EFFECTS OF SEROTONIN (5-HYDROXYTRYPTAMINE) ON MUSCLE VASCULAR RESISTANCE

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ABSTRACT

EFFECTS OF SEROTONIN (5-HYDROXYTRYPTAMINE)
ON MUSCLE VASCULAR RESISTANCE

By

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Knowledge about the cardiovascular effects of serotonin in some of the vascular beds has grown in recent years, but the effects of serotonin (5-hydroxytryptamine) on vascular resistance in the muscle bed have not yet been established. Therefore, serotonin was infused at six different rates into isolated, collateral-free, innervated and denervated gracilis muscles of dogs. Vascular resistance was artificially raised in muscles with low initial resistance and artificially lowered in muscles with high initial resistance. Both natural flow and constant flow experiments were conducted.

Serotonin was found to consistently lower the total vascular resistance in initially high and artificially high resistance muscles. Serotonin consistently raised total vascular resistance in initially low and artificially low resistance muscles.

The decrease in resistance caused by serotonin in muscles with high initial resistance could be reversed to an increase in resistance when the resistance was subsequently lowered by metabolically induced vasodilation. This implies that the muscle's response to serotonin does not depend upon neurogenic tone, but rather upon the level of total muscle vascular resistance at the time serotonin is infused.

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To my parents, Mr. and Mrs. Alexander Meier

and to my wife, Jan

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INTRODUCTION

Serotonin is synthesized in the brain (White, Handler and Smith, 1964) and intestines (Humphrey and Toh, 1954) from tryptophan (Stacey, 1959; Gailitis and Sheiber, 1960; Enerback, 1965; Warner, 1967). It is distributed throughout the body, primarily by the platelets (Stacey, 1959; Whelan, 1959; Marshall, 1966).

Serotonin's effects within the circulatory system are many and varied. It cannot be classified as either a pressor or depressor agent, since it may elevate, lower, or not affect blood pressure (Haddy, 1960). Many differences in vascular response to serotonin seem to be correlated with differences in pre-existing levels of neurogenic tone (Page, 1952, 1953; Page and McCubbin, 1956; Haddy, 1960; Garattini and Valzelli, 1965; Haddy and Scott, 1966; Emerson *et al.*, 1968). Chemoreceptor stimulation also plays a role in vascular responses to serotonin (Page, 1952; McCubbin, Green and Salmoiraghi, 1956; Braun and Stern, 1961; Woolley and Shaw, 1962; Hamberger, Ritzen and Wersall, 1966). Serotonin causes a variety of responses in the different vascular beds (Takacs and Vajda, 1963; Garattini and Valzelli, 1965), including opposite effects in parallel skin and muscle beds (Daugherty *et al.*,1968; Emerson *et al.*, 1968).

REVIEW OF LITERATURE

A REVIEW OF SEROTONIN AND ITS EFFECTS IN THE CARDIOVASCULAR SYSTEM

I. INTRODUCTION.

A serum vasoconstrictor agent was known to exist over a hundred years ago (Heinzelman and Weisblat, 1951; Spies and Stone, 1952). But until 1948, Scientists were unable to isolate and study this vasoconstrictor agent, which later became known as "serotonin" (Rapport, Page and Green, 1948; Rapport, 1949; Heinzelman and Weisblat, 1951; Reid, 1952).

II. DISCOVERY AND EARLY DEVELOPMENT.

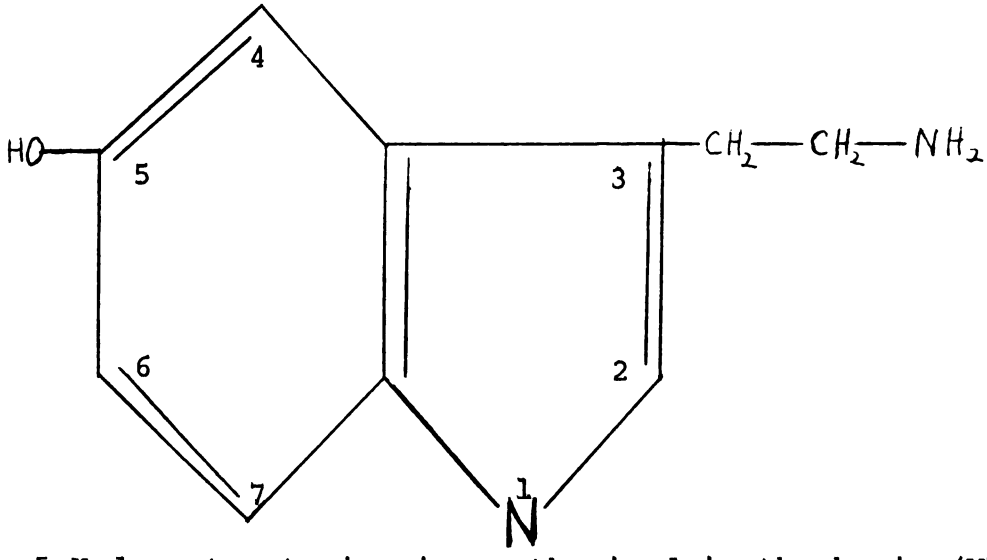
The existence of a potent vasoconstrictor agent in the sera of mammals was reported as early as 1868, by Ludwig and Schmidt, two German medical doctors (Heinzelman and Weisblat, 1951; Spies and Stone, 1952). Rapport, in 1948, described this agent as a "vasoconstrictor which is present in serum and defibrinated blood and which appears in connection with platelet destruction and the clotting process (Rapport, Page and Green, 1948)." Rapport was first to isolate this agent when he made a stable preparation of it in the form of a nitro-barbiturate complex. After further purification, it was shown on chemical grounds to be

