 mmHg during PGE_1 infusions (1-10 ng/kg/min). Heart rate usually increased with blood pressure, but the heart rate did not always increase during the pressor response. Intravenous PGE_1 infusions of 1-10 ng/kg/min failed to demonstrate a pressor response. Larger intravenous infusions (1000-10,000 ng/kg/min) resulted in hypotension and intra-carotid PGE_1 infusion rates was above 100 ng/kg/min produced hypotension.

Fujimoto (1977) observed that intracerebroventricular PGE_2 (1.4-140 ug/kg) increased mean arterial blood pressure up to 25% in a dose related manner in anesthetized rats. Three anesthetic regimens (urethane, pentobarbital or chloral hydrate-chloralose) were used and the PGE_2 pressor effect was consistently seen.

Brus et al. (1979) examined temperature and arterial pressure effects of intracerebroventricular injected PGE_2 and PGF_{2alpha} in the urethane anesthetized rat. Central PGE_2 injection (1 or 10 ug) caused increased body temperature (1.5 ^{o}c) and a 45 mmHg increase in arterial pressure. In previous studies, these authors had shown central hyperthermic and hypertensive responses to centrally injected PGE_2 and PGF_{2alpha}. After injection of 5,6-dihydrotryptamine, which causes degeneration of central serotoninergic neurons, the hyperthermic and pressor effects of centrally injected PGF_{2alpha} were abolished. However, centrally injected PGE_2 continued to cause hypertensive and hyperthermic effects. The authors concluded that serotoninergic neurons mediate the hyperthermic and hypertensive effects of centrally injected PGF_{2alpha}, but not those of centrally administered PGE_2.

Feuerstein et al. (1982) reported the arterial pressure, heart rate and blood catecholamine concentrations prior to and during lateral ventricular and intrahypothalamic PGE_2 injection (60 and 600 ng/kg) in halothane anesthetized rats. Arterial pressure and heart rate both increased. The arterial pressure increased 17 mmHg in association with a heart rate increase of 120 beats/min at the lower dose of PGE_2. With the higher dose, an increase in arterial pressure of 28 mmHg occurred,

associated with a heart rate change of 175 beats/min. At the lower dose of PGE_2, both plasma norepinephrine and epinephrine doubled. The larger dose of PGE_2 caused a tripling of the plasma concentrations of these catecholamines. Intravenous injection of 0.18 ug/kg of PGE_2 caused no hemodynamic alteration, while a brief hypotensive response was observed after 600 ng/kg PGE_2 was injected intravenously. Because plasma norepinephrine increased, the authors concluded that the arterial pressure and heart rate responses observed during central PGE_2 injections were not the result of adrenomedullary catecholamine release, but were consistent with activation of the sympathetic nervous system.

The previous seven reports demonstrate pressor responses to PGE_1 or PGE_2 in anesthetized dogs, cats and rats. Various anesthetics, experimental preparations, and routes and lengths of PGE administration were used. Pressor responses were evident in pentobarbital anesthetized dogs. However, this response was elicited only with ug/kg doses of PGE. Pressor responses were observed in the chloralose anesthetized cat at ng/kg/min PGE doses. The resulting pressor response in the cat was variable, with some animals showing a small response and others a much larger one. Central administration of PGE in the rat also created a pressor response at ng/kg/min doses, regardless of the anesthetic used. The effect of centrally administered PGE on heart rate was not always reported. However, where heart rate was reported, central PGE administration caused heart rate not to change or to increase.

Anesthetized Animals; No Reported Pressor Response to Central PGE

Nakano and McCurdy (1967) examined the cardiovascular effects of PGE_1 in pentobarbital anesthetized dogs. Arterial pressure, heart rate and myocardial contractile force were unchanged by intra-carotid PGE_1 injection (0.1 ug/kg), while peripheral resistance fell by 40%. Intravenous PGE_1 injection (.25-4.0 ug/kg) decreased arterial pressure and increased heart rate.

Lavery et al. (1970) infused PGE_1 (9-60 ug/min) into chloralose anesthetized dogs via venous, and carotid and vertebral arterial routes. No significant changes in arterial pressure were observed. Tachycardia occured with vertebral infusions. Heart rate increases of smaller magnitudes were seen when PGE_1 was infused by the carotid arterial and femoral venous routes.

Holmes (1970) perfused the lateral ventricles of chloralose anesthetized dogs with PGE_1 (100 ng/ml, 0.5 ml/min) for two hours. Respiratory depth increased but arterial pressure did not change.

McQueen and Belmonte (1974) studied afferent carotid sinus and afferent chemoreceptor nerve responses. PGE_2 injected into the carotid sinus (0.4-8.0 ug/kg) and femoral vein (1.5-7.4 ug/kg) of pentobarbital anesthetized cats. PGE_2 injections by either route decreased blood pressure in a dose dependent fashion. Associated with the fall in blood pressure was a similar reduction in baroreceptor nerve activity. No pressor effects occured after carotid PGE_2 injection. Intravenous PGE_2 injections caused a dose dependent increase in chemoreceptor

discharge. The increase in discharge rate was associated with a fall in blood pressure. Additionally, in vitro studies of the isolated carotid body showed no increases in discharge frequency when PGE_2 was placed in the bath. The authors concluded that PGE_2 caused no direct effects on baroreceptor or chemoreceptor discharge rate and that any observed changes were due to the fall in blood pressure.

Yamamoto et al. (1976) studied vasopressin release during ventriculo-cisternal PGE_2 perfusion in the urethane-chloralose anesthetized dog. Plasma vasopressin doubled with PGE_2 perfusion rates of 153 ng/min (0.2 ml/min); blood pressure did not change. Smaller PGE_2 infusion rates (76 ng/min) had no effect on either variable. The authors concluded that central PGE_2 may have a role in the control of vasopressin release.

Spira et al. (1978) examined carotid vascular and hemodynamic responses to intra-carotid infusion of autocoids, including PGE_1, in pentobarbital anesthetized monkeys. Common carotid PGE_1 infusions (1-1000 ng/kg/min, 5 min) caused a dose-related decrease in external carotid vascular resistance. Internal carotid vascular resistance decreased during carotid PGE_1 infusions (1-100 ng/kg/min) and increased during higher dose carotid PGE_1 infusions (100 and 1000 ng/kg/min). No changes in blood pressure were reported. However, from data graphically presented for one animal, arterial pressure increased approximately 20 mmHg immediately after PGE_1 infusions (20 ng/kg/min). The authors concluded that minimal carotid dilation appeared to be the only direct effect of PGE_1 infusion.

Although the previous six papers do not report pressor responses to centrally infused PGE in anesthetized dogs, cats and monkeys they are explainable with the previous group of observations. Anesthetics, method of PGE administration and PGE dose rate may explain the failure to observe pressor responses in these studies. Chloralose anesthetized cats responded with increased blood pressure to very small sustained infusions of PGE_1 (Gyang, 1973), while pentobarbital anesthetized cats failed to respond to ug/kg PGE_2 injections (McQueen and Belmonte, 1974). Spira et al. did not report PGE_1 pressor effects, but the presented data indicates a pressor effect. Furthermore, any centrally mediated pressor response to PGE is potentially complicated by direct peripheral hypotensive effects of PGE (Kaplan et al., 1969; Rinchuse and Deubin, 1976; McQueen and Belmonte, 1974). Massive central PGE administration may not have resulted in pressor responses due to the overwhelming peripheral vasodilatation. In general, the effect of large doses of PGE appeared to be depressor in both sets of literature.

PGE Pressor Responses Reported in Conscious Animals.

Hull (1975) demonstrated that intra-carotid infusions of PGE_2 (5-20 ng/kg/min) would produce dose related increases in arterial pressure in conscious sheep. At PGE_2 infusion rates above 20 ng/kg/min, pyrexia was also observed. The largest intra-carotid PGE_2 infusion rate (60 ng/kg/min) resulted in a 30% increase in mean blood pressure. Pretreatment with indomethacin did not attenuate febrile or pressor effects. No hemodynamic or febrile effects were observed when PGE_2 was infused intravenously (1000 ng/kg/min). The author concluded that

intra-carotid PGE_2 evoked central pressor and febrile responses in conscious sheep and that the febrile response had a higher dose threshold.

Leksell (1976) described pressor responses to centrally infused PGE_1 in conscious goats. PGE_1 was infused (30 ng/kg/min) into the lateral ventricular system; arterial pressure, water intake, vasopressin release and renal sodium excretion were observed. PGE_1 infusion increased arterial pressure in association with increased water intake, vasopressin release and naturesis. The author concluded that PGE_1 may contribute to volume homeostasis through central mechanisms.

Hoffman and Schmid (1979) studied cardiovascular and diuretic responses to intracerebroventricular PGE_2 injections (.05-5000 ng) in unanesthetized rats. Central PGE_2 injections evoked a dose dependent increase in arterial pressure and heart rate lasting up to two hours after PGE_2 injection. Pretreatment with phenoxybenzamine blocked the pressor effects of PGE_2, and pretreatment with propranolol blocked the associated tachycardia. Propranolol alone did not block the PGE_2 pressor effect. PGE_2 injection in water loaded rats resulted in antidiuresis and pressor responses. Median eminence lesions abolished the antidiuretic, but not the pressor, response to PGE_2. Intravenous infusions resulted in hypotension. The authors concluded that central PGE_2 increases arterial pressure through activation of alpha adrenergic neurons, and that antidiuretic effects operate through the median eminence.

Hoffman and Valigura (1979) studied cardiovascular and hyperthermic effects of centrally injected PGE_2 (0.5-500 ng) in conscious rats. Lateral ventricular PGE_2 injections caused a dose dependent increase in arterial pressure (20 mmHg), heart rate (100 beats/min) and body temperature (1.0 ^{o}C). Pretreatment with phenoxybenzamine abolished the PGE_2 pressor response, but not the febrile or heart rate effects. The authors concluded that the pressor and febrile responses resulting from central PGE_2 were separate and that the pressor response was effected via alpha adrenergic receptor stimulation.

Hull and McCracken (1979) studied the cardiovascular effects of PGE_2, PGF_{2alpha}, 13, 14-dihydro 15-keto PGE_2 and 15-keto PGE_2 infusions (1.9-1500 ng/kg/min) in conscious and anesthetized sheep. These data were published in abstract form and unpublished figures are presented in this dissertation in Appendix A. This study formed the preliminary observations for this dissertation. Prostaglandins were infused into the carotid artery, jugular vein and thoracic aorta. Arterial pressure, heart rate and cardiac output were the studied variables. Intra-carotid PGE_2 infusion increased arterial pressure in a dose dependent fashion, with a maximal increase of 30 mmHg. The onset of the arterial pressure rise occurred within one to three minutes and the maximum blood pressure response was apparent within ten minutes. The arterial pressure return to control values was slower than the rise in arterial pressure in response to PGE_2 infusion. With the larger intra-carotid PGE_2 infusion rates, the return to control pressure took an hour. Heart rate increased 5 to 10% during the first two minutes of the intra-carotid PGE_2 infusion and then was not statistically

different from control values. Cardiac output was not statistically altered during the intra-carotid PGE_2 infusions.

Aortic PGE_2 infusion decreased arterial pressure and increased heart rate and cardiac output. Jugular PGE_2 infusions (1500 ng/kg/min) had no effect on blood pressure.

Jugular PGE_2 infusion did not alter heart rate or cardiac output. PGF_{2alpha}, 13, 14-dihydro 15-keto PGE_2 and 15-keto PGE_2 had no hemodynamic effect by any route or dose. Anesthesia (barbiturate and halothane) abolished intra-carotid PGE_2 pressor responses. The authors concluded that intra-carotid PGE_2, and not the principal metabolites of PGE_2, increases arterial pressure through increased total peripheral resistance and that the effect is centrally mediated.

Kondo et al. (1979a) described arterial pressure responses to central PGE_2, PGI_2, PGF_{2alpha} and indomethacin in conscious rats. Intraventricular catheters were inserted prior to the study and the rats were studied in the conscious state. Central injections of PGE_2 (100-1000 ng/kg) produced a dose dependent pressor effect with a maximal rise in arterial pressure of 60 mmHg. PGI_2 (1.25-10 ug/kg) evoked a dose dependent hypotension. PGF_{2alpha} (0.3-20 ug/kg) did not significantly alter arterial pressure. Centrally administered indomethacin (0.6-40 ug/kg) did not alter arterial pressure. The authors concluded that exogenously administered prostaglandins may influence blood pressure, but prostaglandins synthetized in the rat brain do not have a large role in regulation of blood pressure.

Kondo et al. (1979b) studied the central and peripheral cardiovascular effects of bradykinin and PGE_2 injections. Intracerebroventricular injection of PGE_2 (30-300 ng/kg) into conscious rats produced a graded pressor effect that was maximal (60 mmHg increase) at the largest dose of PGE_2. Intravenous injection of PGE_2 (2.5-10 ug/kg) resulted in a dose dependent hypotension. Pretreatment with indomethacin did not alter the pressor or depressor responses. Central bradykinin (1-4 ug) injections similarly increased arterial pressure. Intravenous bradykinin (3-13 ug/kg) injections lowered arterial pressure. Pretreatment with indomethacin markedly attenuated the central bradykinin pressor effect and did not alter the intravenous bradykinin depressor response. The authors concluded that central PGE_2 and bradykinin resulted in pressor effects and that the bradykinin pressor effect was mediated by PGE_2 synthesized in the central nervous system.

Skarnes and McCracken (1980) provided evidence that endogenous synthesis of PGE causes a pressor effect in conscious sheep. They injected non-lethal amounts of endotoxin and studied the hemodynamic and febrile responses, as well as plasma PGE concentrations, in jugular and carotid blood. Twenty minutes after injection of endotoxin, body temperature increased, arterial pressure increased and PGE appeared in jugular and carotid blood. The PGE carotid-jugular difference was small, with peak PGE concentration differences about 80 pg/ml. Intra-carotid PGE_2 infusions (20-40 ng/kg/min) increased body temperature and blood pressure. Administration of indomethacin alone, prior to endotoxin injection resulted in no elevation of body

temperature or blood pressure. Indomethacin pretreatment prior to intra-carotid PGE_2 infusions failed to block the PGE_2 pressor and febrile responses but did block the endotoxin induced pressor and febrile responses. Intra-carotid infusion of PGF_{2alpha} (20-400 ng/kg/min) did not alter blood pressure or body temperature. The authors concluded that exogenous injection of endotoxin stimulates PGE generation within the head. The generated PGE develops a sufficient concentration to cross the blood-brain barrier inducing pressor and pyrexic responses through a central action.

Skarnes et al. (1981) studied blood pressure and temperature responses to intra-carotid infusions of PGE_2, PGD_2, PGF_{2alpha}, PGI_2 and non-lethal injections of endotoxin. They measured plasma PGE concentrations in the jugular vein, carotid artery, femoral vein and femoral artery. Intra-carotid PGE_2 infusions (40 ng/kg/min) caused pressor and pyrexic responses. Pretreatment with indomethacin did not alter arterial pressure or temperature and did not attenuate the pressor or febrile responses to intra-carotid PGE_2 infusion. Intravenous (jugular vein) infusion of PGE_2 did not alter arterial pressure, heart rate or animal temperature. Intra-carotid infusion of PGF_{2alpha} (20-400 ng/kg/min) and intra-carotid PGI_2 infusion (100 ng/kg/min) did not alter blood pressure or body temperature. Intra-carotid infusion of PGD_2 (40 ng/kg/min) increased blood pressure, but did not change body temperature. After a twenty to thirty minute delay, intra-carotid endotoxin injection caused increases in arterial pressure, temperature and femoral artery and vein and carotid artery and jugular vein concentrations of PGE and PGF. Venous prostaglandin

concentrations (PGE and PGF) were higher than arterial concentrations, indicating release of PGE and PGF from structures in the head and leg. The authors concluded that synthesis of PGE is not unique to the cerebral circulation.

Hoffman et al. (1981) studied central cardiovascular effects of PGE_2, PGF_{2alpha} and PGI_2 in unanesthetized rats. Arterial pressure and heart rate comparisons were made after lateral cerebral ventricular (0.5-500 ng) and intravenous (1.0 ng) injection of each prostaglandin. Central PGI_2 resulted in a dose dependent hypotension with a significant tachycardia apparent at the largest dose. Central PGE_2 caused a dose dependent pressor response with a maximal increase of 14 mmHg. The heart rate increased progressively, with a 90 beat per minute increase observed at the largest PGE_2 dose. Central PGF_{2alpha} also evoked a pressor and tachycardic effect, with each arterial pressure and heart rate response about one half that observed with central PGE_2 injection. PGI_2 and PGE_2 both caused a hypotensive response when administered intravenously. Heart rate increased 100% and 30%, respectively. Intravenous PGF_{2alpha} resulted in a small pressor response (9%) and bradycardia (-25%). The authors concluded that central hemodynamic effects of PGE_2 and PGF_{2alpha} occur at very small concentrations and that these hormones do not have similar actions when administered peripherally.

Takahashi and Bunag (1981) investigated blood pressure, heart rate and sympathetic nerve activity after central PGE_2 injection in conscious and anesthetized normotensive and hypertensive rats.

Intracerebroventricular injection of PGE_2 (1 ug) into normotensive rats increased arterial pressure (26 mmHg) and heart rate (89 beats/min). Anesthetized (urethane) normotensive rats showed similar responses, with increases in blood pressure (20 mmHg), heart rate (53 beats/min) and sympathetic nerve firing (24%, inferior nerve bundle merging from the coeliac ganglion). Central PGE_2 injection also increased arterial pressure (50 mmHg) and heart rate (104 beats/min) in hypertensive rats. Anesthetized hypertensive rats showed increased arterial pressure (52 mmHg), heart rate (66 beats/min) and sympathetic nerve firing (50%). Removal of sympathetic vasomotor tone through cervical section of the spinal cord did not abolish the central PGE_2 pressor effect, but decreased the immediate hypertensive response. The authors concluded that central PGE_2 increased arterial pressure through initial sympathetically mediated mechanisms, but that a later release of a humoral pressor substance was involved.

Okuno et al. (1982) demonstrated that central PGE_2 increases arterial pressure, plasma catecholamines, plasma arginine vasopressin and plasma renin activity in conscious rats. Intracerebroventricular PGE_2 (1 ug/kg) injection increased arterial pressure (30 mmHg), plasma norepinephrine (400%) and plasma epinephrine (600%) and tripled plasma renin activity. Administration of captopril prior to central PGE_2 injection did not alter the pressor effect, but pretreatment with a vasopressin antagonist or phenoxybenzamine attenuated the PGE_2 pressor effect. The authors concluded that central PGE_2 increases arterial pressure through activation of the sympathetic adrenergic autonomic system and is dependent on renin and vasopressin release.

The previous twelve reports describe centrally mediated pressor responses to PGE (11 reports with PGE_2) in conscious sheep, goats and rats. The PGE_2 pressor effect is partly mediated through activation of alpha adrenergic sympathetic neurons (Hoffman and Schmid, 1979; Hoffman and Valigura, 1979; Takahashi and Bunag, 1981 and Okuno et al., 1982). There is also a humorally mediated component to the pressor response (Okuno et al., 1982). Other groups have described humorally mediated antidiuresis in goats (Leksell, 1976) and in rats (Takahashi and Bunag, 1981 and Okuno et al., 1982). Only one report (an abstract) described measuring cardiac output during intra-carotid PGE_2 administration; the conclusion was that Cardiac output was unchanged in conscious sheep (Hull and McCracken, 1979), but central PGE administration caused increases in heart rate in conscious sheep (Hull, 1975; Hull and McCracken, 1979) and rats (Hoffman and Schmid, 1979; Hoffman and Valigura, 1979; Hoffman et al., 1981 and Okuno et al., 1982). Intravenous PGE decreased blood pressure in rats, but did not cause hypotension in conscious sheep, even at high doses (Hull, 1975; Hull and McCracken 1979 and Skarnes et al. 1981). Pyrogen induced pressor effects are associated with PGE synthesis in both the head and the leg. (Skarnes et al., 1981). This study showed that after injection of sub-lethal amounts of endotoxin, blood pressure increased in parallel with increases in vascular PGE. This unique observation indicates that endogenous PGE synthesis as well as exogenous PGE administration is capable of increasing blood pressure. Indomethacin injection to block PG synthesis prior to the endotoxin injection blocked the pressor effect.

Statement of Objectives

1). Does intra-carotid PGE_2 infusion alter arterial blood pressure and heart rate in conscious sheep, calves and dogs?

2). Do the effects of intra-aortic and systemic venous PGE_2 infusions differ from intra-carotid PGE_2 infusions in these conscious animals?

3). If arterial pressure is increased during intra-carotid PGE_2 infusion, does total peripheral resistance increase, cardiac output increase or both? Are renal, iliac and superior mesenteric regional resistances increased by intra-carotid PGE_2 infusion?

4). Does intra-carotid PGE_2 alter arterial blood gases (pO_2 and pCO_2) and plasma pH; i.e. does intra-carotid PGE_2 stimulate pulmonary ventilation and thus alter blood gas composition through direct chemoreflex activation, and there by also raise arterial pressure?

5). Is the arterial baroreflex altered by intra-carotid PGE_2 infusion and if it is, does this response differ from the baroreflex response observed with intra-carotid angiotensin II infusion?

6). The pressure results of central PGE administration in anesthetized animals are conflicting; thus does general anesthesia affect the intra-carotid PGE_2 infusion response?

7). The internal carotid artery origin from the carotid artery is lost in the ontogeny of the sheep. Therefore, do the brains of sheep receive blood flow from the common carotid arteries, i.e. can intra-carotid PGE_2 reach the brain of the sheep.

8). Does beta adrenergic blockade, alpha adrenergic blockade or do the two together affect the intra-carotid PGE_2 infusion response? Does the administration order of the adrenergic blockers affect the intra-carotid PGE_2 response?

9). Are changes in plasma renin activity involved in the response to intra-carotid PGE_2 infusion? Does the plasma renin activity follow a dose response relationship similar to the blood pressure responses with increasing infusion rates of intra-carotid PGE_2? i.e. can plasma renin account for the arterial pressure changes.

10) Does blockade of angiotensin converting enzyme alter the response to intra-carotid PGE_2 infusion?

Materials and Methods

General Conditioning and Behavioral Considerations

All animals used in these studies were obtained from the Laboratory Animal Care Service (LACS) at Michigan State University. LACS is available for surgical and medical veterinary consultation and provides animals which are vaccinated against common diseases. The animals were brought into the experimental quarters (Giltner Hall) at least one week prior to surgery to allow the animals to be accustomed to their surroundings. The animals were acclimated to the specific experimental conditions before experiments were conducted to minimize any later stress. If the animals were bothered by noise, efforts were made to minimize the noise. Dogs and sheep were the most sensitive to noise or people entering the room. In contrast, the calves did not seem to be bothered by these distractions. Each of the experimental protocols was designed so that the length of any experiment was less than ninety minutes. In general, the shorter experiments were conducted first to further condition the animal.

Dogs were trained to lie on a padded table and remain quiet for forty to sixty minutes. Conditioning the dogs usually took two to four days with two or three training periods per day. The sheep were trained to stand in their cage restrained by a halter so that they could not chew on infusion catheters. Conditioning took longer for sheep (1-2 weeks), since the sheep experimental protocol required that a person enter the cage to turn stopcocks on and off. Sheep are by

nature more skittish than dogs. It required many training periods before the sheep were conditioned to tolerate a person entering their cage. It was important not to allow other sheep to eat during the experiment, as sheep are particularly sensitive to other animals eating or bleating. The calves were the easiest animals to train and work with. Like the sheep, the calves were trained to stand tied to the end of their cage by a rope fastened to their halter. After one or two disturbances by people entering the room, no further behavioral or hemodynamic (increased arterial pressure and heart rate) startle responses were noticed. The calves would occasionally object to the halter restraint if the adjacent calf was eating; therefore, this situation was avoided. After the experiments the animals were allowed free access to water. All the feeding was conducted by the LACS personnel. The calves, sheep and dogs were fed standard lab chow, approximately 1.1 kg of grain, 1.4 kg of pellets and 1.0 kg of dry food per day respectively. In general, all animals were studied several hours post prandial.

All animals were maintained on a twelve hour light and dark cycle (lights on 7 am) which minimized the stimulus for seasonal breeders (sheep) to come into estrus. Experiments were usually done during light hours with occasional experiments conducted during the evening.

Each animal was weighed at the start of the study. Sheep had a tendency to become obese and, if weight gain became noticable, the daily food ration was reduced. Because drugs were administered on a weight basis, the weight of the growing calves was estimated.

Approximately one year into the study a large animal scale was purchased and the calves and sheep were weighed at biweekly intervals. Retrospective inspection of the estimated weights showed that these estimates were close to actual values. The experimental duration was on the order of three weeks to two months. At the conclusion of the experimental series the animals were killed by intravenous injections of barbiturate and the carcasses were returned to LACS for disposal or necropsy.

All sheep and dogs required at least one jugular venous catheter and one catheter in each carotid artery. This allowed measurement of arterial pressure, heart rate, and arterial blood gases, carotid infusion of PGE_2 and systemic venous administration of drugs. The calves required only one carotid catheter because a distal aortic catheter was inserted. No jugular catheter was required for venous drug administration because a right atrial catheter was available. In some of these calves electromagnetic flow detectors were placed around the main pulmonary, iliac, renal and superior mesenteric arteries. This instrumentation allowed quantification of cardiac output, regional flows and resistances.

Catheter Construction

Catheters for chronic implantation into sheep, calves and dogs were constructed from Tygon tubing (Norton Co., S-54-HL formulation, O.D. .070", I.D. .040") and inserted using the method of Herd and Barger (1964). Daily catheter care is critical in chronically instrumented animals. The catheters were drained of blood and saline daily and

refilled with a heparin-saline (Rugby) solution (1000 IU/ml).

Sheep

Fourteen adult female mixed breed sheep weighing between 45 and 70 kg (mean weight 65 kg) were used in the study. Prior to surgery the sheep were fasted for twenty four hours. Water was removed during the last twelve hours. Each sheep was anesthetized with intravenous sodium thiamylal (10 mg/kg or to effect, Bio-tal, Bio-Ceutic Labs) an ultra short acting barbiturate and intubated with a cuffed endotracheal tube. Halothane (Fluothane, Halocarbon Labs.) in oxygen (1-3 %, 4 liters/min total flow) was administered with a semi-closed circuit anesthetic apparatus. The sheep were allowed to breathe spontaneously, and a surgical level of anesthesia was maintained during the surgery. Anesthetic level was adjusted by altering the delivered halothane gas concentration. Aseptic surgical techniques were followed throughout the surgery.

Sheep Surgical Preparation

To allow for the measurement of arterial pressure and for drug infusions, the sheep were chronically instrumented with arterial and venous non-occlusive indwelling catheters. For the placement of these catheters into the carotid arteries and jugular veins, the sheep were anesthetized, placed in dorsal recumbancy, the ventral aspect of the neck and shaved, washed, swabbed with a 1% iodine solution and draped. After the ventral midline neck skin incision was made, blunt dissection revealed the common carotid artery slightly lateral to the trachea. The external jugular vein was more lateral than the common carotid

artery. Catheter placement was midway between the manubrium and the angle of the jaw. Each catheter was directed in the direction of blood flow and about one centimeter of tubing remained within the vessel lumen. The sheep was placed on its side and the scapular area shaved, washed and swabbed with an iodine solution. The other end of each catheter was then tunnelled beneath the skin from the neck region and was exteriorized at the prepared scapular area. A solution of heparin in saline (100 IU/ml) was used initially to fill the catheters, and stainless steel pins were used to plug the exteriorized ends of the catheters. Each catheter was tied to a small loop of Tygon tubing which was inserted through the skin at the scapular area. Any excess length of catheter tubing was cut off so that the animal had a minimal chance of chewing the catheters. After the wound clot had formed, a stronger solution of heparin (1000 IU/ml, 0.5ml) was used to fill each catheter. The animal was allowed to regain consciousness and usually walked back to its cage. A dilute solution of hydrogen peroxide (1%) was used to remove any blood on the skin and adjacent wool. A cutaneous ointment (Betadine, povidone-iodine, Purdue Frederick Co.,) was smeared (1-2 ml) over the operative site and catheter exit sites to minimize infection. Post operative antibiotics were administered intramuscularly (Combiotic, Pfizer Labs, 10^7 units/day) for five days.

Dogs; Surgical Preparation

Four adult male mongrel dogs with pleasant dipositions weighing 21 to 30 kg (mean weight 25 kg) were selected for the study. Catheters (Herd-Barger) were placed in each common carotid artery and in an external jugular vein. The pre-surgical preparation and post-operative

care were identical to that described for the sheep. Catheter placement was similar except that the catheters were placed caudal (3-6 cm) to the carotid sinus region. Canvas vests were placed on the dogs to prevent them from pulling or chewing on the catheters. As with the other chronically instrumented animals, each catheter was drained daily of blood and saline and then refilled with a saline-heparin solution (1000 IU/ml, 0.5 ml).

Calves

Eighteen young (ten weeks) male Jersey calves were also used in these experiments. The initial weight of the calves was 45 kg. These animals were acquired from another experiment when a critical piece of chronic instrumentation had failed, making them unsuitable for that study. In a majority of cases, the supra-valvular aortic occluder failed leaving the animal equipped with catheters (right & left atrial, aortic and carotid), electromagnetic blood flow detectors (pulmonary, iliac, renal and superior mesenteric) and a thermistor (YSI Inc., #421) located next to the carotid artery. A smaller proportion of animals became available when a catheter or electromagnetic flow detector failed. Not every animal had all of this instrumentation, but common to all was a pulmonary artery flow detector and catheters (right & left atrial and aortic).

Calf; Surgical Preparation

Twenty four hours prior to surgery, food was removed from the cage. Water was removed twelve hours later. The animal was anesthetized with an ultra short acting barbiturate (Bio-tal, 10 mg/kg iv), or to effect,

and intubated with a cuffed endotracheal tube. General anesthesia was maintained with a halothane-oxygen (1-3 %, 4 liters/min) gas mixture. The left chest was then shaved, washed, swabbed with an iodine solution (1%) and draped for a fourth intercostal thoracotomy. After the initial skin incision, an electrosurgery unit was used to dissect through connective tissue, muscle and fascia. After the intercostal muscles were cut, the animal was changed from spontaneous respiration to positive pressure ventilation. End inspiratory pressure was altered to ensure adequate lung inflation. The ribs were spread open and the pericardium longitudinally opened. Catheters (Herd-Barger) were placed in both atria and in the descending aortic arch. An electromagnetic flow detector (Zepeda Inst., 24 - 28 mm dia) was placed around the pulmonary artery and the pericardium loosely closed. The flow detector leads and the catheters were brought out through the third interspace and the ribs apposed with umbilical tape. A chest tube was inserted via the fifth interspace after which muscle layers and skin were brought together and sewn. Like the sheep and dogs, a small loop of Tygon tubing was inserted in the skin to which the catheters and flow detector wires were attached. After evacuation of fluid and air from the thorax, the animal was removed from the ventilator and was allowed to regain consciousness. Post operative procedures consisted of administering intramuscular antibiotics (Combiotic, Pfizer Labs, 10^7 units/day), daily catheter flushing (Heparin 1000 IU/ml) and intravenous fluids if necessary.

A catheter (Herd-Barger) was inserted into the common carotid artery of the calves using the procedure described for sheep. On some

occasions, both a thoracatomy and carotid catheterization were done simultaneously. In some calves a vagal nerve cooling coil was placed around the sympathaovagal nerve bundle (Chimoskey, 1981) and a bead thermistor (Yellow Springs Inst., #421) was placed next to the carotid artery. The thermistor allowed for the accurate measurement of animal temperature when the nerve cooling coils were not in operation.

Other calves were instrumented with electromagnetic flow detectors on the iliac, renal and superior mesenteric arteries. For this procedure the calf was anesthetized as before and placed on its right side. The surgical approach to the arteries was retroperitoneal with particular care taken not to puncture the rumen. Some of the animals required that a stomach tube be passed to prevent anesthetic bloat to which ruminants are susceptible. After each of the arteries was identified, a flow detector (Zepeda Inst.) of appropriate dimension was fitted and an inflatable occluder (Rhodes Medical Inst.) was placed distal to the detector. Inflation of the occluder was later used to establish a temporary zero flow condition. The flow detector leads and occluder tubes were brought out of the skin just dorsal to the skin incision and secured to a loop of Tygon tubing inserted in the adjacent skin. Post operative recovery and treatment were as previously described.

Recording Instrumentation

Physiologic information was recorded by one of three machines. For sheep and dog experiments, a Grass polygraph (Model 7D) was used to transcribe mean arterial pressure, phasic arterial pressure, and heart

rate (calculated from the systolic intervals). The calf experiments utilized an eight channel Gould-Brush recording system and/or an eight channel Hewlett-Packard instrumentation tape recorder.

All arterial pressures were measured with Gould-Statham transducers (P23ID). Zero pressure was referred to heart level. The frequency response of the catheter-manometer system was calculated for each experimental system and was greater than 15 hz (Appendix B). The frequency response of the mechanical recording system was greater than 100 hz (Gould-Brush), or 60 hz (Grass Inst.). In order to maintain the frequency response of the arterial pressure catheter, a low rate infusion of heparinized saline was delivered through the arterial pressure catheter (0.3 ml/min, 10 IU/ml) via a Harvard infusion pump. Care was taken to remove air bubbles, kinks and excess catheter length which can degrade phasic pressures.

Arterial pressure was recorded on each system with a gain of 10 mmHg /10 mm pen deflection. In most cases, electronic zero supression was utilized so that the animal's arterial pressure appeared at mid scale. The gain of each blood pressure transducer was calibrated at weekly intervals using a mercury manometer (0 and 100 mmHg). At the start and end of each experiment an electronic arterial pressure calibration was performed. This involved opening the blood pressure transducer to atmospheric pressure and then pushing the bridge calibrate switch on the polygraph. This procedure insured that the electronic gain of the polygraph was constant and that no zero pressure drift had occurred. Phasic arterial pressure was always recorded and

the averaged (root mean square, time constant 2 sec.) arterial pressure was displayed and recorded on an adjacent channel.

Heart rate was recorded on the Grass polygraph at a scale of 15 beats per minute equalling 10 mm (zero offset 60 beats/min). An average for a thirty second period was established by eye and recorded. During conditions where the cardiotachometer (Grass Inst., 7P4F) output was noisy or when the Gould-Brush system was used, heart rate was calculated manually. This procedure involved counting arterial pressure systolic pressure peaks over a thirty second period and expressing the result on a per minute basis.

Flow Detector Calibration

Electromagnetic flow detectors (Zepeda Inst.) were implanted at various sites (main pulmonary artery, renal artery, superior mesenteric artery and femoral artery) in the calves. These detectors have a calibration curve which has been demonstrated to be linear (Astley et al., 1979). Flow detector calibration entails observing a zero flow signal and then a condition where a specific volume flow rate is observed. The calibration procedure for the pulmonary artery flow detector was performed in situ after a stable electromagnetic flow trace had been observed.

The pulmonary artery flow detector was calibrated after at least one week of recuperation following the implantation of the detector. Cardiac output was determined by the cardio-green technique (Wood, 1962) while the mean pulmonary electromagnetic flow signal was

recorded. A simultaneous reading of mean pulmonary blood flow (Zepeda Inst, SWF-4RD) and measured (cardiogreen technique) cardiac output was used to establish a calibration factor for a particular detector in a particular animal. Specifically, the pulmonary blood flow signal was displayed on a Gould-Brush recording system and the measured diastolic flow taken to be zero flow. The Zepeda demodulator was switched to mean output (electronic average, root mean square) and the polygraph gain noted (volts/cm). The dye-dilution procedure entailed injection of a small known mass (0.5 ml) of indocyanine dye (5 mg/ml) into the jugular vein or right atrium. The changing concentration of dye which occurred in the distal aorta was then observed. An extracorporeal densitometer (Gilson Inst.) was used to record dye concentration as blood was removed from the aorta at a known rate (Harvard Inst. infusion/withdrawal pump). The integral of the dye concentration was determined with semi-logarithmic extrapolation to remove any recirculation artifact. Plasma dye concentrations were later determined by the addition of known masses of indocyanine dye to known volumes of blood which were then passed through the densitometer at the previously established withdrawl rate. Several dye dilution curves (2-4) were performed and the cardiac output calculated. The average voltage output as measured by the electromagnetic flow detector at the time of dye injection was divided by the cardiac output as determined by dye-dilution which established a detector calibration factor.

A more direct method was used to calibrate the renal, iliac and superior mesenteric flow detectors. The animals were anesthetized with sodium thiamyal (Bio-tal, Bio-Ceutic Labs) to effect, intubated with a

cuffed endotracheal tube and maintained on a halothane-oxygen gas mixture (1-3 % halothane, 3-4 liters/min O_2). The abdomen of the animal was opened in order to expose the flow detectors and occluders. Teflon catheters (O.D. .157", I.D. .125") were inserted into the artery distal (2-4 cm) to the flow detector in question. After all three vessels were cannulated, the animal was intravenously heparinized (10,000 IU). Timed collections of blood (30-60 sec) were made from each vessel into a graduated cylinder and the simultaneous electromagnetic flow detector voltage was observed on the polygraph. This gave a calibration factor which allowed retrospective calibration of previously determined electromagnetic flow data. The blood was returned to the animal (intravenous) and the other detectors calibrated in a similar fashion. After all calibrations were made, the animal was killed with an overdose of barbiturate anesthetic.

At the post-mortem examination, the position of the catheters, occluders and flow detectors was confirmed. In particular, the position of the aortic arch catheter was verified to insure that it was located distal to the cephalic arterial branches off the aortic arch. This allowed confidence in the assumption that aortic PGE_2 infusion affected the distal trunk and was not simultaneously perfusing the cranial circulation.

Data Collection and Statistical Treatment

In all of the experimental series, data were obtained during steady state experimental conditions. Although a single value is tabulated as the descriptor for each experimental condition, each of these values

(arterial pressure, heart rate, cardiac output, calculated resistances, blood gases, and temperature) was actually the mean of five values. Two plasma renin determinations were made in each experimental period. Values of the experimental variables were determined every two minutes during the latter half of each control period and again after any experimental responses had steadied. On occasion, one of the periodic determinations would occur when the animal sneezed, coughed, urinated or defecated. In this case the observation was not taken and an additional minute was allowed for the data collection. The readings were averaged and this value was considered to be representative of the variable for that control or experimental period. This use of "nested" data allowed for greater precision and accuracy when testing small magnitude changes for statistical significance.

The use of each animal as the experimental control allowed use of the paired t-test. Linear regression analysis and analysis of variance (randomized block design) was performed to evaluate the effects of varying infusion rates of intra-carotid PGE_2 on arterial pressure and plasma renin activity. Duncans test was used to compare experimental results from the analysis of variance test. Where possible, experimental series and procedures were randomized. In all experimental series, changes between control and experimental periods were analyzed and the null hypothesis was tested. Mean responses together with the standard errors of the means will be presented in the results section. The statistical confidance limit of .05 was accepted.

Drugs

Prostaglandin E_2 (PGE_2, UpJohn) was made up in 100% ethanol at 1 mg/ml, aliquoted into 0.5 ml teflon cryogenic tubes and stored at -20 ^{o} centigrade (C). Angiotensin II (Hypertensin, 87 % purity, Ciba-Geigy) was dissolved in saline at 1 mg/ml and stored in cryogenic tubes at -20 ^{o} C. Just prior to an experiment, the required volume of stock PGE_2 or angiotensin was removed with a Hamilton syringe and mixed with sterile saline (0.9%, Cutter Labs.) for infusion. In all cases the volume infusion rate was 0.3 ml/min. If more than one hour delay was anticipated, the solution was stored on ice in the experimental room.

Phentolamine HCl (Regitine, Ciba-Geigy), captopril (SQ 14,225 Squibb) and d,l-propranolol (Sigma) were stored as powder in a desiccator at 2 ^{o}C. On the day of the experiment, the required mass was removed and mixed with sterile saline (20 ml). On occasion, alkylinization (10 N NaOH, 1-2 drops) of the phentolamine was necessary to insure complete dissolution.

Phenylephrine (Neo-Synephrine, Winthrop), isoproterenol (Isuprel, Breon) and heparin (Rugby) were available in sealed containers and were stored at room temperature.

The drugs were considered to come from aseptic sources and sterile drug delivery materials (syringes, needles, catheters etc.) were consistently used.

Experimental Protocols; Overview

Nine experimental series were performed. The first nine series directly addressed the ten objectives as listed at the end of the literature review. The results section presents these data in sequence. A number of experiments were conducted which are not presented in the text of this dissertation. In several cases, negative results were obtained and in the others a small number of animals was used and were not appropriate for statistical analysis. These experiments may provide information for future research in the area and the reader is directed to Appendix C for a brief rationale and presentation of these experiments.

Series One

Heart rate and arterial pressure responses to intra-carotid PGE_2 were studied in conscious sheep, calves and dogs. The experimental hypothesis was that intra-carotid PGE_2 infusion (10 ng/kg/min) would increase heart rate and arterial pressure when compared to a control period. The protocol allowed a twenty minute control period followed by a twenty minute intra-carotid PGE_2 infusion. Data was collected after a stable control period was established and again after a steady state hemodynamic response was observed during the PGE_2 infusion.

Series Two

The second series contrasted the intra-carotid PGE_2 pressor response with the effects of PGE_2 infusions into alternate vascular sites. Two subgroups were tested. In the first subgroup the hemodynamic effects (arterial pressure and heart rate) of intra-carotid

PGE_2 (10 ng/kg/min) infusions, right atrial PGE_2 (400 ng/kg/min) infusions and distal aortic PGE_2 (200 ng/kg/min) infusions were compared in four conscious calves. In the second subgroup the effects of intra-carotid PGE_2 (10 ng/kg/min) and jugular PGE_2 (400 ng/kg/min) infusions were individually compared in four conscious dogs and six conscious sheep.

Series Three

The third series further tested the hemodynamic response to intra-carotid PGE_2 (10 ng/kg/min) infusion within two subgroups. In the first subgroup the ability of intra-carotid PGE_2 (10 ng/kg/min) to alter arterial pressure, heart rate, cardiac output and calculated total peripheral resistance (TPR) was measured in five calves. In these and subsequent experiments, vascular resistances were calculated as mean arterial pressure divided by the regional flow in question. In the second subgroup the peripheral resistance changes occurring in the iliac, superior mesenteric and renal beds were examined during intra-carotid PGE_2 infusions in six calves. Similar to the first subgroup, the experiment tested the ability of intra-carotid PGE_2 infusion to alter regional resistances.

Series Four

The fourth series examined changes in arterial blood gases and plasma pH during intra-carotid PGE_2 infusions (10 ng/kg/min) in four calves, six sheep and four dogs. The purpose was to determine whether intra-carotid PGE_2 infusion triggered a chemoreflex which would cause a respiratory alkylosis in addition to arterial pressure changes. Five

arterial blood samples (2 ml) were anaerobically withdrawn into oiled glass syringes during the control period and then during the second half of the intra-carotid PGE_2 infusion (when arterial pressure was significantly elevated). Blood samples were drawn two minutes apart and each was immediately placed on ice. Blood gases and pH were determined (Corning, Model 165/2) within one-half hour of blood removal.

Series Five

The baroreflex responses during intra-carotid PGE_2 infusion (10 ng/kg/min) and during intra-carotid angiotensin II infusion (10 ng/kg/min) were examined in the fifth series. This series was prompted by the observation that, during intra-carotid PGE_2 infusion, the arterial pressure was significantly increased and heart rate was unchanged or increased. This suggested a "resetting" of the baroreflex. Infusion of angiotensin II also increases arterial pressure with small alteration in heart rate. Accordingly, baroreflex responses to intra-carotid infusions of PGE_2 and angiotension II were compared to a control period and to each other. Baroreflex response was assessed by infusing a peripheral vasoconstrictor, phenylephrine (1-2 ug/kg/min), intravenously and observing arterial pressure and heart rate changes.

The determination of baroreflex sensitivity or "gain" relies on Marey's observation (1859) that heart rate and arterial pressure vary reciprocally. Robinson et al. (1966) showed that by plotting heart rate changes as a function of mean arterial pressure, a measure of

baroreflex sensitivity can be determined from the slope of the relationship.

In this study phenylephrine was infused (1 ug/kg/min, sheep and calves; 2ug/kg/min, dogs) to increase arterial pressure to approximately the same level as was observed during intra-carotid PGE_2 infusion. After a steady state was achieved for arterial pressure and heart rate, recording was continued for ten minutes and the phenylephrine infusion was stopped. The animal was rested until heart rate and arterial pressure were back to control levels. Intra-carotid PGE_2 (10 ng/kg/min) was then infused and data taken after a hemodynamic steady state occurred. While the intra-carotid PGE_2 infusion was maintained, the phenylephrine infusion was restarted using the same infusion rate of phenylephrine as before. After ten to twenty minutes, a new steady state was developed during which data were recorded. This protocol was repeated four times in each animal.

Baroreflex sensitivity was calculated by graphically plotting heart rate against arterial pressure during: (a) control, (b) intravenous phenylephrine, (c) intra-carotid PGE_2 infusion and (d) intra-carotid PGE_2 infusion plus intravenous phenylephrine. The slope of the line connecting the control point to the phenylephrine infusion point represents the reflex change in heart rate per unit change in arterial pressure over the pressure range tested. Similarly, the slope of the line between the intra-carotid PGE_2 infusion point and the intra-carotid PGE_2 infusion plus phenylephrine infusion point represents the ability of the baroreflex to alter heart rate for

another additional incremental increase in arterial pressure.

Series Six

The effect of intra-carotid PGE_2 infusion during general anesthesia was examined in a sixth series. The calves were initially anesthetized with thiopental (Bio-tal) to effect, intubated and connected to an anesthesia machine (1-1.5% halothane in oxygen, 4 liters/min total flow). Approximately one hour after induction of anesthesia, intra-carotid PGE_2 was infused at 100 ng/kg/min and arterial pressure was recorded.

Four sheep were anesthetized with intravenous chloralose (0.1 gm/kg) and urethane (0.5 gm/kg). Intra-carotid PGE_2 was subsequently infused at 100 ng/kg/min. Care was taken not to excite these animals and anesthesia was initiated with each animal standing in its cage. After the animal was transferred to the lab, the rest of the anesthetic was delivered and the PGE_2 infused.

At the end of the previous sheep experiments, a bolus (0.5 ml) of crystal violet dye (20 mg/ml, 50% ethanol in saline) was rapidly injected into the carotid catheter and the animal was immediately killed with an overdose of barbiturate anesthetic. The cranium was removed and the extent of staining was observed in each hemisphere. The extent of extra-calvarial dye staining in the ear and eye tissues was similarly observed.