an indole derivative, 5-hydroxytryptamine (Rapport, 1949; Heinzelman and Weisblat, 1951). Rapport and his associates injected the substance intravenously into a dog and found that it increased arterial pressure, and then they decided, "We should like provisionally to name it 'serotonin', which indicates that its source is serum and its activity is one of causing constriction (Rapport, Page and Green, 1948)." 5-Hydroxytryptamine was first synthesized in August, 1951, by Hamlin and Fischer (1951). At about the same time Heinzelman and Weisblat (1951) also synthesized 5-hydroxytryptamine from 5-benzyloxyindole and converted it to the creatinine sulfate salt. Shortly thereafter, Reid and Rand (1951) prepared serotonin from beef serum and ran a series of experiments with it in an assortment of animals and found that serotonin caused a rise in pulmonary and systemic arterial pressures in the cat; contraction of isolated arteries of sheep, dogs and oxen; constriction of the guinea pig jejunum and uterus, rat uterus, nictitating membrane and pupillary sphincter of the cat; broncho-constriction of the vessels of the hind limb and kidney of the cat (Reid and Rand, 1951). In 1952, Stone and Spies (1952) injected 0.25-0.50 mg of serotonin into patients and noticed a sharp rise in systolic and diastolic blood pressures.

In the ensuing years, interest and research in the area of serotonin mushroomed rapidly.

III. STRUCTURE AND METABOLISM OF SEROTONIN.

Serotonin (5-hydroxytryptamine) has the following structural formula (White, Handler and Smith, 1964):



5-Hydroxytryptamine is synthesized in the brain (White, Handler and Smith, 1964) and in the intestines (Humphrey and Toh, 1954). In vivo, tryptophan is hydroxylated to 5-hydroxytryptophan, apparently by phenylalanine hydroxylase, and then decarboxylated by the enzyme 5-hydroxytrytophan decarboxylase to yield 5-hydroxy-tryptamine, or serotonin (Stacey, 1959; Gailitis and Sheiber, 1960; Enerback, 1965; Warner, 1967). Serotonin is converted by mono-amine oxidase to an inactive compound called 5-hydroxy-3-indolacetic acid, which is excreted in urine at 2-7 mg/day in man

(Borges and Bessman, 1957; Gailitis and Sheiber, 1960; White, Handler and Smith, 1964). It has been estimated that about three per cent of dietary tryptophan is metabolized via this pathway (White, Handler and Smith, 1964). Alcohol inhibits serotonin metabolism by depressing its biotransformation via oxidation and conjugation (Rosenfeld, 1960). Gursey and Olson (1960) have presented evidence which indicates that intravenous injection of 2 g/kg of ethanol results in decreased serotonin and norepinephrine levels in rabbit brain stems. Tryptophan-deficient rats also showed a significant decrease in serotonin content in all tissues analyzed (Gal and Drewes, 1962). Moran, Uvnas and Westerholm (1962) report that allicin, an SH group inhibitor, and ninhydrin, an NH_2 group inhibitor, also intervene with serotonin metabolism.