Series Seven

The effects of adrenergic blockers (propranolol and phentolamine) on the intra-carotid PGE_2 pressor response were tested in sheep and calves. Three subsets of experiments were performed. The effects of beta blockade (propranolol), alpha blockade (phentolamine) and the two administered sequentially were determined in the first subgroup of control experiments. In the second subgroup intra-carotid PGE_2 was infused and, after the pressor responses were observed, the adrenergic blockers were delivered. In the third subgroup the adrenergic blockers preceded the intra-carotid PGE_2 infusion. This subgroup tested the ability of the adrenergic blockers to prevent the PGE_2-induced pressor response.

In the first subgroup the efficacy of beta block was tested. After a twenty minute control period, an isoproterenol bolus (5 ug) was injected intravenously and the arterial pressure and heart rate responses were noted. After the animal's hemodynamic values returned to control, propranolol (1 mg/kg) was intravenously injected over a thirty second period. At subsequent five minute intervals, 25 ug of isoproterenol was intravenously injected and the hemodynamic responses were noted. No hemodynamic alterations to 25 ug of isoproterenol were noted for sixty minutes after this dose of propranolol. This established a "window" of time where the response to 25 ug of isoproterenol was absent and beta block apparent. No behavioral responses were apparent in response to propranolol. The animals did mildly object (stamping) to the hypotension and tachycardia eventually elicited by isoproterenol challenges as the beta adrenergic block wore

off.

Phentolamine was tested for alpha adrenergic blocking capacity using phenylephrine as the agonist. Phenylephrine was intravenously injected (400 ug) in the control period. After a return of hemodynamic values to control, phentolamine (1 mg/kg) was intravenously injected over a one minute period. Intravenous challenges of phenylephrine (1000 ug) were subsequently injected at ten minute intervals. Hemodynamic responses observed during the first forty to sixty minutes were 20% of the control response or less, after which a gradual return to control phenylephrine responses was observed.

Creating a stronger alpha block, with no immediate hemodynamic responses to phenylephrine, required intravenous doses of 2 mg/kg phentolamine. These larger doses often adversely affected the animal; diarrhea, hypotension, tachycardia and behavioral changes (straining at the rope, stamping etc.) were often observed. The 1 mg/kg dose of phentolamine was therefore selected despite the less than complete alpha blockade condition.

The behavioral changes, hypotension and tachycardia in response to phentolamine were largely averted by previously establishing a beta adrenergic block with propranolol. In these experiments, propranolol was injected as before and, after ten minutes, phentolamine was administered. Prior to either adrenergic blocker the respective agonists were administered and the hemodynamic responses noted for comparison at the end of the experiment. Thirty minutes later, the

larger doses of the agonists were administered. The hemodynamic results of the combined blockade were quite similar to those obtained after either adrenergic blocker alone.

The order of adrenergic blockers was reversed in four sheep and two calves (alpha and then beta). The hemodynamic effects (hypotension and bradycardia) of phentolamine were abrogated by the beta block, but the animal continued to be disturbed (straining, stamping etc.). Therefore, this order of drug administration was not used in any further protocols.

The ability of adrenergic blockers to alter the **ongoing** pressor effect of intra-carotid PGE_2 infusion was tested in the second subgroup. Four sheep and four calves were individually infused with intra-carotid PGE_2 (10 ng/kg/min). After the intra-carotid PGE_2 pressor effect had been established and stabilized, the adrenergic blockers were injected. The experiment questioned whether a beta and/or alpha adrenergic blocker would **attenuate** the intra-carotid PGE_2 pressor effect. As before, the respective agonists were administered to each animal during the control period. After waiting an additional ten to twenty minutes, an intra-carotid PGE_2 infusion (10 ng/kg/min) was started and maintained throughout the experiment. Twenty minutes after the start of the intra-carotid PGE_2 infusion (when the pressor effects were evident and stable) propranolol (1 mg/kg) was injected. Ten minutes were then allowed for hemodynamic stabilization and data collection after which phentolamine (1 mg/kg) was injected. An additional ten minutes were allowed for stabilization, and data was

collected. Before the intra-carotid PGE_2 infusion was turned off, the efficacy of adrenergic blockade (alpha and beta) was tested as before.

In the third subgroup, adrenergic blockers were administered **prior** to the start of the intra-carotid PGE_2 infusion in four sheep and four calves. The purpose was to determine whether these blockers would **prevent** the pressor effect associated with intra-carotid PGE_2 infusion. In this subgroup, the animals were studied first in a control period, after beta adrenergic blockade, after beta and alpha adrenergic blockade and finally during intra-carotid PGE_2 infusion. Since the effects of beta and alpha adrenergic blockade lasted through the intra-carotid PGE_2 infusion, no additional amounts of propranolol or phentolamine were administered. Prior to the control period and at the termination of the experiment, the beta and alpha adrenergic agonists were administered to allow comparison between adrenergic block periods and control responses.

Series Eight

Plasma renin activity (PRA) was determined in an eighth series of experiments. Two subsets of experiments were conducted. In the first subgroup, PRA was determined in four conscious sheep during a control setting and during intra-carotid PGE_2 infusion (10 ng/kg/min). The second subgroup studied PRA when varying doses of intra-carotid PGE_2 (saline vehicle control, 20, 40, 80, and 200 ng/kg/min) were infused into four conscious sheep. The physiological questions were whether infusion of intra-carotid PGE_2 was associated with changes in PRA and whether the elevated arterial pressure was associated with an elevated

PRA.

In the first subgroup of experiments, samples of jugular venous blood (2 ml) were removed during the last two minutes of the control period and during the latter half of the intra-carotid PGE_2 infusion period (2 samples). The second subgroup differed from the first in that increasing dose rates of PGE_2 were infused at sequential twenty minute intervals. In these experiments, venous blood was removed in the control period as before and during the second half of each incremental PGE_2 infusion period (2 samples). In all cases the blood was immediately put into precooled test tubes which had been prepared with ethylenediaminetetraacetic acid (10.5 mg) and placed on ice. Within one hour the blood was centrifuged and the plasma removed. The samples were then stored at -20 ^{o}C until PRA analysis was performed.

Plasma renin analysis was done with commercially available materials (New England Nuclear) and followed the method of Haber et al. (1969). This method uses a radioimmunoassay technique to measure angiotensin I. By comparing the amount of angiotensin I generated during plasma incubation to amounts of angiotensin I pre-existing in control tubes, a measure of plasma renin activity is derived.

Series Nine

The results from the previous series suggested an involvement of the renin-angiotensin system in the intra-carotid PGE_2 pressor response. Therefore, a ninth series was designed to prevent the involvement of this system during intra-carotid infusion of PGE_2.

Captopril, a converting enzyme inhibitor, was injected intravenously (2 mg/kg) in four sheep prior to intra-carotid PGE_2 infusion (10 ng/kg/min).

Injection of captopril was not associated with marked behavioral effects, although the hemodynamic effects were similar to phentolamine (hypotension and tachycardia). The efficacy of captopril as an angiotensin conversion blocker was tested by injecting angiotensin I (1 ug) prior to the captopril injection and then retesting with a larger dose of angiotensin I (10 ug) after captopril injection.

RESULTS

Series One

Figure 1 shows heart rate and arterial pressure from conscious dogs, sheep and calves (n=4,7,5 respectively) during a control period and during intra-carotid PGE_2 infusions.

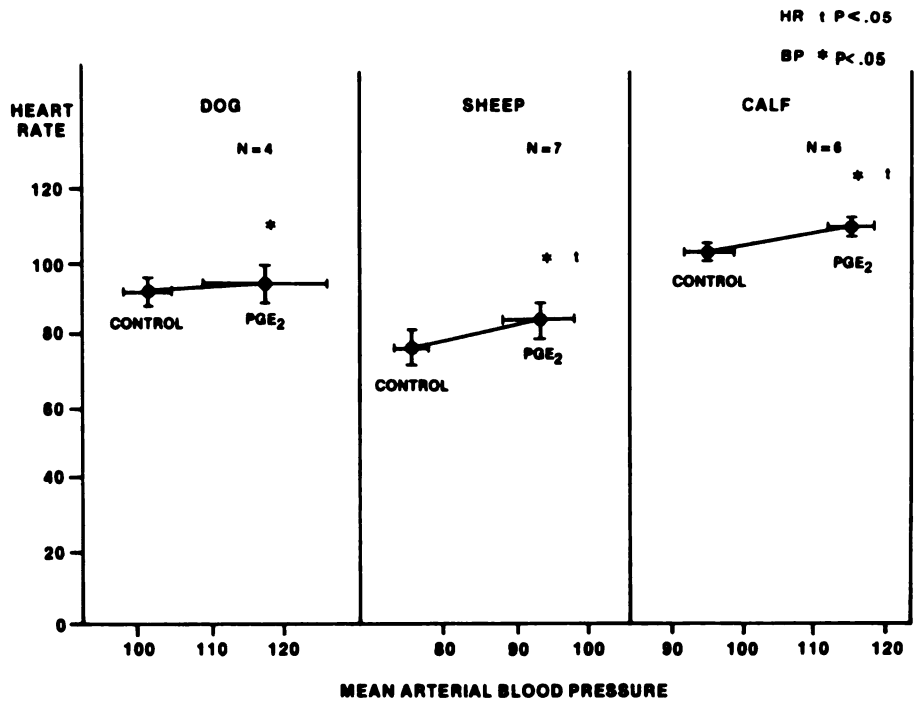

Figure 1: Arterial pressure and heart rate in conscious dogs, sheep and calves during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

Intra-carotid PGE_2 infusion (10 ng/kg/min) significantly increased arterial pressure when compared to control values: (dog: 17%, p=.0038; sheep: 24%, p=.0012; calf: 32%, p=.0003). Heart rate also increased

significantly during intra-carotid PGE_2 infusion in calves (7%, p=.016) and sheep (11%, p=.01). Increases in heart rate during intra-carotid PGE_2 infusion in dogs were considerably smaller (3%) and were not significant. The heart rate of individual sheep and calves usually increased during intra-carotid PGE_2 infusion. However, each dog's heart rate was more variable and would occasionally decrease.

Intra-carotid infusion of vehicle (saline) did not significantly alter arterial pressure or heart rate in the dogs (n=4) and calves (n=5). Intra-carotid infusion of saline did not alter arterial pressure in five conscious sheep and significantly decreased heart rate (p=.034) by less than two beats per minute when compared to control heart rate.

Intra-carotid PGE_2 infusion did not alter the animals' behavior. The sheep and calves were often observed ruminating and the dogs would calmly lie on a padded table. In general, the behavior of the animals was consistent in each of the experimental series unless noted otherwise. As the animal became more used to the experimental protocols, the hemodynamic responses usually became more consistent with less variability observed.

During the intra-carotid PGE_2 infusion, some calves responded with a local erythema on the ear ipsilateral to the carotid infusion site. The affected ear felt warmer to the touch than the contralateral ear, but the animal did not appear to be in pain. More often, the calves had a noticable erythema of periorbital tissues with lacrimation.

Again, the erythematous response was always on the same side of the calf as the PGE_2 infusion site and would disappear 10-30 minutes after the intra-carotid PGE_2 infusion was terminated. The erythema was not seen in the sheep and dogs during intra-carotid PGE_2 infusions.

Series Two

Infusion of PGE_2 into the venous system and into the aorta did not evoke a pressor response; the pressor response occurred only with infusion into the carotid artery (Figure 2).

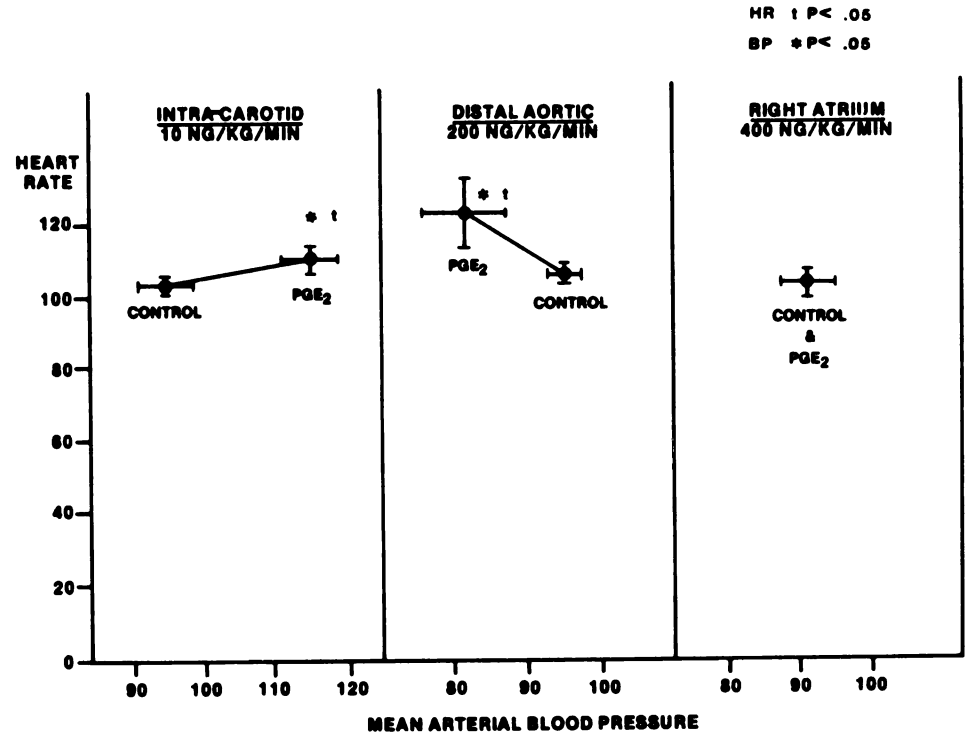

Figure 2: Arterial pressure and heart rate in four conscious calves during control periods and when PGE_2 was infused into the (a) carotid artery, 10 ng/kg/min, (b) distal aortic arch, 200 ng/kg/min and (c) right atrium, 400 ng/kg/min.

Sufficient time was allowed between infusions so that the effects of one infusion were not confounded by another PGE_2 infusion series. The left panel shows that intra-carotid PGE_2 (10 ng/kg/min) significantly increased arterial pressure (p=.0003) and heart rate (p=.016) when compared to the respective control periods.

Distal aortic PGE_2 infusions (200 ng/kg/min) at twenty times the intra-carotid PGE_2 dose rate significantly decreased arterial pressure (15%, p=.012) and significantly increased heart rate (16%, p=.012). Right atrial PGE_2 infusions at forty times the intra-carotid PGE_2 infusion rate failed to significantly alter heart rate or arterial pressure. Infusions of vehicle (saline) into the jugular vein of dogs and sheep failed to significantly alter arterial pressure or heart rate. Aortic infusions of saline in the calves caused heart rate to decrease by two beats per minute (p=.037); arterial pressure was unchanged.

The calves did not show any behavioral changes during right atrial PGE_2 infusions. In contrast, the distal aortic infusion caused irritation and restlessness in the animals. During the time when arterial pressure was decreased, heart rate was increased. The increase in heart rate could be due to the altered animal behavior, a baroreflex response or both.

Jugular PGE_2 infusions at 400 ng/kg/min were administered to 4 conscious dogs and 6 conscious sheep. Table 1 shows arterial pressure and heart rate in the control period and during the venous PGE_2

infusions. No alterations in arterial pressure, heart rate or behavior were observed during the PGE_2 infusions. Vehicle control infusions also failed to alter arterial pressure or heart rate.

Table 1: Arterial pressure and heart rate in four conscious dogs and six conscious sheep in a control period and during jugular vein PGE_2 infusion (400 ng/kg/min). In this and all subsequent tables, data are shown as mean (SEM).

Arterial Pressure mmHg	Heart Rate Beats/min	Period
Dogs		
91.9 (7.0)	92.0 (4.4)	Control
92.4 (7.3)	91.5 (4.2)	Jugular PGE_2
n. sig	n.sig	
Sheep		
77.4 (2.5)	69.1 (5.8)	Control
77.5 (2.4)	70.1 (4.9)	Jugular PGE_2
n. sig.	n. sig.	

Series Three

Intra-carotid PGE_2 infusion increases arterial pressure primarily through increases in total peripheral resistance (Figure 3). Arterial pressure, heart rate, cardiac output and total peripheral resistance were measured in five conscious calves during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min). The increase in arterial pressure during intra-carotid PGE_2 infusion (23%, p=.0003) was approximately equalled by the increase in total peripheral resistance (21%, p=.001). Heart rate and cardiac output both increased (5 & 2%),

and the increases were significant (heart rate, p=.001; cardiac output, p=.009). Nevertheless, Figure 3 indicates that the majority of the arterial pressure rise associated with intra-carotid PGE_2 infusion can be accounted for by the large increase in total peripheral resistance.

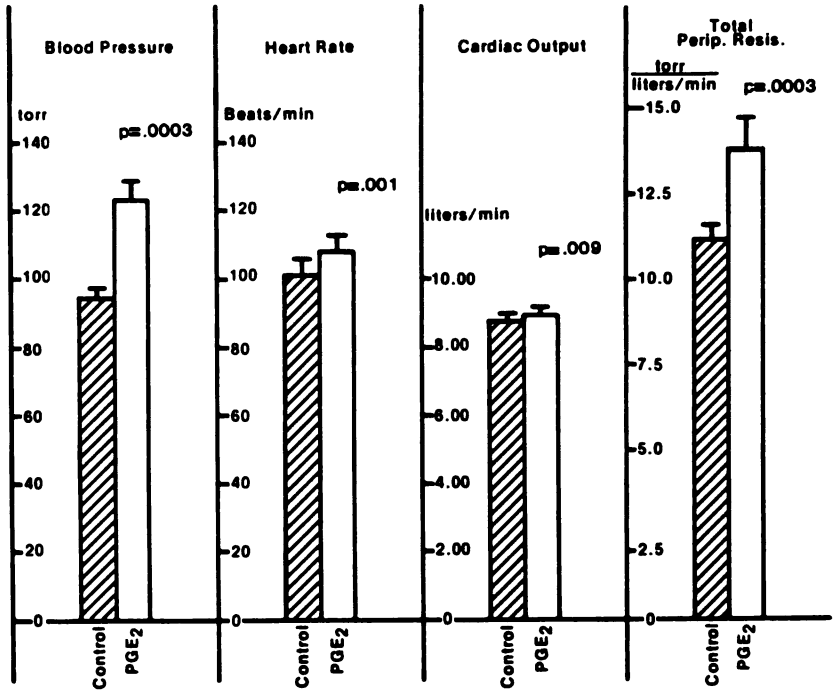

Figure 3: Arterial pressure, heart rate, cardiac output and total peripheral resistance in five conscious calves during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

Figure 4 shows cardiac output, iliac blood flow, renal blood flow, superior mesenteric blood flow and arterial pressure in a conscious calf during a control period and during intra-carotid PGE_2 infusion. This figure was prepared by playing back recorded information (information tape recorder) at high speed onto the polygraph. Zero flow conditions were observed prior to the playback. In this calf, intra-carotid PGE_2 infusion caused a large increase in aortic blood

pressure and minimal changes in cardiac output, renal blood flow and superior mesenteric blood flow. Iliac flow was observed to decrease during intra-carotid PGE_2 infusion. These flow changes indicate that the total peripheral resistance increased with a possible autoregulatory response of the renal and superior mesenteric beds. Iliac resistance increased and contributed to the systemic increase in total peripheral resistance.

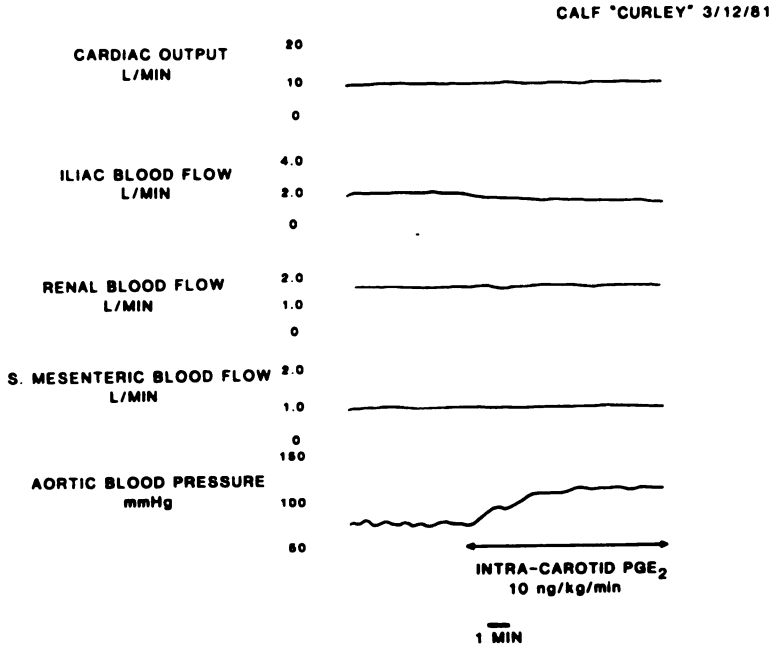

Figure 4: Cardiac output, iliac blood flow, renal blood flow, superior mesenteric blood flow and aortic blood pressure in a conscious calf during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

Figure 5 shows calculated resistances in the iliac, renal and superior mesenteric beds as well as total peripheral resistance during a control period and during intra-carotid infusion of PGE_2 in six conscious calves. The figure indicates that the increase in total

peripheral resistance (21%), which occurred during intra-carotid PGE_2 infusion, was associated with significant increases in iliac resistance (40%, p=.028), superior mesenteric resistance (30%, p=.03) and renal resistance (37%, p=.003). Arterial pressure, although not shown, increased 24% (p=.0003) in this series of animals.

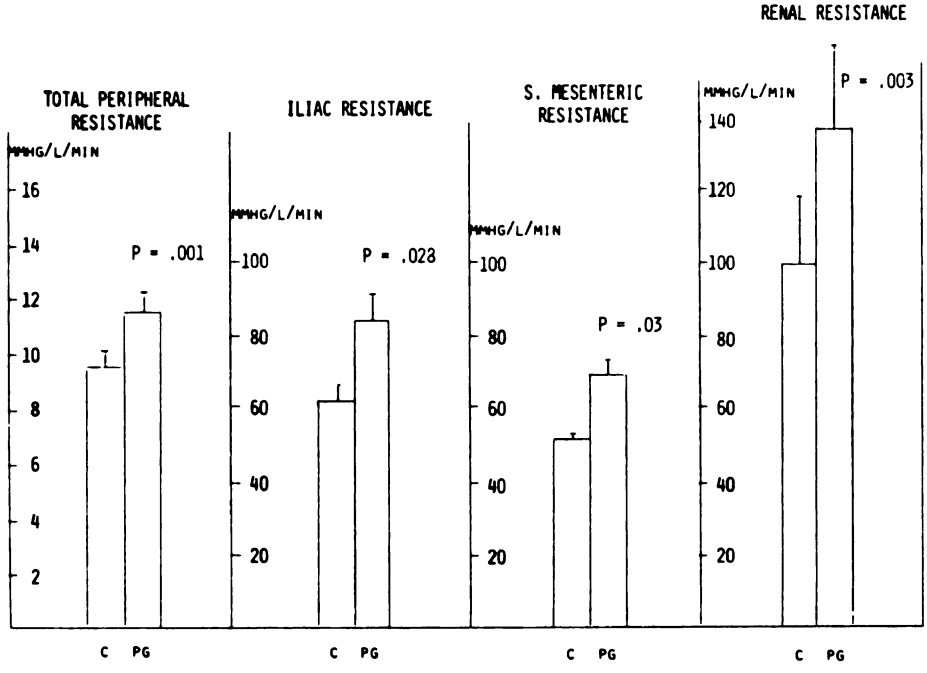

Figure 5: Total peripheral resistance, iliac resistance, superior mesenteric resistance and renal resistance in six conscious calves during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

Each of the regional blood flows was not significantly altered by intra-carotid PGE_2 infusion.