IV. LOCATION OF SEROTONIN IN THE BODY.

Vast amounts of serotonin are found throughout the brain and central nervous system (Woolley and Shaw, 1954; Borges and Bessman, 1957; Stacey, 1959; Woolley, 1960; Burton, 1966; Marchbanks, 1966), where it may play a role in mental processes and nervous transmission (Woolley and Shaw, 1954; Whelan, 1959; Woolley, 1960). Serotonin has also been found throughout the gastrointestinal tract (Reid and Rand, 1951; Rosenberg,

1965; Davenport, 1966). Serotonin has been found in the uterus and placenta (Reid and Rand, 1951; Klinge, Penttila and Tissari, 1964), where it is reported to increase in concentration during pregnancy (Koren, Pfeifer and Sulman, 1965), and also in the spleen (Sanker et al., 1961), pancreas (Tobe, Fujiwara and Tanaka, 1966), kidney (Whelan, 1959), adrenal medulla (Stacey, 1959), and the pineal gland (Giarman and Freedman, 1960). Snyder, Zweig and Axelrod (1964) reported that a major portion of the serotonin in mammalian pineal glands is stored in sympathetic nerve endings. They also reported that a circadian rhythm in serotonin content of the rat pineal gland exists with its peak at noon and trough at about 10 PM, and that it is abolished by removal of the superior cervical ganglia.

V. CARDIOVASCULAR-RELATED ASPECTS OF SEROTONIN.

A. Variability of vascular responses to serotonin.

The circulatory effects of serotonin are variable, depending not only on the species of animals and dose given, but also on variations in the same species under similar conditions (Schneider, 1954; Page and McCubbin, 1956; Kabins, Molina and Katz, 1959; Goodman, 1965). Haddy (1960) stated that "serotonin cannot be classified either as a pressor or depressor agent. Serotonin may

lower, elevate or not affect blood pressure." Serotonin's effects upon blood pressure are so variable that Page and McCubbin (1956) created a new word, "amphibaric," to describe them. Haddy and Scott (1966) called the direct effects of serotonin on arterial and venous resistances "unusual."

B. Transport of serotonin in the blood stream.

DISTRIBUTION IN THE BLOOD

Over half of the blood's supply of serotonin is contained in the platelets (Stacey, 1959; Whelan, 1959; Marshall, 1966). In rabbit platelets, serotonin appears to be bound to the granule fraction, and not to the platelet membrane (Wurzel, Marcus and Zweifach, 1965). Serotonin also is localized in mast cells, either through synthesis or specific uptake (Stacey, 1959; Moran, Uvnas and Westerholm, 1962; Enerback, 1963, 1965). Some serotonin also exists in the plasma (Robertson and Andrews, 1961) and serum (Davis, 1959).

RELEASE FROM MAST CELLS

The release of serotonin from mast cells is believed to be enzymatic and dependent upon pH and temperature (Moran, Uvnas and Westerholm, 1962). Allicin, an SH group inhibitor, and ninhydrin, an NH_2 group inhibitor, block serotonin release from mast cells, but the injection of reserpine facilitates the release of serotonin until only 65% of the normal

concentration remains (Moran, Uvnas and Westerholm, 1962). Cold exposure results in a marked increase in the number of mast cells in the abdominal skin, accompanied by the rapid and continuous excretion of serotonin in the urine (Leblanc, 1963). When mast cells are disrupted *in vitro*, serotonin is released along with histamine, whose concentration in mast cells is about twenty times greater than that of serotonin (Archer, 1961).

ABSORPTION OF SEROTONIN BY PLATELETS

Zucker and Borrelli (1956) found that normal disc-shaped platelets readily absorbed serotonin. Weissbach and Redfield (1960) studied factors affecting the uptake of serotonin by human platelets in an inorganic medium and found that potassium and phosphate are required for maximum uptake of serotonin at a pH of 5.7. At higher pH values, these ions had much less effect. Serotonin is taken up actively by platelets against a concentration gradient of several hundred to one, and its uptake is energy- and temperature-dependent (Burningham *et al.*, 1966). An active transport system at the platelet surface is apparently responsible (Hughes and Brodie, 1959; Bridges and Baldini, 1966). Serotonin uptake, which is facilitated by phosphate and potassium ions (Weissbach and Redfield, 1960; Burningham *et al.*, 1966), is

inhibited by reserpine, fluoride, 2,4-dinitrophenol, chlorpromazine and tyramine (Burningham et al., 1966). Platelets have a very high concentration of adenosine triphosphate (ATP), and the ATP content of platelets and their serotonin uptake are believed to be directly related (Burningham et al., 1966).

Platelets keep the concentration of free serotonin in plasma very low, and at low external concentrations the uptake is almost complete (Humphrey and Toh, 1954). Platelet serotonin is probably accumulated rather than synthesized in situ (Humphrey and Toh, 1954; Garattini and Valzelli, 1965).

RELEASE OF SEROTONIN BY PLATELETS

Serotonin is readily released from platelets under many circumstances (Reid, 1952), and its release apparently involves activation of enzymatic processes (Westerholm, 1966). Serotonin release is inhibited by heparin (Paasonen, 1965; Westerholm, 1966), and also by ninhydrin, and allicin (Westerholm, 1966). Honour and Mitchell (1963) discovered that when an artery is injured, white masses of platelets build up at the site of the injury and embolize. Swank and associates (1963) stated that serotonin greatly increases the tendency of the red blood cells, leucocytes and platelets to aggregate.