Series Four

Arterial blood gases were measured in four conscious calves during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min). Figure 6 indicates that during intra-carotid PGE_2 infusion no differences were observed in arterial pO2, pCO2 or pH when compared to similarly obtained arterial blood gases in a control period.

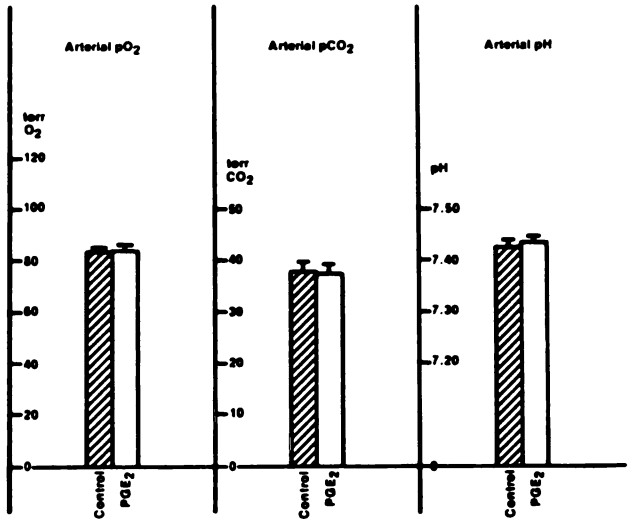

Figure 6: Plasma pO_2, pCO_2 and pH in four conscious calves during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

No significant differences were apparent after comparing each variable obtained during PGE_2 infusion to the corresponding variable observed during a control period. Six sheep and four dogs were similarly tested for direct PGE_2 activation of the chemoreflex and no alterations in arterial blood gases were seen in any individual group that would be consistent with chemoreflex activation (Table 2).

As seen in Table 2, pCO_2 in the sheep and dogs was significantly increased during intra-carotid PGE_2 infusion when compared to the control values. The only other significant change was a decrease in pO_2 in the sheep during intra-carotid PGE_2 infusion. Each of these arterial blood gas alterations can be accounted for by the slow relaxation that occurs in the animals during the experiment. The initial excitement of the experimental setting probably resulted in a transient tachypnea. Neither Figure 6 nor Table 2 shows an alteration in plasma pH during intra-carotid PGE_2 infusion.

Table 2: Plasma pO_2, pCO_2 and pH in four conscious dogs and six conscious sheep during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

pO_2 (mmHg)	pCO_2 (mmHg)	pH	Period
Dog			
78.1 (.57)	31.0 (3.0)	7.434 (.09)	Control
75.6 (2.1)	31.7 (3.3)	7.398 (.20)	Intra-Carotid PGE_2
n. sig	p=.018	n. sig	
Sheep			
90.3 (2.0)	35.7 (1.6)	7.459 (.08)	Control
84.1 (2.6)	37.0 (1.5)	7.440 (.01)	Intra-Carotid PGE_2
p=.002	p=.015	n. sig	

Series Five

Figures 7, 8 and 9 show baroreflex sensitivities in a conscious calf, dog and sheep respectively. Each figure shows heart rate plotted

against arterial pressure during four experimental conditions: (a) control, (b) intravenous phenylephrine infusion, (c) intra-carotid PGE_2 infusion and (d) intra-carotid PGE_2 infusion together with intravenous phenylephrine. The slope of the line connecting the first two conditions (control) and the slope connecting the last two conditions (PGE_2), provide measures of baroreflex sensitivity. The slopes can be compared to reveal the effect of intra-carotid PGE_2 infusion on baroreflex sensitivity.

Figure 7 shows average arterial pressure and heart rate responses from four replications of the protocol in a conscious calf.

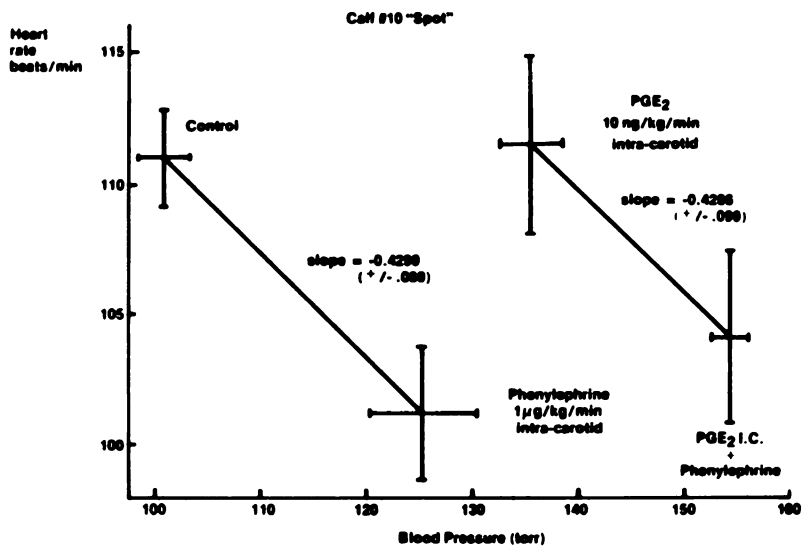

Figure 7: Heart rate, arterial pressure and baroreflex sensitivity in a conscious calf during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

After control heart rate and arterial pressure data were recorded, an intravenous infusion of phenylephrine was administered to increase the

animal's arterial pressure to the range usually observed during intra-carotid PGE_2 infusion. The change in heart rate observed for an increase in arterial pressure is represented by the slope of the line connecting the first two tested conditions (control and phenylephrine). The value of -.43 indicates that this calf responds to phenylephrine infusion with an increased arterial pressure and a reflex slowing of heart rate. After the phenylephrine infusion was terminated, arterial pressure returned to control levels in 20-40 minutes. Intra-carotid PGE_2 (10 ng/kg/min) was subsequently infused and, after the arterial pressure stabilized, intravenous phenylephrine was infused as before. The responses of heart rate and arterial pressure are again represented by the slope of the line connecting the two latter conditions and again averages -.43. These data show that when phenylephrine increased arterial pressure from 100 to 126, the average baroreflex sensitivity was the same as the baroreflex sensitivity obtained during intra-carotid PGE_2 infusion, which increased the arterial pressure from 135 to 155 mmHg. These data indicate that the sensitivity of the baroreflex to phenylephrine infusion does not change during intra-carotid PGE_2 infusion.

Average heart rate and arterial pressure data obtained in four conscious calves using the previously described protocol are shown in Table 3. Phenylephrine infusion significantly increased arterial pressure (28%, p=.0002) and significantly decreased heart rate (11%, p=.001) when compared to the control period. The average baroreflex sensitivity during this control situation was -.39. After the phenylephrine infusion was turned off, the arterial pressure and heart

rate returned to control values. Intra-carotid PGE_2 was infused and arterial pressure significantly increased (28%, p=.0001) when compared to control pressures. Heart rate also increased during intra-carotid PGE_2 infusion (6%), but was not significantly altered in this series of animals. The arterial pressure observed during intra-carotid PGE_2 infusion was not significantly different than the arterial pressure observed during phenylephrine infusion.

Table 3: Arterial pressure, heart rate and baroreflex sensitivity in four conscious calves during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

Arterial Pressure mmHg	Heart rate Beats/min	Period
97.1 (5.3)	102.5 (4.2)	Control
124.3 (8.5)	91.6 (3.5)	Intravenous Phenylephrine
Average baroreflex sensitivity during the control period = -.39 (.04)		
124.2 (8.2)	108.9 (6.0)	Intra-Carotid PGE_2
144.3 (10.1)	100.3 (4.1)	PGE_2 & Phenylephrine
Average baroreflex sensitivity during intra-carotid PGE_2 infusion = -.45 (.12)		

The addition of phenyephrine to the intra-carotid PGE_2 infusion further increased arterial pressure (16%, p=.01) when compared to the PGE_2 infusion period. Heart rate decreased during the second phenylephrine infusion (9%), but was not significantly different when compared to the previous PGE_2 infusion period. The average baroreflex sensitivity calculated during intra-carotid PGE_2 infusion was -.45 which was not significantly different from the average baroreflex response during the control periods (control to phenylephrine).

Baroreflex response data was similarly obtained in conscious dogs and is shown in Figure 8. As was observed in conscious calves, the baroreflex sensitivity in dogs did not markedly change during intra-carotid PGE_2 infusion when compared to control baroreflex sensitivities (Figure 8).

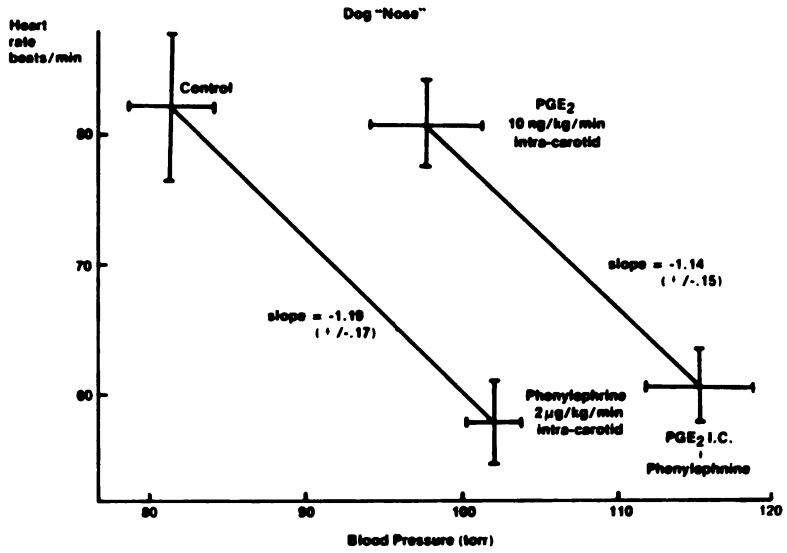

Figure 8: Heart rate, arterial pressure and baroreflex sensitivity in a conscious dog during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

Only two dogs had baroreflex sensitivity characterized and therefore statistical analysis was inappropriate. Each individual dog experiment was replicated four times and showed no marked change in sensitivity between the control period and the sensitivity determined during intra-carotid PGE_2 infusion. Table 4 shows grouped data for the two dogs.

Table 4: Arterial pressure, heart rate and baroreflex sensitivity in two conscious dogs during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

Arterial Pressure mmHg	Heart Rate Beats/min	Period
92.1 (10.9)	89.6 (7.4)	Control
113.0 (11.0)	68.5 (10.6)	Intravenous Phenylephrine

Average baroreflex sensitivity during the control period = -1.07 (.11)

114.3 (16.7)	90.4 (9.6)	Intra-Carotid PGE_2
135.6 (20.4)	72.3 (11.7)	PGE_2 & Phenylephrine

Average baroreflex sensitivity during intra-carotid PGE_2 infusion = -.92 (.23)

An example of an individual sheep baroreflex sensitivity during the control period and during intra-carotid PGE_2 infusion is shown in Figure 9.

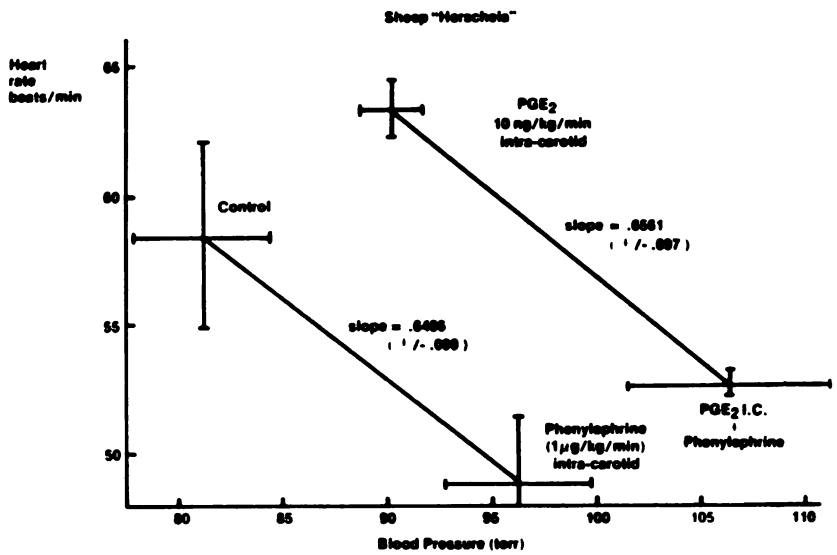

Figure 9: Heart rate, arterial pressure and baroreflex sensitivity in a conscious sheep during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

The protocol, number of replicates and specific infusion rates were identical to the calf experimental design. Figure 9 shows that the sheep has a baroreflex sensitivity during the control period of -.65. During intra-carotid PGE_2 infusion, the average baroreflex sensitivity was -.66 and was not significantly altered when compared to the control period.

Table 5 shows results from five sheep. Intravenous phenylephrine and intra-carotid PGE_2 both significantly increased arterial pressure (23%, p=.001; 20%, p=.007 respectively) when compared to control values.

Table 5: Arterial pressure, heart rate and baroreflex sensitivity in five conscious sheep during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

Arterial Pressure mmHg	Heart Rate Beats/min	Period
78.9 (3.2)	70.1 (5.7)	Control
96.2 (3.4)	55.2 (4.7)	Intravenous Phenylephrine

Average baroreflex sensitivity during a control period = -.77 (.16)

95.0 (1.9)	74.3 (6.0)	Intra-Carotid PGE_2
112.0 (3.9)	66.6 (6.4)	PGE_2 & Phenylephrine

Average baroreflex sensitivity during intra-carotid PGE_2 = -.75 (.15)

The arterial pressure determined during phenylephrine infusion was not significantly different from the arterial pressure observed during intra-carotid PGE_2 infusion. Heart rate decreased with phenylephrine infusion and increased during intra-carotid PGE_2 infusion (18%, p=.004:

9%, n. sig., respectively) when compared to the control period. The calculated baroreflex sensitivity in the control period (control to phenylephrine) averaged -.77. The addition of phenylephrine in the presence of intra-carotid PGE_2 further increased arterial pressure (18%, p=.002) compared to PGE_2 infusion alone. Heart rate decreased (10%, p=.02) when compared to the prior intra-carotid PGE_2 infusion period. The baroreflex sensitivity during PGE_2 infusion averaged -.75 and did not significantly differ from the average baroreflex sensitivity obtained during the control period (-.77).

Taken together, data from these three species indicate that the baroreflex is still operating during intra-carotid PGE_2 infusion. The bradycardia associated with phenylephrine administration is unchanged in the presence of intra-carotid PGE_2 infusion despite a much higher arterial pressure.

Figure 10 shows arterial pressure and heart rate responses obtained in four conscious calves when angiotensin II was the tested compound instead of PGE_2. In this series intra-carotid angiotensin II was infused at 10 ng/kg/min. Otherwise this protocol was identical to the other calf baroreflex protocols. Intravenous phenylephrine by itself increased arterial pressure (30%, p=.0006) and decreased heart rate (12%, p=.0016). The baroreflex sensitivity in this control period averaged -.43. After the phenylephrine infusion was terminated, the animal's arterial pressure and heart rate returned to control values. Intra-carotid angiotensin II was infused and the animal's arterial pressure increased (37%, p=.00001) when compared to the control

pressure. Heart rate did not significantly change. The arterial pressure obtained during angiotensin II infusion was not significantly different from the arterial pressure observed during phenylephrine infusion. The addition of intravenous phenylephrine infusion to the continuing angiotensin II infusion caused a further significant increase in arterial pressure (14%, p=.0005) and a significant decrease in heart rate (5%, p=.0027).

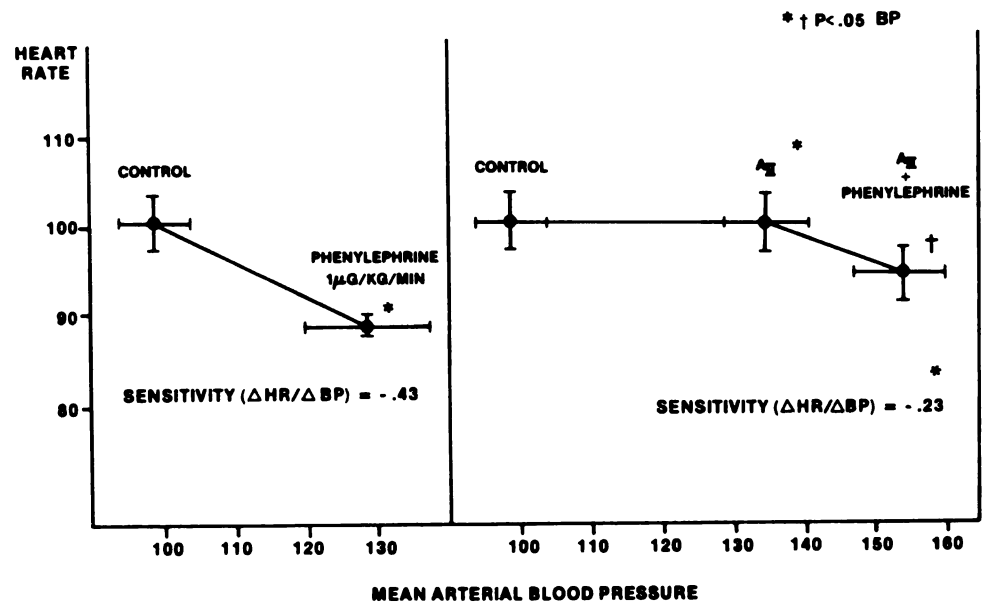

Figure 10: Left Panel: Heart rate, arterial pressure and baroreflex sensitivity during a control period. Right Panel: Heart rate, arterial pressure and calculated baroreflex sensitivity during intra-carotid angiotensin II infusion (10 ng/kg/min). Data obtained in four conscious calves.

The baroreflex sensitivity during angiotensin II infusion averaged -.23 and was significantly different (p=.017) from the average baroreflex sensitivity determined in the control period (-.43). Data from these calves indicate that intra-carotid angiotensin II infusion

decreases the responsiveness of the baroreflex.

Baroreflex sensitivity data from five sheep (Table 6) also show alterations in baroreflex responsiveness during intra-carotid angiotensin II infusion (10 ng/kg/min). Intravenous phenylephrine significantly increased arterial pressure 18% (p=.0007) and significantly decreased heart rate 21% (p=.0026). The average baroreflex sensitivity during the control period was -.81. After the phenylephrine infusion was terminated, intra-carotid angiotensin II was infused, increasing arterial pressure 34% (p=.0014) and decreasing heart rate 8% (p=.038) when compared to control values. The average arterial pressure observed during angiotensin II infusion was significantly higher (10%, p=.025) than the arterial pressure observed during phenylephrine infusion.

Table 6: Arterial pressure, heart rate and baroreflex sensitivity in five conscious sheep during a control period and during intra-carotid angiotensin II infusion (10 ng/kg/min).

Arterial Pressure mmHg	Heart Rate Beats/min	Period
78.9 (3.2)	70.1 (5.7)	Control
96.2 (3.4)	55.2 (4.7)	Intravenous Phenylephrine
Average baroreflex sensitivity during a control period = -.81 (.25)		
105.4 (1.6)	64.6 (4.5)	Intra-Carotid A II
133.1 (2.1)	54.0 (2.8)	A II & Phenylephrine
Average baroreflex sensitivity during angiotensin II infusion = -.24 (.09)		

The addition of phenylephrine infusion to the ongoing angiotensin II infusion caused a further increase in arterial pressure 26% (p=.0009) when compared to the prior period and a decrease in heart rate 16% (p=.005). The average baroreflex sensitivity during angiotensin II infusion period was -.24, a significant decrease (p=.024) from the baroreflex sensitivity observed during the control period, -.81.

Table 7 shows data obtained in a control period and during angiotensin II infusion in two dogs. Baroreflex sensitivity was reduced during angiotensin II infusion when compared to the control period (-.49 to -1.2). Because of the small number of dogs, statistical analysis was not performed.

Table 7: Arterial pressure, heart rate and baroreflex sensitivity in two conscious dogs during a control period and during intra-carotid angiotensin II infusion (10 ng/kg/min).

Arterial Pressure mmHg	Heart Rate Beats/min	Period
84.2 (4.1)	84.0 (7.1)	Control
119.4 (11.0)	56.0 (9.0)	Intravenous Phenylephrine
Average baroreflex sensitivity during the control period = -1.22 (.71)		
122.7 (7.4)	63.4 (6.6)	Intra-Carotid A II
136.5 (8.6)	56.9 (10.1)	A II & Phenylephrine
Average baroreflex sensitivity during angiotensin II infusion = -.49 (.29)		

The data indicate that intra-carotid PGE_2 and angiotensin II infusions affect the baroreflex in different ways. Both PGE_2 and angiotensin II infusions increase arterial pressure with little alteration in heart

rate. However, the sensitivity of the baroreflex does not change during intra-carotid PGE_2 infusion, whereas intra-carotid angiotensin II infusion markedly decreases baroreflex sensitivity.

Series Six

Intra-carotid PGE_2 was administered to four calves prior to and during general anesthesia. The calves were studied after initially anesthetizing them with an ultra-short barbiturate anesthetic and then establishing gas anesthesia with halothane. These data are presented in Figure 11.

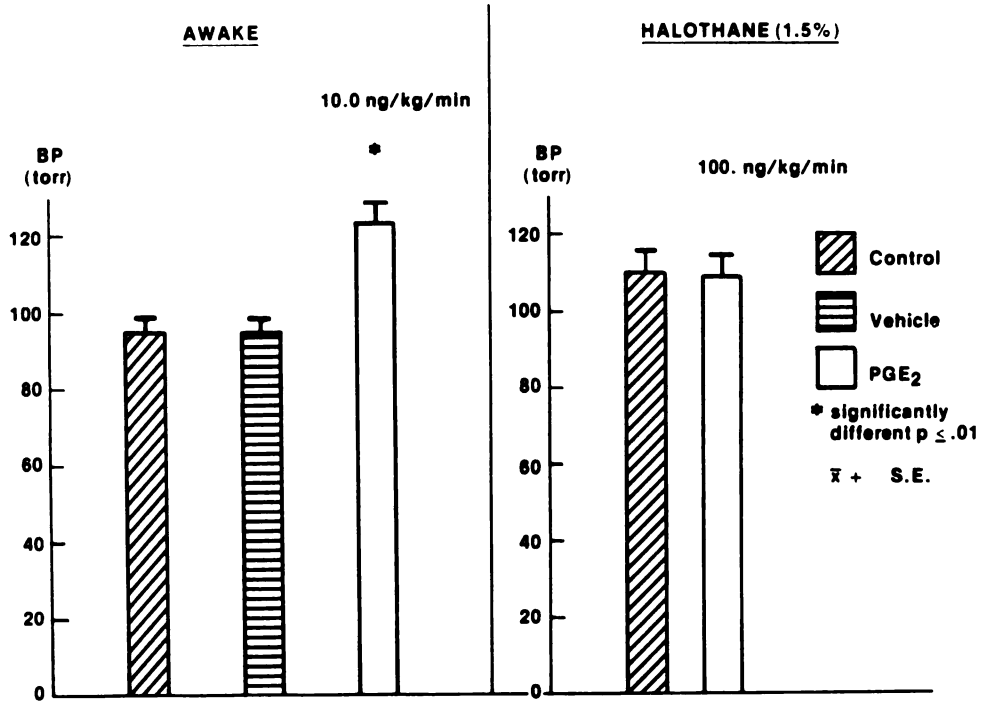

Figure 11: Left Panel: Arterial pressure in four conscious calves during: (a) control, (b) vehicle control infusion and (c) intra-carotid PGE_2 infusion, 10 ng/kg/min. Right Panel: Arterial pressure in four anesthetized (halothane) calves during: (a) control and (b) intra-carotid PGE_2 infusion, 100 ng/kg/min.