Differential centrifugation of homogenates of human platelets revealed that amino acids and potassium were largely free, but that serotonin was bound within granules

(Buckingham and Maynert, 1964). Platelets are not capable of synthesizing serotonin (Garattini and Valzelli, 1965), so most platelet serotonin probably originates in the enterochromaffin cells of the intestinal mucosa (Paasonen, 1965).

C. Effects of serotonin on the heart.

Intravenous infusion of serotonin usually results in tachycardia (Maxwell et al., 1959; Noble and Nanson, 1959; Peskin and Miller, 1962), increased right ventricular work (Maxwell et al., 1959), and increased cardiac output (Takacs and Vajda, 1963).

D. Effects of serotonin on systemic blood pressure.

PRESSOR AND DEPRESSOR EFFECTS

Hamilton (1966) calls serotonin "one of the most potent vasoconstrictors yet recognized." It must be remembered, however, that serotonin's effects vary, depending not only on the species of animals and doses given, but also on variations within the same species and under similar conditions (Schneider, 1954; Page and McCubbin, 1956; Kabins, Molina and Katz, 1959; Goodman, 1965). Injection of large doses of serotonin usually produces a pressor response in the systemic circuit (MacCanon and Howath, 1954; Braun and Stern, 1961). Page and McCubbin (1956) compared the effects of injection versus infusion of serotonin in the femoral veins of dogs. They found that a quick injection of serotonin

resulted in a pressor effect, whereas intravenous infusion at a slower rate, but with the same amount of serotonin, resulted in a sustained fall in arterial blood pressure.

Other researchers have also found serotonin infusion to result in decreased arterial blood pressure and systemic vascular resistance (Maxwell et al., 1959; McGaff and Milnor, 1962). MacCanon and Howath (1954) found that in all their observations, both pulmonary and femoral arterial pressures began to rise within 3-6 seconds after the rapid injection of 500 ug of serotonin creatinine sulfate. The pressures reached maximum values in 12-20 seconds, and then decreased to control within 1-7 minutes. The mean recovery time was 2.75 minutes.

BIPHASIC BLOOD PRESSURE RESPONSE TO SEROTONIN

Gailitis and Sheiber (1960) injected 0.5-2.0 mg of serotonin into humans and reported a biphasic response: a brief fall in blood pressure followed by an overshoot for 1-5 minutes, accompanied by an increase in pulse rate. Reid (1952) also found that when he injected 36 ug of serotonin into dogs, there was an initial transient fall in blood pressure, succeeded by a pressor response which was followed in turn by a depression lasting for several minutes. The initial steep drop in systemic blood pressure was believed to be due primarily to vasoconstriction in the pulmonary circulation. When Reid intravenously injected 10-110 ug of

serotonin, the pressure in the pulmonary artery rose and, simultaneously, the pressure in the carotid artery fell. At the same time there was a fall in pulmonary venous pressure and a rise of pressure in the right atrium. Reid concluded that the rise of pressure in the pulmonary artery was due to an increase in pulmonary resistance and was not cardiac in origin. Serotonin liberates adrenaline from the suprarenal glands (Reid, 1952; Garattinin and Valzelli, 1965), and this may contribute to the pressor effect of large doses given intravenously (Reid, 1952; Reid and Rand, 1952; Garattini and Valzelli, 1965). But the drug also has an independent pressor action, since similar changes in blood pressure occur in adrenalectomized animals (Reid, 1952; Page and McCubbin, 1953). The secondary depressor phase may be due to decreases in either the peripheral resistance or the left ventricular output (Reid, 1952). The reduced peripheral resistance may be the result of a direct vasodilator action or an indirect action mediated through the nervous system, or it may be the result of the liberation of a vasodilator agent (Reid, 1952). Some of the depressor effects of serotonin may be the result of its ability to release histamine (Page and McCubbin, 1956; Moore, Normell and Eiseman, 1963; Goodman, 1965).

OPPOSITE RESPONSES IN LARGE AND SMALL VESSELS

Haddy (1960) has stated, "It is clear that the calibers of arteries, veins and arterioles can actively change in opposite directions." Some evidence indicates that serotonin actively constricts large vessels at the same time that it actively dilates small vessels (Haddy, Fleishman and Emanuel, 1957; Haddy, 1960). Haddy (1960) found that when he administered serotonin into the brachial artery in amounts small enough to have no noticeable effect on systemic arterial and venous pressures, the small artery pressures fell while the pressures in the small veins rose in the dog forelimb, with no net change in blood flow through the limb. Histamine, which is released during the administration of serotonin (Page and McCubbin, 1956; Haddy, 1960; Moore, Normell and Eiseman, 1963; Goodman, 1965), might possibly be involved in the arteriolar dilation (Page and McCubbin, 1956; Haddy, 1960), since the administration of histamine causes the arterioles to dilate and the veins to constrict (Haddy, 1960). Haddy (1960) has also stated that "local antagonism between serotonin and the adrenalines, whether occurring at the molecular or physiologic level, might well account for the observed arteriolar dilation." Whelan (1959) and Chacalos (1963) have also reported small vessel dilation following the administration of serotonin.

FACTOR OF NEUROGENIC VASCULAR TONE

Spinal cord transection at the sixth cervical vertebra and removal of the cord in dogs resulted in a definitely augmented response to serotonin, marked by a large and prolonged initial fall in arterial pressure (Page, 1952). Serotonin has been observed to lower blood pressure in animals with neurogenic hypertension and elevate pressure in animals with neurogenic hypotension (Page and McCubbin, 1956; Haddy, 1960). After a series of experiments on the dog foreleg, Haddy and associates (1959) described this response by stating,

> Serotonin antagonizes extremes of vascular tone induced by neurogenic means. It produces net dilation when the bed is constricted and net constriction when the bed is dilated. This bi-directional response derives ultimately from the fact that changes in nervous activity change calibers of small vessels without greatly altering calibers of large vessels, and that serotonin produces small vessel dilatation at the same time that it constricts large vessels. When small vessels are highly constricted, serotonin dilates small vessels more than it constricts large vessels. The net effect is "dilatation."

The most important mechanism controlling vascular reactivity to serotonin is probably the degree of autonomic nervous activity (Page, 1953; McCubbin, Kaneko and Page, 1962). McCubbin and associates (1962) found that when they stimulated the lumbar sympathetic trunk, both large and small arteries constricted; when serotonin was injected during stimulation, the formerly constricted small arteries relaxed, but the formerly constricted large arteries constricted even more. The net effect was a decrease in total vascular resistance, caused by the dilation of the small arteries and veins.

ROLE OF CHEMORECEPTORS IN SEROTONIN RESPONSE

Chemoreceptor stimulation appears to play a role in the rise of both pulmonary artery and pulmonary venous pressures after the administration of serotonin (Braun and Stern, 1961). Serotonin receptors apparently have a high degree of specificity since tryptamine and 5-hydroxytryptamine (serotonin) do not even activate the same receptors (Woolley and Shaw, 1962). Experiments performed with DBMC (N-dimethylamide-N-benzyl-m-metoxycinnamamide) indicate the existence of two types of serotonin receptors: DBMC-sensitive and DBMC insensitive (Wurzel, 1966). Serotonin is known to activate aortic and carotid chemoreceptors (Page, 1952; McCubbin, Green and Salmoiraghi, 1956; Braun and Stern, 1961). Serotonin causes a pronounced increase

in chemoreceptor impulse traffic in the carotid sinus nerve when given in amounts of 12 ug into the common carotid artery (McCubbin, Green and Salmoiraghi, 1956). Serotonin is also said to activate chemoreceptors located in the heart and lung (Garattini and Valzelli, 1965). The presence of serotonin in the human carotid body was established by fluorescence microspectrophotometry in 1966 (Hamberger, Ritzen and Wersall, 1966).

SEROTONIN AND SHOCK

Anaphylactic reactions produce a release of serotonin from the platelets and a fall in blood serotonin concentrations (Rosenberg et al., 1959). Borges and Bessman (1957) report that serotonin is liberated from lung tissue, along with histamine, during anaphylaxis.

Serum serotonin concentration falls immediately after administration of endotoxin and remains low throughout the shock state (Rosenberg et al., 1959). Total serotonin levels fall rapidly after the intravenous infusion of E. coli, as do the number of circulating platelets, along with marked changes in platelet morphology (Davis et al., 1960).

E. Effects of serotonin in specific vascular beds.

CORONARY VESSELS

Intravenous infusion of serotonin at 2.3 ug/kg/min in the rat results in a large increase in blood flow and decrease in coronary vascular resistance (Takacs and Vajda,

1963). Increases in coronary blood flow of 79% (Maxwell et al., 1959) and 134% (Hashimoto et al., 1964) have been reported following intravenous serotonin infusion.

FORELIMB VESSELS

Haddy (1960) administered serotonin into the brachial artery of dog forelimbs in doses small enough to have no noticeable effect upon systemic arterial and venous pressures, and found that the pressures in the small arteries fell and those in small veins rose, with no net change in blood flow through the limb. When serotonin (0.25-16 ug/min) was infused into the arteries of human forearms, the results were vasoconstriction, decreased blood flow, flushing and delayed cyanosis (Garattini and Valzelli, 1965).