During anesthesia, intra-carotid PGE_2 infusion did not increase arterial pressure when compared to an anesthesia control period. Intra-carotid PGE_2 infusion (10 ng/kg/min) prior to anesthesia caused a significant increase in arterial pressure when compared to control periods or to saline vehicle infusions (29%, p=.0009). During anesthesia (barbiturate/halothane), intra-carotid PGE_2 infusion (100 ng/kg/min) into the same calves failed to significantly alter the animals' arterial pressure when compared to an anesthetic control period. During the anesthetic control period and during the latter intra-carotid PGE_2 infusion (100 ng/kg/min), the animal's arterial pressure was significantly increased (16%, p=.003) when compared to the unanesthetized control period.

Arterial pressure and heart rate were also measured in four sheep first while conscious, then after anesthesia (chloralose/urethane) and finally, while still anesthetized, during intra-carotid PGE_2 infusion (100 ng/kg/min) (Table 8).

Table 8: Arterial pressure and heart rate in four sheep during: (a) control, sheep conscious, (b) anesthesia, chloralose/urethane and (c) during anesthesia and intra-carotid PGE_2 infusion, 100 ng/kg/min.

Arterial Pressure mmHg	Heart Rate Beats/min	Period	Signif to Control BP	Signif to Control HR
78.2 (3.9)	68.1 (8.1)	Control	--	--
99.4 (3.6)	83.3 (13.5)	Anesthesia	.00009	n. sig
99.3 (3.9)	90.6 (19.3)	Anest., + PGE_2	.00009	n. sig

Both chloralose/urethane anesthesia and intra-carotid PGE_2 infusion

during chloralose/urethane anesthesia (100 ng/kg/min) significantly increased arterial pressure (27%). While anesthetized, the animal's arterial pressure and heart rate did not significantly change during the intra-carotid PGE_2 infusion (100 ng/kg/min). Heart rate was non-significantly increased in the anesthesia period and during the intra-carotid PGE_2 infusion period which reflects the large heart rate variability associated with anesthesia. The sheep tested in this experiment all previously reacted to intra-carotid PGE_2 infusion (10 ng/kg/min) with pressor responses similar to those previously shown.

Crystal violet dye appears in the brain of sheep after dye injection into a carotid artery. In the latter part of the anesthesia study, 10 mg of crystal violet dye (0.5 ml) was rapidly injected into the carotid catheter and the animal was immediately killed with an overdose of barbiturate anesthetic (Bio-tal). The timing of the two injections was calculated so that minimal recirculation of dye would occur. Several extra-calvarial structures and tissues were stained, including the ear, facial skin, facial muscles, tongue and periorbital tissues. The extra-calvarial staining was most prominent on the side ipsilateral to the dye injection. A much smaller extent of staining was observed on the contralateral side. Similar results were found when the brain was examined for dye. The ipsilateral cerebral hemisphere was heavily stained. The contralateral hemisphere, as with the extra-calvarial structures, was also stained, but to a much smaller extent than the hemisphere ipsilateral to the dye injection. Visualization of the Circle of Willis at the base of the brain indicated very strong staining of the internal carotid artery on the dye injection side. Faint staining was apparent in the contralateral

internal carotid artery indicating that some recirculation occurred.

Series Seven

The following four figures and seven tables show mean arterial pressure and heart rate responses to various adrenergic blockers, singly and in combination. These studies were performed in conscious sheep and calves. The effects of intra-carotid PGE_2 infusion (10 ng/kg/min) on arterial pressure and heart rate were assessed in the presence of propranolol and phentolamine.

Figure 12 shows arterial pressure and heart rate responses in seven conscious sheep during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min). These sheep show the typical hemodynamic effects of intra-carotid PGE_2 infusion, which are increased arterial pressure (24%, p=.0012) and increased heart rate (10%, p=.009). The magnitude of the responses is similar to that previously shown.

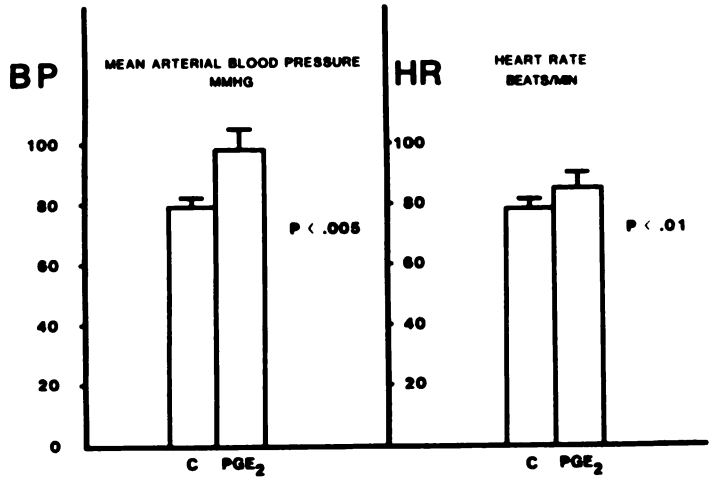

Figure 12: Arterial pressure and heart rate in seven conscious sheep during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

Figure 13 shows the arterial pressure and heart rate effects of intravenous propranolol (1 mg/kg) in four conscious sheep.

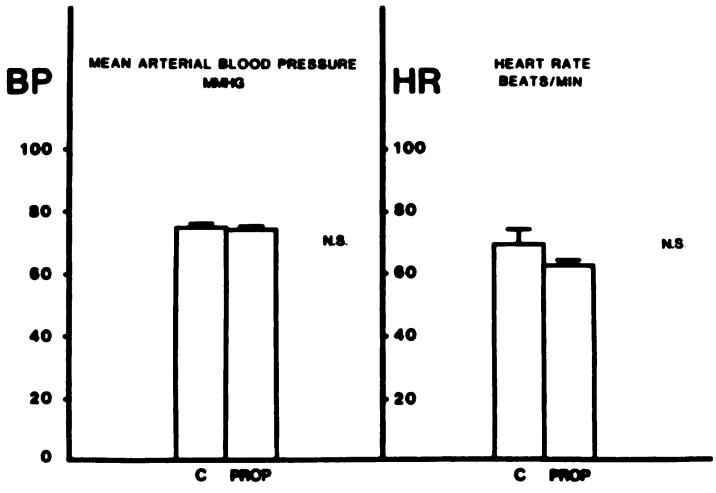

Figure 13: Arterial pressure and heart rate in four conscious sheep during a control period and during beta adrenergic blockade (propranolol, 1 mg/kg).

Both arterial pressure and heart rate showed a tendency to decrease, with a larger heart rate change being observed. Because of the variability in these responses, neither arterial pressure nor heart rate changes approached significance. In this and subsequent experiments using propranolol, pharmacologic beta blockade was assured at the end of each experiment by injecting 25 ug of intravenous isoproterenol. This resulted in less than a 10 beat/min heart rate response. Prior to propranolol administration, the intravenous injection of 5 ug of isoproterenol decreased arterial pressure 10-20 mmHg and increased heart rate 60-80 beats/min. The animals displayed no behavioral alterations during beta adrenergic blockade.

Figure 14 shows the effects of intravenous phentolamine (1 mg/kg) in four conscious sheep.

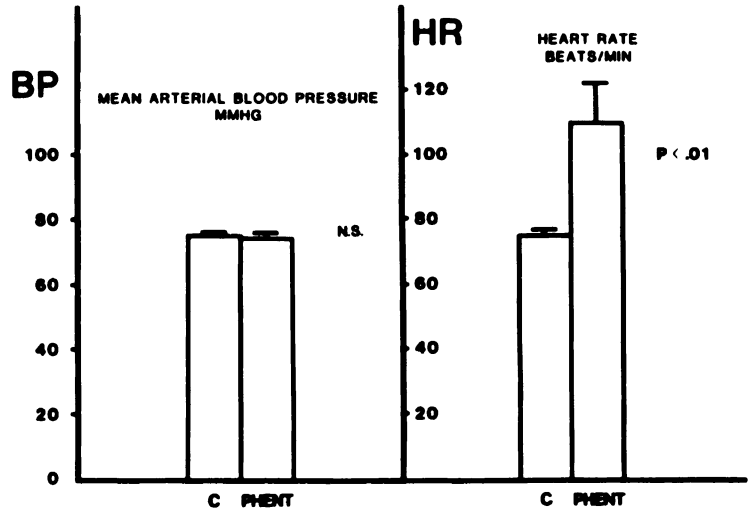

Figure 14: Arterial pressure and heart rate in four conscious sheep during a control period and during alpha adrenergic blockade (phentolamine, 1 mg/kg).

As with the beta blockade, no significant arterial pressure changes were evident when alpha adrenergic blockade was compared to control values. Alpha adrenergic blockade resulted in a large and significant increase in heart rate (55%, p=.003). In this and subsequent experiments involving phentolamine, the efficacy of alpha adrenergic blockade was demonstrated at the end of each experiment. Intravenous injection of 1000 ug of phenylephrine elicited less than a 10 mmHg increase in arterial pressure. Prior to phentolamine injection, the intravenous injection of 400 ug of phenylephrine increased arterial pressure 40-60 mmHg. Diarrhea occurred in many animals in the hour after phentolamine administration. In addition, most animals became restless and arterial pressure became more variable.

The effects of injecting intravenous propranolol and subsequently intravenous phentolamine into four conscious sheep are presented in Table 9.

Table 9: Arterial pressure and heart rate in four conscious sheep during three experimental periods: (a) control, (b) during beta adrenergic blockade (propranolol, 1 mg/kg) and (c) during beta and alpha adrenergic blockade (propranolol, 1 mg/kg and phentolamine, 1 mg/kg).

Arterial Pressure mmHg	Heart Rate Beats/min	Period
75.5 (1.7)	70.0 (4.8)	Control
74.8 (1.0)	64.0 (3.8)	Propranolol
66.8 (4.8)	72.5 (2.8)	Propranolol & Phentolamine

This combination of adrenergic blockade was considered desirable as alpha adrenergic blockade alone triggered a tachycardia which was associated with restlessness in the animals. Introduction of beta adrenergic blockade before alpha blockade abrogated the phentolamine induced tachycardia and prevented the restlessness.

The heart rate and arterial pressure data in Table 9 show that the tachycardia associated with phentolamine (Figure 14) is eliminated when beta adrenergic blockade precedes alpha adrenergic blockade. Heart rate is not significantly different from control heart rate after combined beta and alpha adrenergic blockade. Arterial pressure, although decreased, is also not significantly altered by the combination of propranolol and phentolamine.

Table 10 shows arterial pressure and heart rate responses in four conscious sheep during a control period and during beta adrenergic blockade (propranolol, 1 mg/kg) introduced during a continuing intra-carotid PGE_2 infusion (10 ng/kg/min).

Table 10: Arterial pressure and heart rate responses in four conscious sheep during three experimental periods: (a) control, (b) intra-carotid PGE_2 infusion (10 ng/kg/min) and (c) intra-carotid PGE_2 infusion during beta adrenergic blockade (propranolol, 1 mg/kg).

Arterial Pressure mmHg	Heart Rate Beats/min	Period
73.8 (4.7)	77.5 (7.6)	Control
91.7 (5.6)	83.3 (7.0)	Intra-Carotid PGE_2
91.0 (5.8)	76.9 (4.0)	Intra-Carotid PGE_2 + Propranolol

As before, intra-carotid PGE_2 infusion significantly increased arterial pressure (24%, p=.0004) and heart rate (8%, p=.006) when compared to control values. Introduction of beta adrenergic blockade during the ongoing intra-carotid PGE_2 infusion did not significantly affect the arterial pressure (compared to the arterial pressure obtained with intra-carotid PGE_2 infusion alone). Heart rate decreased 7% when compared to the intra-carotid PGE_2 infusion period and was not significantly different from control heart rate.

Arterial pressure and heart rate were observed in four conscious sheep when intravenous phentolamine (1 mg/kg) was injected during a continuing intra-carotid PGE_2 infusion (10 ng/kg/min) (Table 11).

Table 11: Arterial pressure and heart rate in four conscious sheep during three experimental periods: (a) control, (b) intra-carotid PGE_2 infusion (10 ng/kg/min) and (c) intra-carotid PGE_2 infusion during alpha adrenergic blockade (phentolamine, 1 mg/kg).

Arterial Pressure mmHg	Heart Rate Beats/min	Period
74.4 (4.1)	76.6 (4.1)	Control
94.0 (3.7)	86.9 (1.4)	Intra-Carotid
83.8 (4.0)	130.3 (11)	Intra-Carotid PGE_2 + Phentolamine

As with other intra-carotid PGE_2 infusion experiments, this intra-carotid PGE_2 infusion increased arterial pressure (27%, p=.00007) and heart rate (13%, p=.02) when compared to control values. Alpha adrenergic blockade during the ongoing intra-carotid PGE_2 infusion significantly increased heart rate when compared to the control period (70%, p=.004) and when compared to the intra-carotid PGE_2 infusion period (51%, p=.004). Arterial pressure during the alpha adrenergic blockade period remained significantly increased when compared to the control period (14%, p=.003). However, arterial pressure was significantly decreased after phentolamine injection when compared to the prior intra-carotid PGE_2 infusion period (-11%, p=.0025). There were marked changes in animal behavior after phentolamine injection, typically including teeth grinding and straining at the halter. Diarrhea often occurred in the hour after phentolamine injection.

Because of the marked heart rate and behavioral alterations associated with intravenous phentolamine administration, intravenous propranolol (1 mg/kg) was introduced into the protocol during the

intra-carotid PGE_2 infusion (10 ng/kg/min) and before intravenous phentolamine injection (1 mg/kg) (Table 12).

Table 12: Arterial pressure and heart rate in four conscious sheep during four experimental periods: (a) control, (b) intra-carotid PGE_2 infusion (10 ng/kg/min), (c) intra-carotid PGE_2 infusion during beta adrenergic blockade (propranolol, 1 mg/kg) and (d) intra-carotid PGE_2 infusion during beta and alpha adrenergic blockade (propranolol, 1 mg/kg and phentolamine, 1 mg/kg).

Arterial Pressure mmHg	Heart Rate Beats/min	Period
73.8 (4.7)	77.5 (7.6)	Control
91.7 (5.6)	83.3 (7.0)	Intra-Carotid PGE_2
91.0 (5.8)	76.9 (4.0)	Intra-Carotid PGE_2 + Propranolol
78.7 (6.3)	81.2 (2.5)	Intra-Carotid PGE_2 + Propranolol & Phentolamine

Intra-carotid PGE_2 infusion increased arterial pressure (24%, p=.0008) and heart rate (8%, p=.005) in four conscious sheep. Similar to data shown before, arterial pressure during the continuing intra-carotid PGE_2 infusion was not significantly altered by the propranolol injection. Arterial pressure during these periods remained significantly elevated (22%, p=.0009) when compared to control arterial pressure. A non-significant decrease of 8% in heart rate occurred (compared to the intra-carotid PGE_2 infusion period) and heart rate did not significantly differ from control. The addition of alpha adrenergic blockade to beta adrenergic blockade during intra-carotid PGE_2 infusion lowered arterial pressure to control levels. When compared to the prior beta blockade condition, phentolamine caused a significant decrease in arterial pressure (14%, p=.0008) and a

non-significant increase in heart rate (7%).

Arterial pressure and heart rate responses were subsequently determined in four conscious sheep, as in Table 12, except that the order of adrenergic blockade was reversed. In this experiment, phentolamine injection preceded propranolol injection (1 mg/kg phentolamine, 1 mg/kg propranolol) during the intra-carotid PGE_2 infusion (10 ng/kg/min) period (Table 13).

Table 13: Arterial pressure and heart rate in four conscious sheep during four experimental periods: (a) control, (b) intra-carotid PGE_2 infusion (10 ng/kg/min), (c) intra-carotid PGE_2 infusion during alpha adrenergic blockade (phentolamine, 1 mg/kg) and (d) intra-carotid PGE_2 infusion during alpha and beta adrenergic blockade (phentolamine, 1 mg/kg and propranolol, 1 mg/kg)

Arterial Pressure mmHg	Heart Rate Beats/min	Period
74.4 (4.1)	76.6 (4.1)	Control
94.0 (3.7)	86.9 (1.4)	Intra-Carotid PGE_2
83.8 (4.0)	130.3 (11.0)	Intra-Carotid PGE_2 + Phentolamine
82.5 (5.2)	83.8 (5.6)	Intra-Carotid PGE_2 + Phentolamine & Propranolol

Intra-carotid PGE_2 infusion increased arterial pressure (26%, p=.00008) and heart rate (13%, p=.03). After phentolamine injection, heart rate increased (50%, p=.004) when compared to the preceding intra-carotid PGE_2 infusion period and increased (70%, p=.0002) when compared to control heart rate. After propranolol injection (i.e. during combined alpha and beta adrenergic blockade), arterial pressure did not significantly change from the preceding alpha block period and remained

above control arterial pressure (11%, p=.0003). When compared to the preceding alpha adrenergic blockade period, heart rate significantly decreased (36%, p=.0005) and now was not significantly different than control heart rate.

As had been observed before, alpha adrenergic blockade caused a tachycardia and generally upset the animals. The propranolol injection which followed the alpha adrenergic blockade eliminated the tachycardia, but many of the animals remained restless and agitated. This prolonged excitement may explain why combined alpha and beta adrenergic blockade in this series of experiments did not return arterial pressure to control, in contrast to the results of combined alpha and beta blockade shown in Table 12.

The effects of alpha and beta adrenergic block, when administered prior to intra-carotid PGE_2 infusion, are shown in the following figures and tables. In these experiments, propranolol and phentolamine (1 mg/kg each) were individually injected intravenously into four conscious sheep approximately 10 minutes apart and 15-20 minutes prior to the start of the intra-carotid PGE_2 infusion (10 ng/kg/min) (Figure 15). Arterial pressure was non-significantly decreased (12%), when compared to control, by combined alpha and beta adrenergic blockade. Heart rate during combined beta and alpha adrenergic blockade was not significantly different from control values. During the intra-carotid PGE_2 infusion, arterial pressure was not significantly altered when compared to the prior beta and alpha adrenergic blockade period. However, arterial pressure in this latter period was significantly

lower (9%, p=.02) than control arterial pressure.

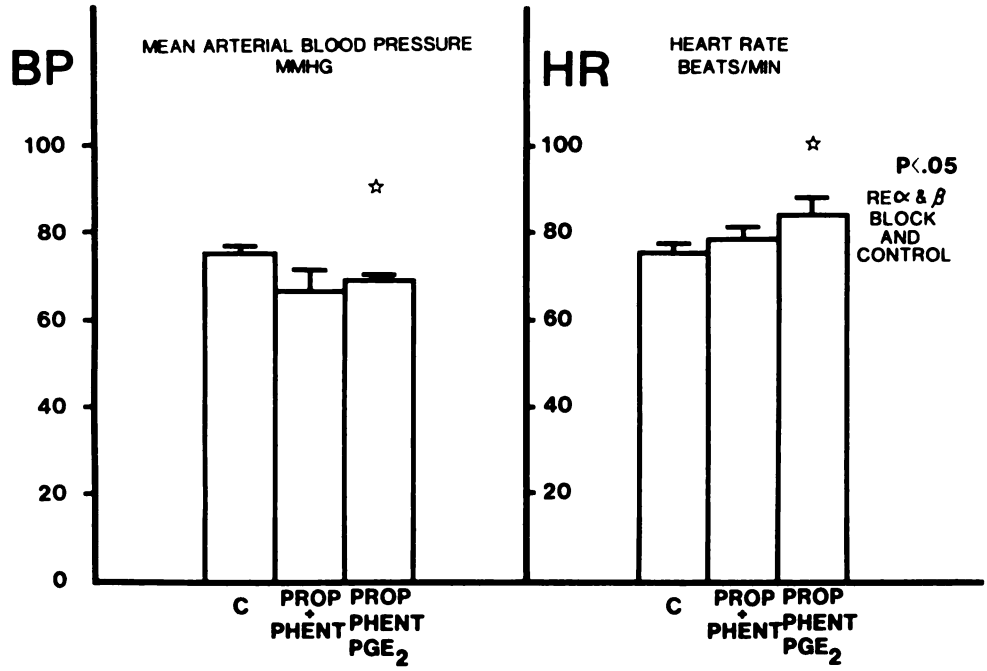

Figure 15: Arterial pressure and heart rate in four conscious sheep during three experimental periods: (a) control, (b) during beta and alpha adrenergic blockade (propranolol, 1 mg/kg and phentolamine, 1 mg/kg) and (c) during intra-carotid PGE_2 infusion (10 ng/kg/min) during beta and alpha adrenergic blockade.

The individual effects of beta and alpha adrenergic blockade and combined beta and alpha adrenergic blockade were determined in four conscious calves during intra-carotid PGE_2 infusion (Table 14). In these experiments intra-carotid PGE_2 (10 ng/kg/min) was infused and, during the continuing PGE_2 infusion, propranolol (1 mg/kg) and then phentolamine (1 mg/kg) were injected. When compared to control values, intra-carotid PGE_2 infusion increased arterial pressure (35%, p=.0001) and non-significantly increased heart rate (6%). Introduction of beta adrenergic blockade during the intra-carotid PGE_2 infusion resulted in

no significant change in arterial pressure or heart rate when compared to the preceding intra-carotid PGE_2 infusion period. Arterial pressure remained significantly elevated above control pressure and heart rate was not significantly altered from control heart rate. The addition of alpha adrenergic blockade significantly reduced arterial pressure (23%, p=.00009) when compared to the previous beta adrenergic blockade period. Phentolamine injection caused a significant increase in heart rate (3%, p=.0001) when similarly compared to the prior propranolol period. Arterial pressure and heart rate did not differ in this beta and alpha adrenergic block period when compared to control values.

Table 14: Arterial pressure and heart rate in four conscious calves during four experimental periods: (a) control, (b) intra-carotid PGE_2 infusion (10 ng/kg/min), (c) intra-carotid PGE_2 infusion during beta adrenergic blockade (propranolol, 1 mg/kg) and (d) intra-carotid PGE_2 infusion during beta and alpha adrenergic blockade (propranolol, 1 mg/kg and phentolamine, 1 mg/kg)

Arterial Pressure mmHg	Heart Rate Beats/min	Period
92.3 (4.3)	97.5 (11.4)	Control
124.8 (6.8)	103.8 (13.4)	Intra-Carotid PGE_2
124.5 (6.6)	95.3 (9.3)	Intra-Carotid PGE_2 + Propranolol
94.8 (6.1)	97.8 (9.4)	Intra-Carotid PGE_2 + Propranolol & Phentolamine

Arterial pressure and heart rate responses of four conscious calves were examined when the adrenergic blockade preceded the intra-carotid PGE_2 infusion (Table 15). In these experiments, beta and alpha adrenergic blockade were in effect prior to and during the

intra-carotid PGE_2 infusion (10 ng/kg/min).

Table 15: Arterial pressure and heart rate in four conscious calves during four experimental periods: (a) control, (b) beta adrenergic blockade (propranolol, 1 mg/kg), (c) beta and alpha adrenergic blockade (propranolol, 1 mg/kg and phentolamine, 1 mg/kg) and (d) intra-carotid PGE_2 infusion (10 ng/kg/min) during beta and alpha adrenergic blockade.