GASTROINTESTINAL VESSELS

Intravenous infusion of serotonin (2.3 ug/kg/min) into the rat resulted in increased blood flow through the gut vessels (Takacs and Vajda, 1963). The topical effect of serotonin in the rat's mesoappendix is said to be about 200 times greater than that of general administration (Garattini and Valzelli, 1965).

HEPATIC VESSELS

Intravenous serotonin infusion causes a slight increase in the blood flow through the liver vessels of the rat (Takacs and Vajda, 1963). In dogs, intravenous infusion of

serotonin increases portal pressure and resistance to hepatic blood flow, but it increases hepatic flow in man (Garattini and Valzelli, 1965).

PULMONARY VESSELS

Serotonin administration produces a marked pressor response in the pulmonary circuit (MacCanon, 1954; Shepherd et al., 1959; Rudolph and Auld, 1960; Vitolo et al., 1962; Rudolph and Scarpelli, 1964; Marshall, 1966) with a rapid increase in pulmonary arterial pressure (Reid and Rand, 1951; MacCanon and Howath, 1954; Maxwell et al., 1959; Aviado, 1960; Gailitis and Sheiber, 1960), along with pulmonary arteriolar and venous constriction (Kabins, Molina and Katz, 1959; Noble and Nanson, 1959) and a resultant increase in pulmonary vascular resistance (Noble and Nanson, 1959; Shepherd et al., 1959; McGaff and Milnor, 1962). The degree of response of the pulmonary vasculature depends upon the state of vasomotor tone (Rudolph et al., 1959).

Haddy (1960) has stated,

> Serotonin is a potent constrictor of the pulmonary vascular bed. Dilator responses are not seen. The pulmonary vascular bed is low tone bed. The arterioles are widely dilated. Therefore, the predicted response would be constriction.

The principal site of increased resistance to flow through the lungs appears to be in the precapillary vessels (Shepherd et al., 1959). McGaff and Milnor (1962) have stated that serotonin infusion reduces pulmonary blood volume by an average of about 26% below control values, which they say is an example of shifting of blood from pulmonic to systemic circuits "by reciprocal changes in the distensibility of these beds." Somlyo and Somlyo (1964) reported significant vasoconstriction in helically cut strips of canine main pulmonary artery. Marshall (1966) states that serotonin may be responsible for many of the effects of embolization of the lungs by blood clot or thrombus. Serotonin is a more effective pulmonary vasoconstrictor than adrenaline or noradrenaline, and the pulmonary hypertension it causes is not influenced by spinal section, vagotomy, adrenalectomy, ganglionic blockade, administration of antihistamines or the administration of reserpine (Garattini and Valzelli, 1965).

RENAL VESSELS

Renal blood flow in rats is decreased by intraperitoneal infusion of serotonin, but increased when serotonin is infused intravenously (Takacs and Vajda, 1963). The renal vessels of cats, guinea pigs, and rabbits are less sensitive to serotonin than those of dogs (Garattini and Valzelli, 1963).

SKELETAL MUSCLE VESSELS

Vasodilation is the usual response of the skeletal muscle blood vessels to serotonin (Goodman, 1965). Takacs and Vajda (1963) report an increase in blood flow in skeletal muscles following the intraperitoneal injection of 10 mg/kg of serotonin.

Emerson and associates (1968) investigated the local effects of serotonin on resistance to blood flow in 20 isolated innervated gracilis muscles of dogs; six of the muscles were perfused at constant flow and 14 at natural flow. Small vein and perfusion pressures were measured in both groups, and venous outflow was also measured in the natural flow experiments. Intra-arterial infusion of serotonin at 2-100 ug/min had variable effects on perfusion pressure and no effect on small vein pressure in the constant flow experiments. Emerson lowered vascular resistance in the muscle by stimulating the cut gracilis motor nerve, and found that serotonin always raised perfusion pressure but still had no effect upon small vein pressure. In the natural flow experiments, infusion of 2-100 ug/min of serotonin increased the blood flow in the ten experiments in which the initial resistance was above 10 mmHg/ml/min per 100 grams of muscle weight. In the four experiments with initial resistances below 10, blood flow fell in two and did not change in the

other two. In each experiment, venous pressures paralleled flow changes. It was concluded that "the response of the innervated gracilis muscle was irregular and perhaps in part related to the initial level of resistance. Muscle veins apparently do not respond actively to serotonin (Emerson et al., 1968)."