Arterial Pressure mmHg	Heart Rate Beats/min	Period
93.0 (4.1)	97.5 (10.6)	Control
93.3 (4.6)	94.0 (8.8)	Propranolol
90.8 (4.4)	94.0 (6.5)	Propranolol + Phentolamine
95.0 (3.9)	103.8 (8.5)	Propranolol + Phentolamine & Intra-Carotid PGE_2

Propranolol (1 mg/kg) did not significantly alter arterial pressure or heart rate when compared to control values. After intravenous phentolamine injection, arterial pressure was significantly decreased (2%, p=.03) when compared to control values and heart rate was not significantly altered. Intra-carotid PGE_2 infusion increased arterial pressure (5%, p=.017) when compared to the preceding beta and alpha adrenergic blockade period. When compared to the same period, heart rate significantly increased (10%, p=.01). When compared to control values, arterial pressure was significantly (2%, p=.025) increased during intra-carotid PGE_2 infusion during beta and alpha adrenergic blockade. When similarly compared, heart rate was also significantly increased(6%, p=.027).

The data in tables 9-15 and figures 12-15 show that, in conscious calves and sheep, regardless of adrenergic block order, the effects of pressor doses of intra-carotid PGE_2 are negated by alpha adrenergic blockade. Beta blockade is useful in conjunction with alpha adrenergic blockade, as it prevents the tachycardia that is associated with phentolamine injection. Beta adrenergic blockade alone did not alter the pressor effect of intra-carotid PGE_2 infusion. The tachycardia noted during alpha adrenergic blockade appeared in parallel with the restlessness if those animals were not previously beta blocked.

Series Eight

Intra-carotid PGE_2 increases plasma renin activity (PRA) in sheep and calves. Table 16 shows arterial pressure, heart rate and PRA activity during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min) in five conscious sheep.

Table 16: Arterial pressure, heart rate and plasma renin activity in five conscious sheep during a control period and during intra-carotid PGE_2 infusion (10 ng/kg/min).

Arterial Pressure mmHg	Heart Rate Beats/min	Plasma Renin Activity ng/ml hr	Period
74.8 (2.6)	74.4 (4.3)	1.28 (.34)	Control
89.6 (3.6)	82.0 (1.0)	2.86 (.69)	Intra-Carotid PGE_2
p=.001	p=.10	p=.038	

When compared to control values, intra-carotid PGE_2 infusion increased arterial pressure (20%, p=.001), did not significantly change heart rate and increased PRA (124%, p=.038).

Figure 16 shows data from four sheep during intra-carotid PGE_2 infusion (10 ng/kg/min). The change in arterial pressure from control to that observed during intra-carotid PGE_2 infusion is shown on the ordinate. The change in PRA observed from the control period to the intra-carotid PGE_2 infusion period is shown on the abscissa. The increases in arterial pressure resulting from intra-carotid PGE_2 infusion were correlated with relatively small changes in PRA and are associated with a low slope indicating a small influence on blood pressure changes. The PRA not associated with a large correlation coefficient (r=.83), but indicate some degree of association.

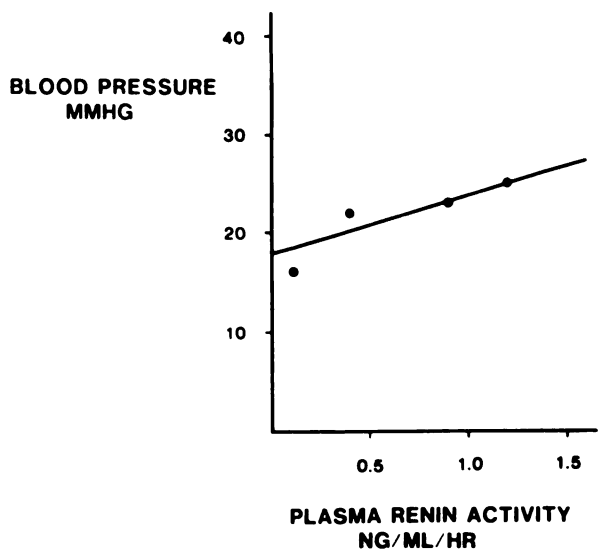

Figure 16: Changes in plasma renin activity (experimental - control) plotted against changes in arterial pressure (experimental - control) observed in four conscious sheep during intra-carotid PGE_2 infusion (10 ng/kg/min).

Figure 17 shows the arterial pressure and PRA responses of an individual sheep during an increasing intra-carotid PGE_2 infusion (saline vehicle control, 5, 20, 80 and 200 ng/kg/min). The figure indicates that both PRA and arterial pressure increase with increasing

intra-carotid PGE_2 infusion rates.

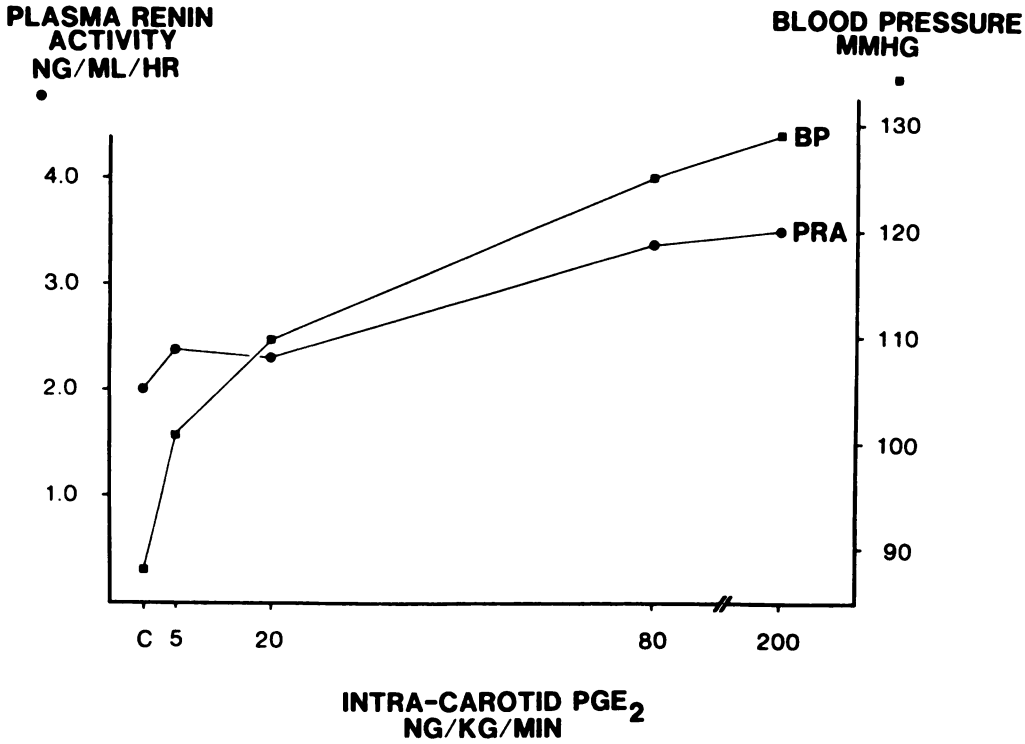

Figure 17: Arterial pressure and plasma renin activity in a conscious sheep during a control period and during intra-carotid PGE_2 infusions (5, 20, 80 and 200 ng/kg/min).

Table 17 shows the PRA and arterial pressure in four conscious sheep during increasing intra-carotid PGE_2 infusions.

Table 17: Arterial pressure and plasma renin activity in four conscious sheep during a saline vehicle control period and during intra-carotid PGE_2 infusions (5, 20, 80 and 200 ng/kg/min).

Arterial Pressure mmHg	Plasma Renin Activity ng/ml hr	Period	Signif BP	Signif PRA
75.8 (4.1)	1.41 (.56)	Saline Control	---	---
87.8 (5.9)	2.55 (.96)	PGE_2 5 ng/kg/min	signif.	n. sig.
99.0 (3.5)	2.36 (.88)	PGE_2 20 "	signif.	n. sig.
106.8 (6.2)	2.38 (.61)	PGE_2 80 "	n. sig.	n. sig.
109.5 (6.9)	2.63 (.80)	PGE_2 200 "	n. sig	signif.

The associated arterial pressure responses obtained during the intra-carotid PGE_2 infusions are shown alongside the PRAs determined during the PGE_2 infusion periods. At each increased PGE_2 infusion rate, arterial pressure is significantly increased over control (paired t). This same data when tested by analysis of variance indicates that the PGE_2 infusions at 80 and 200 ng/kg/min are not significantly different from each other but are significantly different from all other infusion levels. At each increasing PGE_2 infusion level plasma renin activity is also increased over control (paired t). However, analysis of variance indicates significance only at the highest PGE_2 infusion rate when compared to any other infusion level.

Figure 18 shows the preceding arterial pressure and plasma renin activity values plotted against each other.

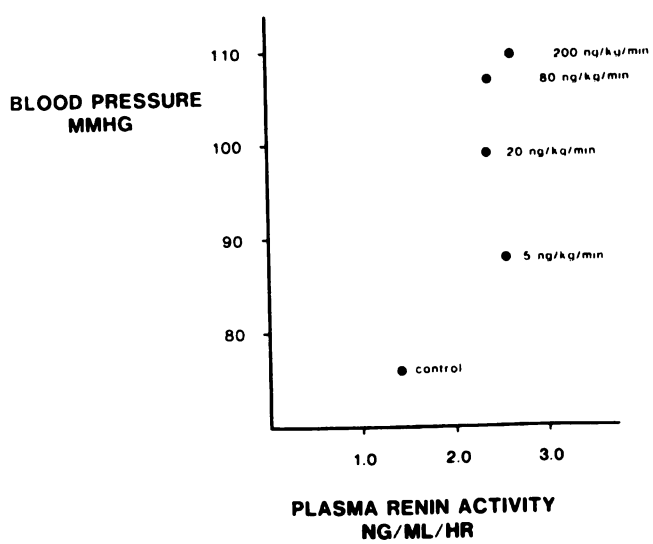

Figure 18: Arterial pressure and plasma renin activity in four conscious sheep during a saline control period and during intra-carotid PGE_2 infusions (5, 20, 80 and 200 ng/kg/min).

A correlation coefficient of .77 indicates that at each increasing intra-carotid PGE_2 infusion rate, there is some associated change in plasma renin activity.

Series Nine

Figure 19 shows arterial pressure and heart rate responses before and after intravenous captopril (2 mg/kg) in four conscious sheep. The efficacy of converting enzyme blockade was confirmed at the end of the experiment by intravenous injection of 10 ug of angiotensin I, with no pressor response resulting. Captopril injection caused a decrease in arterial pressure (11%, p=.006) and an increase in heart rate (11%, p=.008).

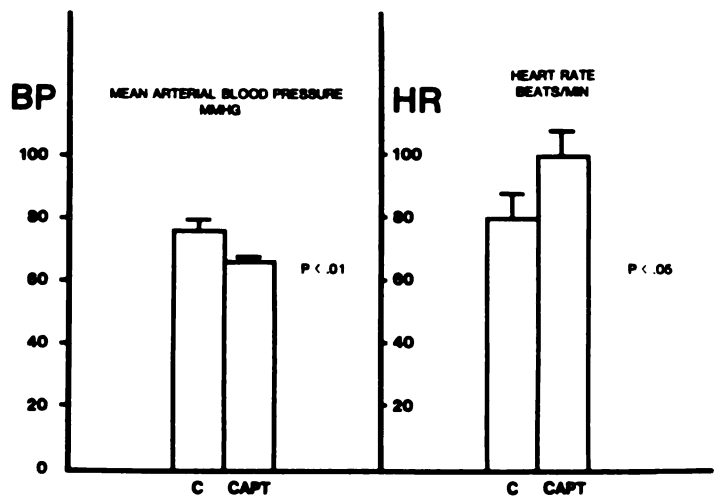

Figure 19: Arterial pressure and heart rate in four conscious sheep during a control period and during angiotensin converting enzyme blockade (captopril, 2 mg/kg).

The arterial pressure and heart rate responses after captopril injection were similar to those observed after phentolamine, but the animals did not become restless and no diarrhea was observed.

Arterial pressure and heart rate responses in four conscious sheep were determined during a control period, after injection of captopril (2 mg/kg) and during intra-carotid PGE_2 infusion (10 ng/kg/min) after captopril injection (Table 18).

Table 18: Arterial pressure and heart rate in four conscious sheep during three experimental periods: (a) control, (b) angiotensin converting enzyme blockade (captopril, 2 mg/kg) and (c) intra-carotid PGE_2 infusion (10 ng/kg/min) during angiotensin converting enzyme blockade.

Arterial Pressure mmHg	Heart Rate Beats/min	Period
75.2 (2.4)	86.2 (7.8)	Control
67.2 (0.5)	95.9 (9.7)	Captopril
80.5 (3.9)	113.1 (2.7)	Intra-Carotid PGE_2 after Captopril

Intra-carotid PGE_2 infusion during angiotensin converting enzyme blockade significantly increased arterial pressure (7%, p=.0003) and significantly increased heart rate (31%, p=.0018) when compared to control values. When compared to the prior converting enzyme blockade period, intra-carotid PGE_2 infusion significantly increased both arterial pressure (19%, p=.004) and heart rate (18%, p=.04).

Figure 20 shows arterial pressure during a control period, during intra-carotid PGE_2 infusion alone, during angiotensin converting enzyme

blockade alone and during intra-carotid PGE_2 infusion during converting enzyme blockade. These data were obtained from the four conscious sheep. The experiments were not always done in the sequence shown, but are presented in this manner for clarity.

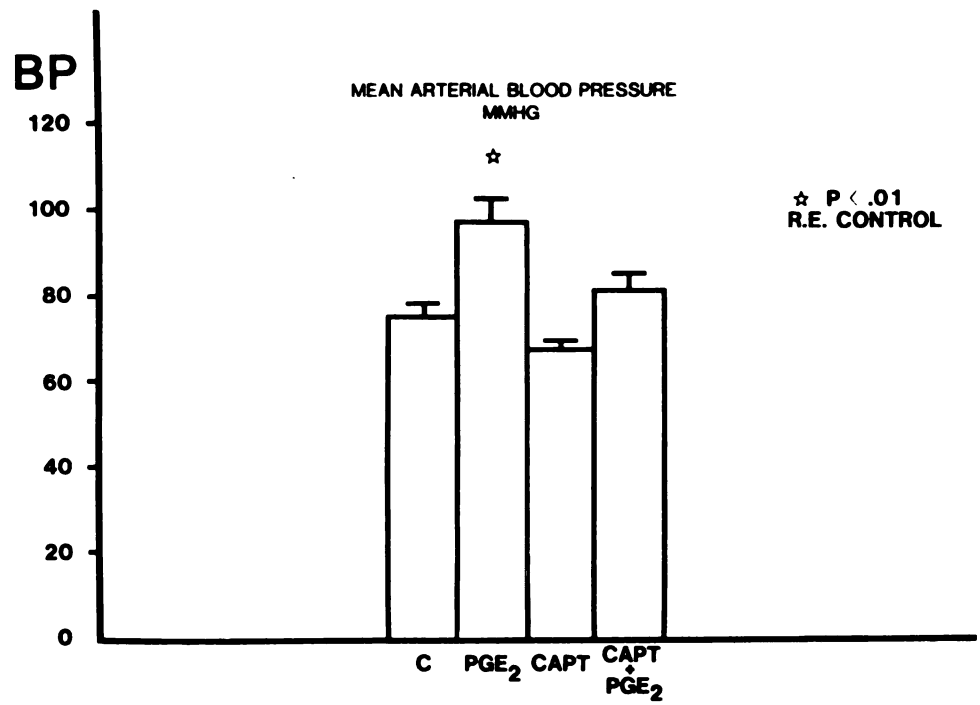

Figure 20: Arterial pressure in four conscious sheep during four experimental periods: (a) control, (b) during intra-carotid PGE_2 infusion (10 ng/kg/min), (c) during angiotensin converting enzyme blockade (captopril, 2 mg/kg) and (d) during intra-carotid PGE_2 infusion during angiotensin converting enzyme blockade.

As has been previously shown and discussed, intra-carotid PGE_2 infusion significantly increases arterial pressure when compared to control values. Several days later these same animals were given captopril and intra-carotid PGE_2 was infused during the subsequent converting enzyme blockade period. As can be seen, arterial pressure did increase, but it did not significantly change when compared to control. When compared to the prior captopril period, intra-carotid

PGE_2 infusion significantly increased arterial pressure. The increase in arterial pressure was significantly different ($p=.024$) from the arterial pressure observed during intra-carotid PGE_2 infusion alone. Therefore, from a statistical viewpoint captopril eliminated the intra-carotid PGE_2 pressor effect. However, intra-carotid PGE_2 infusion significantly increased arterial pressure after captopril injection. The apparent statistical discrepancy is caused by the significantly decreased arterial pressure caused by captopril injection. Therefore, from a physiological viewpoint, blockade of converting enzyme attenuated the intra-carotid PGE_2 pressor effect, but did not completely eliminate it.

Discussion

The results of the experiments presented in this dissertation can be summarized as follows. Intra-carotid PGE_2 infusion (10 ng/kg/min) increased blood pressure approximately 25 mmHg in conscious sheep, calves and dogs. Intra-carotid PGE_2 infusion markedly increased total peripheral resistance in conscious calves including increases in iliac resistance (40%), renal resistance (37)% and mesenteric resistance (30)%. Cardiac output, although significantly increased by 2%, did not substantially contribute to the blood pressure increase in these calves. The heart rate increase associated with intra-carotid infusion was smaller in percentage terms than the blood pressure increase and, in the dog, was not significant. In the conscious calf, intra-aortic PGE_2 infusion (200 ng/kg/min) decreased blood pressure (15%) and increased heart rate (16%). In all three species, systemic venous administration of PGE_2 (400 ng/kg/min) caused no discernible hemodynamic effects. Intra-carotid PGE_2 infusion in the three species did not cause alterations in pO_2, pCO_2 or pH; therefore, there is no reason to suspect chemoreflex activation. The sensitivity of the cardiac component of the baroreflex was not modified by intra-carotid PGE_2 infusion in the three species. Intra-carotid infusion of angiotensin II (10 ng/kg/min) substantially reduced baroreflex sensitivity. Anesthetized calves and sheep showed no increase in blood pressure during intra-carotid PGE_2 infusion despite infusion rates which were ten times larger than those which caused pressor effects in the same conscious animals. Alpha adrenergic blockade prevented the

intra-carotid PGE_2 pressor effect. Beta adrenergic blockade did not alter the PGE_2 pressor effect. Plasma renin activity was doubled during intra-carotid PGE_2 infusion. During incremental intra-carotid PGE_2 infusions (20-200 ng/kg/min), blood pressure consistently increased at each higher infusion level; small incremental increases in plasma renin activity also occurred. Blockade of converting enzyme resulted in an attenuation of the intra-carotid PGE_2 pressor response.

The following discussion will consider each of the objectives as stated at the end of the literature review. First, the results of the experiments on anesthetized animals will be compared with the previous reports of central PGE hemodynamic effects during anesthesia. Then the remaining objectives will be discussed approximately in the order presented in the Statement of Objectives.

In contrast to several reports (Carlson and Oro, 1966; Kaplan et al., 1969; Gyang et al., 1973; Rinchuse and Deuben, 1976; Fujimoto, 1977; Brus et al., 1979; and Feuerstein et al., 1982), the present study did not show a pressor response to intra-carotid PGE_2 infusion in anesthetized animals. Anesthetized calves (halothane after barbituate) and anesthetized sheep (chloralose/urethane) were given intra-carotid PGE_2 infusions (100 ng/kg/min) at ten times the dose rate that caused substantial blood pressure increases in the same conscious animals. The differences between the present study and previous studies could potentially be accounted for by the use of different species, the effect of PGE_1 in contrast to PGE_2, the PGE dose rate, the method of PGE administration and the type of anesthesia used. However no clear

pattern emerges from the literature to account for the discrepancies.

The largest pressor responses to centrally infused PGE were observed in the anesthetized rat. Fujimoto studied central PGE_2 pressor responses in the rat and consistently observed blood pressure increases (25%) during urethane, pentobarbital or chloral hydrate-chloralose anesthesia. The author used much larger doses of PGE_2 (1.4-140 ug/kg) than were used in the present experiments; PGE_2 was injected into the cerebroventricular system. Rinchuse and Deuben presented similar evidence for central PGE_1 pressor responses in the urethane anesthetized rat. These authors used a much smaller PGE dose (1-10 ng/kg/min), and infused PGE_1 into the carotid artery. The resulting PGE pressor response was similar in magnitude to the pressor responses to intra-carotid PGE_2 shown in this dissertation. Brus et al., using the urethane anesthetized rat, presented data showing a 45 mmHg blood pressure increase after cerebroventricular PGE_2 injection. The PGE_2 doses used in their study were quite large, 1-10 ug, and were administered by bolus injection. Feuerstein et al. similarly injected PGE_2 into the cerebroventricular system in halothane anesthetized rats. The resulting pressor response was similar to that observed in this dissertation; however the PGE_2 doses were administered by injection and were in the ug/kg range.

Gyang et al. reported blood pressure increases (10-48 mmHg) in the chloralose anesthetized cat during intra-vertebral PGE_1 infusion. The dose and infusion duration of PGE_1, as well as the blood pressure response, were similar to those presented in this dissertation for

conscious animals.

A pressor effect of central PGE_1 administration in the pentobarbital anesthetized dog was reported by Carlson and Oro and by Kaplan et al. Carlson and Oro reported small (6 mmHg) pressor responses to intra-carotid PGE_1 during 1.2 ug/kg/min infusions. Kaplan et al. demonstrated a much larger pressor effect (40-50 mmHg) with intra-carotid injections of PGE_1 (5-10 ug).

Several other studies involving barbiturate anesthesia have failed to show a central PGE pressor response. Nakano and McCurdy (1967) studied pentobarbital anesthetized dogs during intra-carotid PGE_1 injections and did not report a pressor response. McQueen and Belmonte (1974) also failed to show pressor responses in pentobarbital anesthetized cats during intra-carotid PGE_2 injections. Spira et al. (1978) infused PGE_1 into the carotid arteries of pentobarbital anesthetized monkeys and failed to report a pressor effect. Hull and McCracken (1979) observed that halothane anesthesia abolished a central PGE_2 pressor effect in sheep. These sheep were initially anesthetized with a short acting barbiturate.

In this dissertation, calves were initially anesthetized with an ultra-short acting barbiturate and intra-carotid PGE_2 infusion was begun approximately one hour after the start of halothane administration. This mode of anesthesia resulted in a blood pressure that was significantly above control (conscious) levels. Sawyer et al. (1971) has shown that the anesthetic properties of barbiturates include

a sympathomimetic effect and that halothane anesthesia alone is associated with vasodepressant effects. Despite the sixty minute wait after barbiturate injection to infuse PGE_2 some residual barbiturate pressor effect appears to be present during the halothane anesthesia period. Therefore, intra-carotid PGE_2 infusion may have been unable to increase blood pressure above the already increased level. Despite a PGE_2 infusion dose ten times that which caused a pressor response in the conscious animal, no hemodynamic response was observed.