SKIN VESSELS

Skin blood flow decreases after intradermal, intravenous or intra-arterial injection of serotonin (Garattini and Valzelli, 1965) as a result of constriction of skin vessels (Reid, 1952; Noble and Nanson, 1959; Daugherty et al., 1968; Emerson et al., 1968). Demis, Davis and Lawler (1960) studied various responses to intradermally administered serotonin in 50 normal humans and found that all of them developed local erythemia, a prominent protracted flare, and constriction of large subcutaneous veins, although the small vessels showed no marked changes. Emerson and associates (1968) studied the local effects of serotonin on resistance to blood flow in 8 isolated denervated hindpaws of dogs perfused at constant flow. They found that intra-arterial infusion of serotonin at 2-4 ug/min always raised small vein and perfusion pressures in the paw. When they raised vascular resistance by sympathetic nerve stimulation, perfusion and small vein pressures were again raised and to about the same extent. It was thus concluded that "serotonin

regularly raises total and venous resistances in the denervated hindpaw both before and during sympathetic nerve stimulation (Emerson et al., 1968)." Daugherty and colleagues (1968) made a comparison of the effects of intrabrachial and intravenous administration of serotonin on forelimb blood flow in the dog. Serotonin was infused at a variety of rates, and pressures and flows were only measured after they became stable. Almost no change in total flow from the limb was observed during intrabrachial or intravenous administration of serotonin. However, a shift in flow from the cephalic (skin) vein to the brachial (muscle) vein occurred with no net change in total outflow during intrabrachial administration in constant inflow perfused limbs. This suggests that serotonin affects the two parallel beds in opposite directions, increasing vascular resistance in the skin and possibly decreasing it in the muscle (Daugherty et al., 1968). This provides another good example of the variability of responses to serotonin.

F. Serotonin antagonists.

Reserpine releases serotonin from all tissues in which it is found (Stacey, 1959), and causes brain cells to lose their ability to retain serotonin, which is released and destroyed by monoamine oxidase (Gailitis and Sheiber, 1960). Reserpine also causes the release of serotonin from platelets and the

intestine, and results in increased excretion of 5-indol-acetic acid (Borges and Bessman, 1957). Thorazine antagonizes the cardiovascular actions of serotonin and also decreases serotonin-induced peristalsis (Gailitis and Sheiber, 1960). Heparin effectively antagonizes the effects of serotonin on the pulmonary vascular bed and on the bronchial wall muscle (Noble and Nanson, 1959).

MATERIALS AND METHODS

A total of 52 dogs were used in these studies. The animals were anesthetized with sodium pentobarbital (30 mg/kg), anticoagulated with sodium heparin (3 mg/kg), and ventilated with a Harvard constant volume respirator via an intratracheal tube. A femoral artery was cannulated for arterial blood pressure; all pressures were measured with Statham pressure transducers and a Sanborn recorder.

Natural Flow.

Twenty dogs were used for natural flow studies. The right gracilis muscle was completely isolated and all blood vessels, with the exception of the gracilis artery and vein, were cut between ligatures or cauterized. The origin and insertion of the muscle were also ligated. A small branch of the gracilis artery and vein were cannulated for infusion and large vein pressure measurement, respectively. All other branches on these vessels were ligated. The gracilis vein was cannulated and allowed to flow into a reservoir. Blood was returned with a Sigmamotor pump to a cannulated femoral vein of the opposite leg. When the flow remained constant, serotonin (100 ug/ml) was infused at 2, 5, 10, 20, 50 and 100 ug/min into the gracilis artery. Infusion was started at the lowest rate and progressively increased to the highest in each experiment. Thirty-second flows were taken at each

level of infusion, and the rate was not raised until a steady-state ensued. After recovery from the effects of serotonin, control flows were again taken and isotonic saline was infused at the same rates as a volume and dilutional control. Acetylcholine was infused into all the muscles at a rate of 5-10 ug/min to test their response to a known vasodilator. Total resistance per 100 grams of muscle was calculated by using the following formula:

$$R_T = \frac{P_{A_S} - P_{LV}}{FLOW} \times \frac{MUSCLE\ WEIGHT}{100} = \frac{mmHg}{ml} \times MIN \times 100g$$

R_T = Total resistance per 100 grams of muscle

P_{A_S} = Mean systemic arterial blood pressure

P_{LV} = Large vein pressure

Constant Flow.

Thirty-two dogs were used for constant flow studies. The set-up was the same except blood was pumped from a femoral artery into the gracilis artery via a Sigmamotor pump and at a constant rate. Serotonin was infused behind the pump at the same rates as in the preceding group. Perfusion pressure was measured from the arterial inflow tubing distal to the pump. The same rates of infusion were used as in the natural flow series and only steady-state values were recorded.