Other investigators have also failed to show central PGE pressor responses during chloralose anesthesia. Lavery et al. (1970) infused PGE_1 into the carotid and vertebral arteries in chloralose anesthetized dogs and failed to show a pressor response. No barbiturates were used in the latter study and morphine was used as a pre-anesthetic. Yamamoto et al. (1976) perfused the ventricular-cisternal system with PGE_2 in the chloralose/urethane anesthetized dog and failed to show a pressor effect. Here again, no barbiturate anesthetic was used and morphine was again used as a pre-anesthetic. In the present experiments, no pressor response to intra-carotid PGE_2 was observed in the chloralose/urethane anesthetized sheep.

Halogenated and barbiturate anesthetics are known to depress central reflex mechanisms (Goodman and Gilman) and baroreflex mechanisms in particular (Kirchheim, 1976). Chloralose anesthesia depresses these reflexes to a lesser degree; however, substantial alterations in cardiovascular regulation are still apparent (ibid.). Thus, whereas no conclusive explanation is available, several other

possibilities exist other than an initial barbiturate sympathomimetic effect to explain the failure of intra-carotid PGE_2 infusion to increase blood pressure in the anesthetized calves and sheep. The initial barbiturate anesthetic that was given to the calves may have depressed the ability of the central nervous system action to respond to intra-carotid PGE_2 infusion. Halothane anesthesia alone may specifically prevent the central PGE_2 pressor response in calves, even though it does not in rats. Lastly, halothane anesthesia may have shifted the PGE_2 pressor dose response relationship greater than 10 times. If this were the case, the pressor effect of centrally administered PGE_2 might not be evident.

No premedication was used in the chloralose/urethane anesthetized sheep. Specific care was taken not to excite the sheep and each animal was anesthetized while in its own pen. Intra-carotid PGE_2 was infused after the sheep were transported to the lab. Chloralose/urethane anesthesia statistically increased the blood pressure in these sheep when compared to control pressure (unanesthetized). The increase in blood pressure during anesthesia (27%) resulted in a blood pressure that was generally greater than that observed during intra-carotid PGE_2 infusion (10 ng/kg/min) in the same conscious sheep. Except for a barbiturate sympathomimetic effect, the inability of the sheep to respond to intra-carotid PGE_2 during chloralose/urethane anesthesia thus could possibly be explained by mechanisms which are similar to those proposed above for the calves.

Intra-carotid PGE_2 infusion (10 ng/kg/min) increased blood pressure

approximately 25% in conscious sheep. The dogs showed a smaller pressor response (17%) and calves showed a larger pressor response (32%). Intra-carotid PGE_2 increased heart rate in the sheep and calves, whereas no consistent heart rate change was observed in the dogs. The blood pressure and heart rate responses were highly dependent on the behavioral state of the animal. Several forms of stress interfered with the PGE_2 pressor response. It was generally observed that the control blood pressure and heart rate were elevated immediately after surgery or after the animal was transferred to a new cage. Intra-carotid PGE_2 infused during such periods would not increase blood pressure to a higher level. However, after several days, the resting blood pressure would decline and the PGE_2 pressor response became consistent.

No alterations in blood pressure were observed over a one hour period during intra-carotid saline infusions in calves and dogs. However, the sheeps' heart rate statistically decreased by less than 2 beats/min during saline infusion. This small heart rate change is not unexpected since some initial excitement may have been caused when the catheters and infusion lines were connected.

Although no prior literature reports intra-carotid PGE_2 pressor effects in conscious calves and dogs, the literature contains many reports of pressor effects of PGE in conscious sheep, goats and rats. Hull (1975) showed that intra-carotid PGE_2 infusion increased blood pressure in conscious sheep at infusion rates greater than 5 ng/kg/min. In later studies, Hull and McCracken (1979) described that

intra-carotid PGE_2 initially increased heart rate 5-10%, with increases in blood pressure of up to 30% observed in conscious sheep. Skarnes and McCracken (1980) and Skarnes et al. (1981) showed similar heart rate and blood pressure changes during intra-carotid PGE_2 infusions. Leksell (1976) infused PGE_1 into the lateral ventricular system of conscious goats and observed a 25% increase in blood pressure. Several reports have characterized the heart rate and blood pressure response to central PGE administration in the conscious rat. Hoffman and Schmid (1979), Hoffman and Valigura (1979), Hoffman et al. (1981) and Takahashi and Bunag (1981) showed dose dependent increases in blood pressure (14-26 mmHg) and heart rate (80-100 beats/min) with intracerebroventricular PGE_2 injection. Kondo et al. (1979a and 1979b) and Okuno et al. (1982) showed even larger blood pressure increases (60 mmHg and 30 mmHg, respectively) after intracerebroventricular PGE_2 injection.

Conscious rats react differently from other species in their hemodynamic response to central PGE_2. In conscious rats, a large increase in heart rate was consistently observed after central PGE_2 administration, with some literature reporting changes of 100 beats/min. When expressed as a percentage change from control heart rate, these changes are still larger than the heart rate increases that were observed in conscious dogs, sheep and calves. The blood pressure increases in rats during central PGE_2 administration were approximately of the same magnitude as the blood pressure changes seen in the conscious dogs, sheep and calves. Although a majority of the PGE pressor responses were observed after intracerebroventricular

injection, at least one study reported blood pressure increases when PGE was injected into the carotid artery (Rinchuse and Deuben, 1976).

PGF_{2alpha} has been shown to appear in cerebrospinal fluid after carotid artery injection. At physiological blood pH, prostaglandins are highly ionized and, without a transport mechanism, large amounts would not be expected to cross the blood brain barrier membranes and appear in cerebrospinal fluid after intra-carotid injection. McCracken, Bovaird and Kolb (unpublished observations) have evidence that PGF_{2alpha} crosses into cerebrospinal fluid and that PGF_{2alpha} injected into cerebrospinal fluid appears in jugular venous blood. These experiments showed that PGF_{2alpha} appeared in cerebrospinal fluid within three minutes after intra-carotid injection. A similar delay was observed in the appearence of jugular vein PGF_{2alpha} after cerebrospinal PGF_{2alpha} injection. PGF_{2alpha} and PGE_2 are chemically similar and perhaps cross through the blood brain barrier in similar ways. Prostaglandins could cross into cerebrospinal fluid through porous areas of the blood brain barrier or via a specific transport mechanism, although a specific prostaglandin transport mechanism has not been demonstrated. Therefore, intra-carotid PGE_2 infusion and intracerebroventricular PGE_2 injection may influence blood pressure through similar mechanisms.

The pressor effect observed during intra-carotid PGE_2 infusion was not observed when larger PGE_2 infusion rates were administered via systemic venous or intra-aortic routes in conscious animals. PGE_2 has been shown to be a potent vasodilator when infused into the arterial

supply of peripheral vascular beds (Vane and McGiff, 1975). This PGE_2 vasodilation effect was even observed in the conscious calves during intra-carotid PGE_2 infusion. After the start of the intra-carotid PGE_2 infusion, a marked erythema was observed in the ear and facial tissues during the time period when systemic arterial pressure was increased. This response was always observed ipsilateral to the PGE_2 carotid infusion site. The vasodilation would cease when infusion of intra-carotid PGE_2 was stopped. Thus it appears that PGE_2 dilated the extracalvarial structures supplied by the common carotid artery. In these same conscious calves, intra-aortic PGE_2 infusion significantly decreased blood pressure (15%) and increased heart rate (16%). This depressor effect is again consistent with a peripheral vasodilatory action of PGE_2. The increase in heart rate associated with intra-aortic PGE_2 infusion was double the increase observed during intra-carotid infusion. The heart rate increase during intra-aortic infusion probably results from baroreflex effects as well as a change in the calves' behavioral state. The conscious animals did not tolerate intra-aortic PGE_2 infusion well; they became excited and moved about. In contrast, no changes in behavior were noted during intra-carotid PGE_2 infusions. Hull and McCracken (1979) similarly described that during intra-aortic PGE_2 infusion in sheep blood pressure decreased and heart rate increased.

PGE_2 infused into the aorta should not recirculate and have a central effect, since lung degradation of blood-borne PGE_2 is virtually complete (Piper and Vane, 1970). For the same reason, right atrial infusions of PGE_2 in calves did not alter any of the observed

hemodynamic or behavioral parameters, despite the fact that the dose of PGE_2 infused into the right atrium was forty times the dose necessary to cause a pressor effect when infused into the carotid artery. Neither were hemodynamic responses observed during systemic venous infusion in conscious sheep and dogs. Hull (1975), Hull and McCracken (1979), Skarnes and McCracken (1980) and Skarnes et al. (1981) have also infused similar or larger doses of PGE_2 into the jugular vein of conscious sheep with no hemodynamic effects.

In contrast, conscious rats show a hypotensive response to intravenous PGE_2 administration (Hoffman and Schmid, 1979; Hoffman and Valigura, 1979; Kondo et al., 1979b and Hoffman et al., 1981). Similar hypotensive responses have been observed in anesthetized rats (Rinchuse and Deuben, 1976; Feuerstein et al., 1982 and McQueen and Belmonte, 1974). These hypotensive responses to intravenous PGE_2 suggest that the rat lung does not completely clear PGE_2 from systemic venous blood and that the resulting peripheral vasodepressor response overwhelms the central pressor response. These observations make interpretation of other rat data more complicated, as the central pressor effect may be confounded with a directionally opposite peripherally mediated effect. Data obtained in anesthetized dogs and cats may be similarly confounded since intravenous PGE evoked **pressor** hemodynamic effects in two studies (Gyang et al., 1973 and Lavery et al., 1970). These results suggest that (a) degradation of PGE_2 by the lungs may be compromised in anesthetized animals and (b) the central pressor effect may sometimes dominate the peripheral depressor effect.

If the lungs did not clear PGE_2 from the circulation in conscious animals, a pressor effect might be expected during systemic venous infusion of PGE_2 at 400 ng/kg/min. If lung degradation of blood borne PGE_2 was 90% efficient, sufficient quantities of PGE_2 would exist in the aortic outflow to produce a net pressor effect. This conclusion results from data showing that small depressor effects occur when PGE_2 is infused into the aorta at a dose of 200 ng/kg/min, while a directionally opposite, and much larger, pressor response occurs with intra-carotid PGE_2 infusion at 10 ng/kg/min. In addition it is assumed that 20% of cardiac output goes to the brain. Therefore, the lack of a pressor response during systemic venous PGE_2 infusion at large dose rates indicates virtually complete clearance of PGE_2 by the lungs.

The increase in blood pressure during intra-carotid PGE_2 infusion in the conscious calves was principally caused by an increase in total peripheral resistance. Intra-carotid PGE_2 infusion caused increases in blood pressure (23%), heart rate (5%), cardiac output (2%) and calculated total peripheral resistance (21%). Although the increase in cardiac output was statistically significant, the contribution to the increase in blood pressure from cardiac output is small in comparison to the increase in total peripheral resistance. The technique used to assess cardiac output (electromagnetic flow detection) is capable of resolving small differences in blood flow. When calibrated with a technique of high accuracy (cardiogreen technique), an electromagnetic flow signal has qualities of both precision and accuracy. Only one other report has partitioned the central PGE_2 pressor effect into a cardiac output and a peripheral resistance component. Hull and

McCracken (1979) found no significant alteration in cardiac output during intra-carotid PGE_2 infusion in conscious sheep. Blood pressure was significantly increased, as was calculated total peripheral resistance. The technique used by Hull and McCracken to quantify cardiac output, thermodilution, was not precise enough to resolve changes such as those reported in this dissertation.

Sympathetic stimulation to the heart cannot explain the constant cardiac output observed during intra-carotid PGE_2 infusion. With a 23% increase in blood pressure, the increased afterload would be expected to decrease stroke volume (Burns et al., 1973). However, cardiac output did not fall. Heart rate did not decrease during intra-carotid PGE_2 infusion and in sheep and calves it increased. In calves, the heart rate change was larger than the cardiac output change; therefore calculated stroke volume decreased during intra-carotid PGE_2 infusion. Stroke volume may have decreased due to increased afterload, decreased inotropic state, decreased preload or a combination of these effects. Beta blockade did not alter the PGE_2 pressor effect. Therefore, mechanisms other than inotrophic stimulation are maintaining cardiac output in the face of an increased afterload. A decrease in cardiac parasympathetic tone in the presence of a constant cardiac sympathetic tone would be consistent with the increased heart rate. The increased heart rate would buffer afterload induced reductions in cardiac output. Additionally, Levy (1976) has shown parasympathetic mediation of cardiac contractility. Accordingly, decreased cardiac parasympathetic tone could account for the increased heart rate and the constant cardiac output during intra-carotid PGE_2 infusion.

The increase in vascular resistance during intra-carotid PGE_2 infusion occurred in several vascular beds. Resistance increased in the iliac bed (40%), renal bed (37%) and superior mesenteric bed (30%). Blood flow in each of these beds did not significantly change. Autoregulation could potentially account for the increased resistance with no observed change in blood flow. Kirchheim and Gross (1971) have shown that autoregulation accounted for an increased vascular resistance after bilateral carotid occlusion in conscious dogs. In their study, cardiac output was not increased in the steady state and alpha adrenergic blockade did not attenuate the increased vascular resistance. The authors concluded that cardiac output initially increased and caused a flow induced autoregulation which then buffered the increased cardiac output through an increased afterload. Autoregulation of flow through the measured vascular beds would be consistent with the data reported in this dissertation.

A second explanation for the increased blood pressure during intra-carotid PGE_2 infusion might be that increased regional resistance caused by sympathetic vasoconstriction was the initial perturbation. Increased sympathetic vasoconstrictor tone has been shown to increase blood pressure through increased vascular resistance (Kollai and Koizumi, 1977). These authors showed direct sympathetic nerve recordings and associated blood flow alterations. In the presence of constant blood flow, blood pressure would increase in direct proportion to the increase in resistance. In this dissertation iliac blood flow was generally observed to fall during intra-carotid PGE_2 infusion. The

decrease in iliac blood flow did not reach statistical significance because of response variability. If a decrease in iliac flow occurred and cardiac output did not change, flow had to increase in some vascular bed. Therefore, regional flow may have increased in some beds with autoregulation resulting.

Differentiation between these two mechanisms is difficult due to the concomitent vascular changes. However, since alpha adrenergic blockade reduced the blood pressure during intra-carotid PGE_2 infusion, it is unlikely that autoregulation was occurring.

Because PGE_2 was infused in close proximity to the carotid body, it is necessary to consider the possibility that intra-carotid PGE_2 infusion caused blood pressure to increase via chemoreflex activation. Direct carotid body stimulation has been shown to increase blood pressure and heart rate (Comroe and Schmidt, 1938; Heistad et al., 1976). Stimulation of the carotid body reflex would cause sympathetic adrenergic vasoconstriction, an increase respiratory depth and volume, a decrease in plasma pCO_2 and an increase in plasma pH (ibid; Parker et al., 1975). However, no significant alterations were observed in pO_2, pCO_2 and blood pH in conscious calves and dogs during intra-carotid PGE_2 infusion. The sheep responded to intra-carotid PGE_2 infusion with significant changes in pO_2 and pCO_2. The blood pO_2 decreased and the pCO_2 increased. Each of these changes was directionally opposite to that which would be expected if a chemoreflex was being triggered. These changes can be explained by the excitement often experienced by sheep at the start of an experiment, which may have caused a slight

tachypnea. As the experiment continued and the sheep relaxed, a decrease in pO_2 and an increase in pCO_2 might be expected. No alterations were noted in blood pH during intra-carotid PGE_2 infusion in the sheep. Yet, in each of these species, blood pressure significantly increased during intra-carotid PGE_2 infusion. McQueen and Belmonte (1974) measured carotid body discharge during proximal intra-carotid PGE_2 infusion. No changes in carotid body afferent nerve discharge frequency were observed that were attributable to direct PGE_2 stimulation of the carotid body. Chemoreflex activation causes large increases in respiratory rate and marked alterations in conscious animal behavior (Vatner et al., 1980). No substantial alterations in animal behavior were observed. Therefore, a PGE_2 induced chemoreflex is unlikely.

The hemodynamic response to intra-carotid PGE_2 infusion was characterized by an increase in blood pressure with no alteration or an increase in heart rate. This elevated heart rate in the presence of an increased blood pressure suggested that a "resetting" of the cardiac component of the baroreflex occurred during intra-carotid PGE_2 infusion. The baroreflex is a homeostatic mechanism which operates to minimize or buffer alterations in blood pressure. Cardiac output and total peripheral resistance are the factors influenced by the baroreflex. Cardiac output is altered by heart rate and stroke volume, while peripheral resistance is governed by sympathetic adrenergic nerve activity (Kirchheim, 1976). A typical baroreflex response to a sudden elevation in blood pressure is a slowing of the heart (decreased cardiac output) and decreased sympathetic vasoconstrictor activity.

These two actions serve to buffer the original alteration in blood pressure. The blood pressure resulting after baroreflex compensation is intermediate between the control state and the blood pressure that would have occurred in the absence of the baroreflex. The "baroreflex" studied in this dissertation is the blood pressure and heart rate relationship. Baroreflex mediated alterations in heart rate can result from altered animal behavior, parasympathetic activity, sympathetic activity or some combination of these events. Baroreflex sensitivity was assessed by infusing phenylephrine and measuring the heart rate change associated with the blood pressure change. Intra-carotid PGE_2 infusion did not significantly alter the baroreflex sensitivity in conscious calves, sheep and dogs over the pressure range tested.

The experiments were not intended to partition the baroreflex into autonomic components, but were designed to study the heart rate and blood pressure interrelationship during intra-carotid PGE_2 infusion. Prior reference has been made to the word "resetting". This is an ambiguous term which generally refers to a condition in which blood pressure is increased and heart rate remains at control levels or increases. For example, blood pressure is said to be "reset" in hypertension when the blood pressure is no longer regulated at some normal range, but, instead stabilizes about a higher pressure. Resetting of the baroreflex is said to occur in this situation because heart rate is not decreased even though blood pressure is increased. The term resetting can have other connotations. However, the term resetting will be used here to describe a normal or elevated heart rate in the presence of a blood pressure increase. The observation that the

baroreflex is reset by intra-carotid PGE_2 infusion does not necessarily imply that the sensitivity of the baroreflex has been altered.

No prior literature exists examining baroreflex sensitivity during intra-carotid PGE_2 infusion. Several implications, however, can be made from the data presented.

The baroreflex was quantified by infusing a pressor substance (phenylephrine) which should act only on the peripheral vasculature to increase blood pressure. This technique has several limitations. First, only a small portion of the baroreflex response is quantified. Secondly, although phenylephrine increased blood pressure 20-25 mmHg in both the control period and during intra-carotid PGE_2 infusion, the starting blood pressure in the two situations differed by the amount that intra-carotid PGE_2 increased blood pressure over the control period. Thus, baroreflex sensitivity was not being measured at the same blood pressure level. Lastly, it remains possible that the ability of the baroreflex to control blood pressure is functioning normally and only the cardiac limb (heart rate) of the baroreflex is affected by PGE_2 infusion.

Despite the limitations implicit in measuring the baroreflex response as was done in this dissertation, there are several strengths in the method. The response of the baroreflex is quantified during a hemodynamic steady state in conscious, unstressed animals. Baroreflex slowing of the heart is affected by both divisions of the autonomic nervous system; the sympathetic division requires approximately 4-6

seconds to act (Kezdi and Geller, 1968). The reproducibility of the animal data allows the small blood pressure changes to be statistically interpretable. These small increments in blood pressure do not saturate the baroreflex response. Larger alterations in blood pressure would allow a larger portion of the blood pressure-heart rate relationship to be quantified. However, because the relationship follows a sigmoidal curve, larger changes in blood pressure might saturate the baroreflex.

Although intra-carotid PGE_2 and angiotensin II infusion had similar effects on blood pressure and heart rate, the baroreflex sensitivities were dramatically different. In the same species and using the same technique, baroreflex sensitivity was decreased during angiotensin II infusion. This observation is consistent with the results of Ismay et al. (1979) and Lee and Lumbers (1981). One explanation for the decreased sensitivity observed during angiotensin II infusion is that intra-carotid angiotensin increased blood pressure to a higher range than intra-carotid PGE_2. As with the PGE_2 baroreflex sensitivity only limited conclusions can be drawn from the angiotensin sensitivity results. Regardless of these interpretations, PGE_2 and angiotensin II affect baroreflex sensitivity in different ways.

Ruminants do not have a bifurcation of the carotid artery with a internal carotid artery (Baldwin and Bell, (1963a,b). Therefore, it was appropriate to confirm that a substance would access brain tissue after being injected into the carotid artery. Examination of the extirpated sheep brain showed that carotid blood does perfuse brain

tissue as shown by extensive dye staining of one lateral cerebral hemisphere. Extracalvarial structures also were stained, indicating that carotid blood does not exclusively perfuse the brain. Dye staining in the brain and in extracalvarial tissues always appeared on the side of the animal where the dye was injected. In the sheep the circle of Willis is formed by the junction of the basilar artery and an internal carotid artery from each side of the animal. The internal carotid artery arises from a carotid rete which is supplied by the arteria anastomotica which in turn arise from the common carotid artery (ibid.). Therefore, a functional vascular conduit delivers common carotid blood to the brain of sheep.

The observation that the intra-carotid PGE_2 pressor effect is blocked by alpha adrenergic blockers is consistent with reports in the literature. Carlson and Oro (1966) observed that pretreatment with a ganglionic blocker would prevent the subsequent pressure increase after intra-carotid PGE_1 infusion in pentobarbital anesthetized dogs. Kaplan et al. (1969) similarly showed that the intra-carotid pressor effects of PGE_1 were blocked by hexamethonium. These two reports indicate that total adrenergic blockade would reduce the blood pressure effects, but did not identify the specific mediation of the response. Hoffman and Schmidt (1979) and Hoffman and Valigure (1979) specifically tested for alpha and beta adrenergic blockade effects during central PGE_2 pressor administration in conscious rats. They observed that phenoxybenzamine blocked the pressor effect and that propranolol blocked the associated heart rate increase, but not the blood pressure increase. These two reports suggest that, in the conscious rat, the increase in blood

pressure associated with intracerebroventricular PGE_2 injection is not caused by an increased heart rate (chronotrophic effect), but rather by an increase in peripheral vascular adrenergic activity. Okuno et al. (1982) similarly observed that pretreatment with phenoxybenzamine would attenuate the pressor effect of central PGE_2 administration.

Takahashi and Bunag (1981) have measured sympathetic nerve discharge (coeliac ganglion) in anesthetized rats after intracerebroventricular PGE_2 injection. They observed increases in blood pressure, heart rate and sympathetic nerve discharge after central PGE_2 administration. These authors also showed that a residual pressor effect was still apparent after removal of sympathetic vasomotor tone by cervical spinal cord section. They concluded that an initial increase in sympathetic adrenergic tone elevated blood pressure, but that the increase was sustained by a later release of a humoral pressor substance. Okuno et al. (1982) also suggested alternative pressor mechanisms in the mediation of the central PGE_2 pressor response. In these experiments plasma renin and catecholamines were quantified after cerebroventricular PGE_2 injection. Associated with the blood pressure increase was a 400% increase in plasma epineprine, a 600% increase in plasma norepinephrine and a 600% increase in plasma renin activity. In addition, pretreatment with an argine vasopressin antagonist attenuated the PGE_2 induced pressor response. Feuerstein et al. (1982) similarly showed increases in plasma catecholamines after central PGE_2 injection in anesthetized rats. By comparing the relative increases in epinephrine and norepinephrine, the authors concluded that adrenomedullary activation

was not the cause of the increased catecholamines and that activation of the sympathetic nervous system was occurring. This dissertation showed alpha adrenergic but not beta adrenergic involvement in the PGE_2 pressor response.

Plasma renin activity was observed to increase during intra-carotid PGE_2 infusion in conscious calves and sheep. The increase in plasma renin activity was not large, but could be involved in the pressor response since the larger pressor effects were observed together with larger plasma renin changes. The calves showed larger pressor responses and plasma renin increases, while the sheep responded with smaller pressor and renin changes. These changes in plasma renin activity could be species specific, with the larger increases associated with calves having no specific relation to the PGE_2 induced pressure increase. However, when incrementally increasing intra-carotid PGE_2 infusions were performed, the plasma renin activity generally increased at each infusion level along with the blood pressure increases. There were differences in the responses of blood pressure and plasma renin activity to increasing doses of PGE_2. At each incremental PGE_2 infusion rate, blood pressure was observed to increase consistently, while the plasma renin activity, although increased, appeared to plateau at intermediate infusion rates. These interpretations are complicated for a number of reasons. Increased sympathetic activity causes renin release at the kidney (Seymour, 1981). However, a renal arterial blood pressure increase causes decreased renal renin release (Fray, 1978). These two opposing signals for renin release could result in small renin changes that have

negligible vasoconstrictive effects. Secondly, plasma renin activity is an index for the generation of angiotensin II. The plasma renin assay measures angiotensin I levels which are converted by kinase II to angiotensin II. Although angiotensin I has vasoconstrictive properties, angiotensin II is more potent and might function to increase blood pressure during these experiments. Therefore, this assay does not allow us to measure the hormone (angiotensin II) of interest.

The effects of captopril also support an involvement of the renin-angiotensin system in the PGE_2 pressor response. Captopril prevents conversion of angiotensin I to angiotensin II by inhibiting converting enzyme (kinase II) (Ondetti et al., 1977). Captopril injection attenuated the intra-carotid PGE_2 pressor response. This observation is consistent with a measured small increase in plasma renin activity during intra-carotid PGE_2 infusion. At larger intra-carotid PGE_2 infusion rates (200 ng/kg/min) the plasma renin increase although statistically significant, was not markedly different than the increase observed during lower intra-carotid PGE_2 infusion rates. However, a higher blood pressure (20 mmHg) was observed at the higher PGE_2 infusion rate than at 10 ng/kg/min. Although plasma renin activity was shown to have some correlation with the blood pressure increase caused by intra-carotid PGE_2 infusion, this does not imply dependence.

Blockade of the converting enzyme may have effects other than blockade of the renin-angiotensin system. Converting enzyme is also

responsible for the degradation of bradykinin. Bradykinin has been shown to have cardiovascular effects and, in particular, a central pressor effect has been demonstrated (Kondo et al., 1979a). However, since captopril injection caused a reduction in the PGE_2 pressor response, it is unlikely that bradykinin caused a diminished central effect. Bradykinin does have peripheral vasodilator effects (Kondo et al., 1979b) and, if bradykinin degradation was reduced, increased bradykinin may have had an increased peripheral vasodilator effect which buffered the centrally mediated PGE_2 pressor effect. The results of this dissertation cannot distinguish between these possibilities.

For PGE_2 to have a functional role in blood pressure control an endogenous synthesis and release mechanism must be identified. PGE was first identified in the brain of the ox by Samuelsson (1964) and more recently in other species (Wolfe, 1979). PGE has also been identified in sheep lungs (Anggard and Samuelsson, 1963) and PGE synthesis has been shown to occur in the guinea pig lung (Anggard, 1965). Prostaglandins, and particularly PGE, have been shown to be released by polymorphonuclear leukocytes and macrophages (Davidson et al., 1980) and also during inflammatory responses (Zurier and Sayadoff, 1975). More recently, the spontaneous release of PGE_2 has been reported in the dog brain (Holmes, 1970) and a similar release of PGF_{2alpha} has been shown in sheep cerebrospinal fluid (McCracken et al., 1979).

A central brain prostaglandin mechanism has been implicated in behavior (Holmes and Horton, 1968), thermoregulation (Milton and Wendlandt, 1971), volume homeostasis (Anderson and Leksell, 1975) and

food intake (Wolfe and Coceani, 1979). It has been proposed that many of these changes are mediated through intermediate modulation of central cyclic nucleotides at hypothalamic sites (Malik, 1978). Avanzino et al. (1966) have demonstrated that PGE_1 has a specific excitatory effect on brain stem neurons.

Prostaglandins have been implicated in the regulation of blood pressure (McGiff and Vane, 1975; Dusting et al., 1979). It was proposed that prostaglandin synthesis might regulate vascular resistance. Prostaglandins of the E series were proposed to control vasodilatation and F series were thought to cause vasoconstriction. An alteration in the synthesis or degradation of either "regulatory prostaglandin" would then upset the prostaglandin pressure regulatory system with hypotension or hypertension resulting. Intrarenal infusion of PGE_2 has been shown to alter kidney function, water balance and blood pressure (Hockel and Cowley, 1979).

The intravenous injection of non-lethal amounts of bacterial endotoxin have been shown to increase PGF and PGE concentration in the conscious calf and sheep. Anderson et al. (1975) injected endotoxin into conscious calves and showed increased PGF in the pulmonary vein which was coincident with substantial increases in pulmonary artery pressure. A similar endotoxin injection into conscious sheep causes increases in PGE and PGF in jugular and carotid blood (Skarnes and McCracken, 1980; Skarnes et al., 1981). In this situation either the lung and/or blood vessels generate PGE or lung inactivation of PGE is compromised. Administration of indomethacin prior to the endotoxin

administration resulted in no hemodynamic changes and no increases in vascular PGE.

PGE_2 may function in pathologic situations to support blood pressure. During endotoxin shock, blood pressure is reduced and plasma PGE is increased (Reichard and Fletcher, 1981; Cowley and Trump, 1982). It is possible that the centrally mediated pressor effect helps to offset the direct peripheral vasodilatation caused by PGE_2. In hemorrhagic shock, endotoxin is released from the gut and is not cleared by the liver. In this situation, secondary generation of PGE may also support blood pressure through a central mechanism.

The pressor response observed during intra-carotid PGE_2 infusion may be part of a thermoregulatory response. A considerable literature has developed in the last decade to implicate PGE in the thermoregulatory process (Cranston, 1979; Wolfe and Coceani, 1979). PGE has been shown to alter sodium and calcium concentrations in the preoptic anterior hypothalamic thermoregulatory area (Cranston, 1979). Coincident with mechanisms to generate heat (shivering), it is possible that central PGE_2 also causes a sympathetically mediated vasoconstriction to reduce heat loss. However, Hoffman and Valigura (1979) have shown that the central PGE_2 pressor and febrile effects are separate. Alpha adrenergic blockade prevented the pressor effect but not the febrile response.

PGE_2 may also mediate volume regulation. Several investigators have shown data consistent with this hypothesis. Vilhard and Hedqvist

(1970) showed vasopressin responses in rats during central PGE_2 administration. Anderson and Leksell (1975) showed increased thirst, vasopressin release and naturesis in conscious goats after central PGE_1 infusion. In their study no blood pressure increase was reported. Leksell (1976) reported pressor responses to centrally infused PGE_1, together with thirst, vasopressin release and naturesis. Okuno et al. (1982) has shown that central PGE_2 increases blood pressure and vasopressin in the conscious rat. Presuming that the major central effect of PGE_2 was to control sodium excretion, these demonstrated changes favor naturesis. Increased blood pressure will increase glomerular filtration and, if urinary volume is increased, a volume stimulus may trigger drinking. Vasopressin release will increase blood pressure through a direct effect on the peripheral vasculature and will concentrate the urine.

Conclusions

1). Intra-carotid PGE_2 infusion increases blood pressure in conscious sheep, calves and dogs. The blood pressure increase averages between 17-32% and is consistently observed.

2). Systemic venous infusion of much larger amounts of PGE_2 caused no observable hemodynamic effects. Aortic PGE_2 infusions caused hypotension in the calves.

3). Blood pressure is increased through alpha adrenergicially mediated increases in vascular resistance. Cardiac output is unchanged.

4). Chemoreflex activation is not involved in the central PGE_2 pressor response.

5). Both intra-carotid PGE_2 and angiotensin II reset the baroreflex. The baroreflex sensitivity during intra-carotid PGE_2 infusion is unchanged. Intra-carotid angiotensin II infusion substantially decreases baroreflex sensitivity.

6). Intra-carotid PGE_2 did not increase blood pressure in calves initially anesthetized with a barbitureate and maintained on halothane anesthesia. Similarly, no PGE_2 pressor response was observed in chloralose/urethane anesthetized sheep.

7). The carotid artery supplies blood to sheep brains.

8.) Beta adrenergic blockade did not prevent the intra-carotid PGE_2 pressor response. Alpha adrenergic blockade eliminated the pressor response.

9.) Plasma renin activity exerts a small role in the PGE_2 pressor response. The blood pressure and plasma renin responses during increasing PGE_2 infusions show substantial differences. Blood pressure increases with larger PGE_2 infusions; plasma renin activity does not.

10. Blockade of the renin angiotensin system attenuates the PGE_2 pressor response.

APPENDICES

APPENDIX A

Unpublished Preliminary Data

Figure 21 shows mean arterial blood pressure in conscious sheep during a control period, during intra-carotid PGE_2 infusion (1.9-190 ng/kg/min) and during a post infusion control period. Each point shows the mean and SEM for five sheep. Arterial pressure was unchanged at the lowest infusion rate and increased 30% at the highest infusion rate. Heart rate was generally elevated 5-10% immediately after the start of the PGE_2 infusion and thereafter was not significantly different from control.

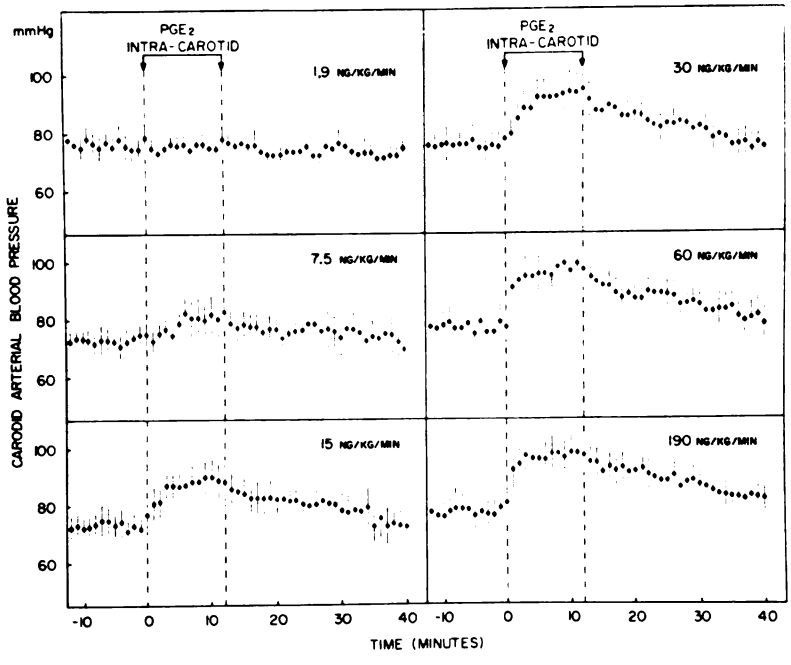

Figure 21: Blood pressure in five conscious sheep during a control period and during intra-carotid PGE_2 infusion (1.9-190 ng/kg/min).

Figure 22 shows blood pressure responses from one conscious sheep during jugular PGE_2 infusions (1500 ng/kg/min) and during intra-carotid PGE_2 infusions (15 ng/kg/min). As shown in Figure 21 intra-carotid PGE_2 increased blood pressure; typically the blood pressure increase during intra-carotid PGE_2 infusion was considerably faster than the blood pressure decay after termination of the PGE_2 infusion. Jugular PGE_2 infusion at 100 times the intra-carotid dose did not alter blood pressure in this or the four other sheep. This is consistent with lung degradation of PGE_2.

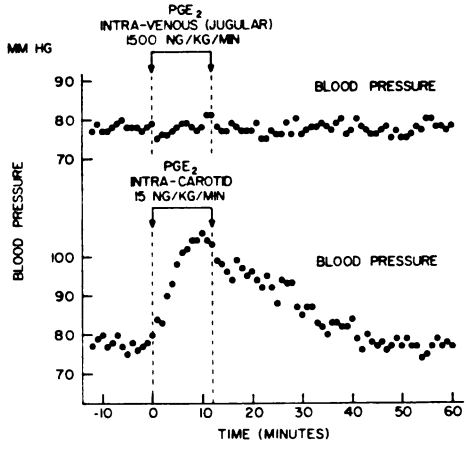

Figure 22: Blood pressure in a conscious sheep during jugular PGE_2 infusion (1500 ng/kg/min) and during intra-carotid PGE_2 infusion (15 ng/kg/min).

Figure 23 shows blood pressure, heart rate and cardiac output in a conscious sheep during a control period and during intra-carotid PGE_2 infusion (15 ng/kg/min). Cardiac output was measured with a thermodilution technique (right heart, Swan-Ganz). In this and other sheep, intra-carotid PGE_2 infusion increased blood pressure without a significant alteration in cardiac output. Therefore, calculated total

peripheral resistance increased.

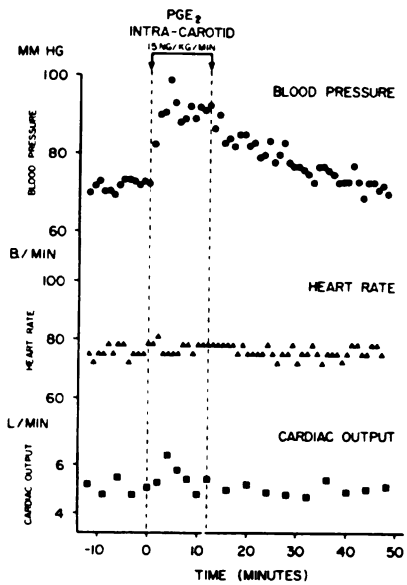

Figure 23: Blood pressure, heart rate and cardiac output in a conscious sheep during intra-carotid PGE_2 infusion (15 ng/kg/min).

APPENDIX B

Frequency Response of Catheter-Manometer Systems

The frequency response of a catheter-manometer system is determined by several factors. The response characteristics of modern blood pressure transducers far exceed that needed to faithfully reproduce a phasic blood pressure pulse at physiologic frequencies. However, it is usually difficult or innappropriate to introduce the transducer itself into the system to be measured and catheters are utilized to allow convenient blood pressure measurement. It is the catheter characteristics that introduce non-linear functions and serve to distort the phasic blood pressure. Therefore the transducer will be treated as an ideal transducer and catheter properties will be addressed.

A natural or resonant frequency can be calculated for a catheter of known elasticity, length and width which is filled with saline. This frequency can be used to indicate the largest frequency that can be faithfully reproduced. Several other factors affect the resonant frequency. The damping or viscous frictional characteristics of the fluid within the catheter will critically affect the frequency response, particularly at the resonant frequency. With an underdamped catheter (highly compliant) a phenomenon called ringing may be apparent. This undesirable oscillation occurs in the system after the original

perturbation has passed. Another manifestation of an underdamped system is a widened pulse pressure. An underdamped catheter can also show very slow output responses to high frequency input signals. A catheter with a narrow lumen will show underdamped characteristics when tested. An ideal catheter has the property of critical damping, responding to an impulse rapidly, with no ringing occurring.

The method used to calculate catheter frequency response in this thesis is often called the "pop test" (McDonald, 1960; Bergel, 1972). Using this technique, a small balloon (tip of surgical glove) is attached to the end of the catheter to be tested. The balloon is expanded with water almost to the bursting point. A blood pressure transducer is connected to the other end of the catheter. The recording system is calibrated such that full scale pen diplacement equals 100 mmHg with the zero pressure setting placed at mid channel. While operating the recording system with mimimal filtering and at a high paper speed (100 mm/sec), the balloon is popped (sharp needle) while the catheter is held as still as possible. The recording system transcribes a series of exponentially decaying waves of a common frequency. By calculating the time between pressure peaks and expressing the wavelength as a frequency, a measure of the resonant frequency is determined.

Mathematically, a step function is delivered to the blood pressure transducer when the balloon is popped. A step function

can be mathematically approximated as an infinite series of increasing frequency sine waves of varying amplitudes and phases. The catheter will not pass the total step function and has a transfer characteristic that will pass sine waves up to the catheter cut-off frequency. At the cut-off frequency the catheter will attenuate higher frequency signals. The cut-off frequency, as measured, is very close to the resonant frequency. By measuring the highest frequency that will pass through the catheter, some measure of the transfer characteristics below that frequency can be assumed. The catheter is considered to have a "flat" frequency response from 0 hz (steady pressures) up to the resonant frequency.

Through the use of fourier analysis it can be shown that a blood pressure pulse can be adequately reproduced by eight to ten harmonics from the fundamental periodic frequency (Bergel, 1972). If an animal has a resting heart rate of 60 beats/min (1 hz), the eighth and tenth harmonics occur at 8 and 10 hz. In order to faithfully reproduce the phasic blood pressure pulse, the frequency response of every element within the catheter to polygraph system must be larger than 8-10 hz. The system response is composed of the catheter, transducer, electronic amplifiers and physical transcription system. In this study the polygraphs (Gould-Brush and Grass) both have a system response greater than 70 hz. The transducer-catheter system is the limiting element. All catheters had a measured frequency response of 15 hz or more. This means that phasic pressures are

faithfully reproduced up to fundamental frequencies of 1.5 - 2 hz or heart rates of 90-120 beats/min. Since mean pressure is calculated from phasic pressures, the phasic blood pressure signal should be as close as possible to the actual animal blood pressure. Blood pressures were measured when heart rate was greater than 120 beats/min. This will introduce more signal distortion than would be observed at lower heart rates. However, since the fidelity characteristics are slowly attenuated at higher frequencies, this does not severely distort the pressure signal.

APPENDIX C

Miscellaneous and Preliminary Observations

A. During the intra-carotid PGE_2 infusions (10 ng/kg/min) shown in figure 1, body temperature was measured in 2 sheep and 2 calves calves. Both species of animals showed a small rise in body temperature (0.2 - 0.8 ^{o}C.) compared to the control period. The sheep temperature elevation was smaller than the calf temperature response. These results are in accord with previous work which indicated that intra-carotid PGE_2 is involved in the febrile process. Hull (1975) showed that intra-carotid PGE_2 infusion increased body temperature in conscious sheep and that the minimum effective dose for this temperature increase was 40 ng/kg/min. At PGE_2 infusion rates above 40 ng/kg/min, animal temperature increased 1 to 2 degrees celcius.

B. Intra-carotid injection or infusion of adenosine (1-1000 ng/kg/min), PGI_2 (10-500 ng/kg/min), nitroglycerine (1-4 ug/kg/min), serotonin (1-160 ng/kg/min), and histamine (1-640 ng/kg/min) failed to increase blood pressure in conscious calves or sheep. In each case, the infusion or injection series was halted when blood pressure decreased or animal behavior changed. Adenosine elicited a vasodilatation of the distal tissues with a marked erythema of the extracalvarial structures. Histamine and

serotonin similarly caused an erythema however, a marked swelling appeared on the infusion side. PGI_2 caused an increase in blood pressure in one calf, but the response could not be duplicated in other calves and sheep. In all cases, no hemodynamic similarity was observed with these vasodilators when compared to the intra-carotid PGE_2 hemodynamic response.

Intra-carotid vasopression infusion (.01 Pressor Unit/kg/min) increased blood pressure 17% and decreased heart rate 67% in one conscious sheep. This hemodynamic response is quite different from the intra-carotid PGE_2 hemodynamic response where heart rate was observed to increase. This experiment was prompted by several reports that vasopressin might be involved in the intra-carotid PGE_2 pressor response. It is suggested that further work attempt blockade of vasopressin receptors during intra-carotid PGE_2 infusions.

C. Intra-carotid bradykinin at doses between 5 and 40 ng/kg/min increased blood pressure and heart rate in two conscious sheep and one conscious calf. The hemodynamic response was qualitativly and quantitatively similar to the intra-carotid PGE_2 response. Intra-carotid bradykinin increased blood pressure and heart rate 12-17% and 3-4% respectively in two conscious sheep. In these sheep, intravenous injection of indomethacin (5 mg/kg) generally decreased blood pressure and increased heart rate when compared to control. After

indomethacin injection, intra-carotid bradykinin did not alter blood pressure although heart rate increased (9-28%). After terminating the bradykinin infusion intra-carotid PGE_2 was infused (10 ng/kg/min) which resulted in increased blood pressure and heart rate 7-21% and 15-19% respectively. Therefore, although intra-carotid bradykinin and PGE_2 have similar hemodynamic effects, the pressor response to bradykinin is blocked by indomethacin. Literature supports intermediate synthesis of PGE from the kidney after perfusion with bradykinin (McGiff et al., 1972).

D. Intra-carotid saralysin administration attenuated the intra-carotid angiotensin II response, but did not alter the intra-carotid PGE_2 pressor response. Several different protocols were observed. In a conscious sheep intra-carotid PGE_2 was infused (15 ng/kg/min) and blood pressure increased from 75-105 mmHg. After stopping the PGE_2 infusion, a bolus of angiotensin (10 ug) was injected and blood pressure increased (80-145 mmHg). Saralysin was infused (1 ug/kg/min) for 30 minutes and another angiotensin II bolus (10 ug) was given and blood pressure increased (85-105 mmHg). During the continuing saralysin infusion, intra-carotid PGE_2 (15 ng/kg/min) was infused and blood pressure increased (85-105 mmHg). In this sheep the hemodynamic response to angiotensin II was substantialy reduced by saralysin (65 mmHg vs 20 mmHg increase) and the intracarotid PGE_2 pressor response was not altered.

In another sheep, the pressor influence of intra-carotid angiotensin II infusion (10 ng/kg/min) was attenuated by saralysin infusion (150 ng/kg/min) and the pressor response to intra-carotid PGE_2 remained evident.

In a third sheep, saralysin (10 ug/min) attenuated the pressor effect of intra-carotid angiotensin II, and intra-carotid PGE_2 (30 ng/kg/min) increased blood pressure.

E. Intra-carotid PGE_2 (10 ng/kg/min) was infused prior to and after intravenous nalaxone injection. Prior to nalaxone, intra-carotid PGE_2 infusion increased blood pressure (55-75 mmHg). Nalaxone (100 ug/kg) injection caused no discernible changes in blood pressure or heart rate. Within 3 minutes of the nalaxone injection, the intra-carotid PGE_2 infusion was restarted and blood pressure increased (55-75 mmHg). Therefore, in this sheep intravenous nalaxone did not alter the intra-carotid PGE_2 pressor response. This experiment was prompted by a suggestion from Dr. Bertrum Pitt that the intra-carotid PGE_2 pressor response might be mediated through central opiate receptors. Dr. Pitt suggested the dose of nalaxone 100 ug/kg, as it markedly attenuates the hemodynamic effects of exogenously administered opiates in conscious dogs in his lab.

F. Baroreflex sensitivity was determined in two calves during an intravenous nitroglycerine infusion (4 ug/kg/min). In these animals the heart rate responses to decreases in blood pressure were plotted and quantified. These experiments were done in order to quantify the baroreflex sensitivity over a wider blood pressure range. This aspect of baroreflex sensitivity measurement was abandoned because of the excitement created when the vasodilator was infused. The calves would strain vigorously against the halter. Blood pressure and heart rate observations became very difficult to determine and were not reproducible. This in in contrast to the reproducibility of the results and the calm behavior of the animals when baroreflex sensitivities were determined with phenylephrine.

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