The following experiments were completed to determine whether the vascular response of a particular muscle to serotonin could be modified by altering the initial vascular resistance. After serotonin infusion was completed in the constant flow muscle preparations, the muscles were denervated. If the pre-denervation resistance was high, the resistance was lowered metabolically by electrical stimulation of the gracilis motor nerves (frequency = 2/sec, duration = 5 milliseconds, voltage = 5 volts). The resultant gracilis muscle contraction always markedly lowered muscle vascular resistance. The study was continued during the muscle contraction. If the pre-denervation resistance was low, the muscle was prevented from contracting by intra-arterial infusion of 2 mg decamethonium. Vascular resistance was then increased by sympathetic nerve stimulation (frequency = 10/sec, duration = 10 milliseconds, voltage = 10 volts). In each of these experiments, serotonin was infused at 50 ug/min until a steady-state ensued. The responses to serotonin at the altered resistance, by metabolic and neurogenic means, were compared to the responses at the initial resting resistance. A paired replicates test was used for statistical evaluation of the data. A "p" value less than 0.05 was considered significant.

RESULTS

In 50 out of 52 constant and natural flow experiments, muscles with an initial total vascular resistance per 100 grams (R_T) greater than 12.3 mmHg/ml/min/100 grams showed a drop in resistance, and those with an initial R_T lower than 12.3 showed a rise in resistance upon infusion with serotonin.

Figure 1 shows that in muscles with low initial resistance ($R_T < 12.3$ units) and perfused at natural flow, serotonin infusion caused a progressive increase in total muscle vascular resistance and decrease in muscle flow ($p < 0.05$). Mean systemic arterial and large vein pressure were unaffected at any rate of infusion.

Figure 2 shows that in muscles with high initial resistance ($R_T < 12.3$ units), serotonin caused a progressive decrease in total vascular resistance and increase in muscle flow. These changes were significantly greater than those produced by equivalent volumes of saline.

Figure 3 shows that in muscles with low initial resistance ($R_T < 12.3$ units) and perfused at constant flow, serotonin infusion caused an increase in total muscle vascular resistance ($p < 0.05$). Mean systemic arterial blood pressure was again unaffected at any rate of infusion.

TABLE 1.

RESISTANCE CHANGES (mmHg/cc/min/100g); NATURAL FLOW EXPERIMENTS WITH LOW INITIAL RESISTANCE:								
Exp. No.	SEROTONIN: CC/MIN (100 ug/cc): Control	0.02	0.05	0.10	0.20	0.50	1.00	Rec.
1.	9.2	10.4	11.8	11.9	12.2	12.6	11.9	14.7
2.	11.5	13.6	14.0	12.7	12.7	12.4	16.4	16.6
3.	11.9	10.6	9.7	10.2	10.6	12.5	11.3	11.2
4.	8.7	8.0	8.3	9.2	9.1	9.1	8.4	10.7
5.	9.2	6.7	6.5	6.3	6.8	7.4	9.6	8.5
6.	12.3	11.3	11.3	13.5	15.0	17.8	20.5	13.2
7.	5.3	5.0	4.2	4.7	5.1	5.6	12.3	7.6
8.	8.2	8.0	8.6	8.6	8.8	10.5	10.6	9.3
Ave.	9.4	9.2	9.3	9.7	10.0	11.0	12.6	11.4
±S.E.	0.82	0.98	1.10	1.09	1.12	1.33	1.39	1.11
Exp. No.	SALINE: CC/MIN Control	0.02	0.05	0.10	0.20	0.50	1.00	Rec.
1.	14.7	15.1	14.8	15.6	15.7	15.7	14.0	17.9
2.	16.6	13.3	12.5	11.3	10.8	9.7	9.4	12.7
3.	12.9	13.1	13.1	11.5	12.6	11.7	10.3	12.2
4.	10.7	10.8	10.1	9.4	9.4	8.8	7.8	9.3
5.	8.0	7.7	7.7	7.3	7.6	7.7	7.3	9.1
6.	13.5	13.1	12.5	13.2	13.6	14.4	14.4	14.2
7.	13.9	15.0	15.0	14.3	12.4	13.7	13.1	14.3
8.	10.6	10.7	10.4	10.4	10.4	10.2	9.8	10.4
Ave.	12.6	12.3	12.0	11.6	11.6	11.5	10.8	12.5
±S.E.	0.96	0.88	0.88	0.95	0.90	1.01	0.97	1.05

Figure 1. Effects of I.A. serotonin infusion in gracilis muscles with low initial resistance. Flow = natural. R_T = total muscle vascular resistance; F = flow; P_{A_S} = mean systemic arterial blood pressure; P_{LV} = muscle large vein pressure.

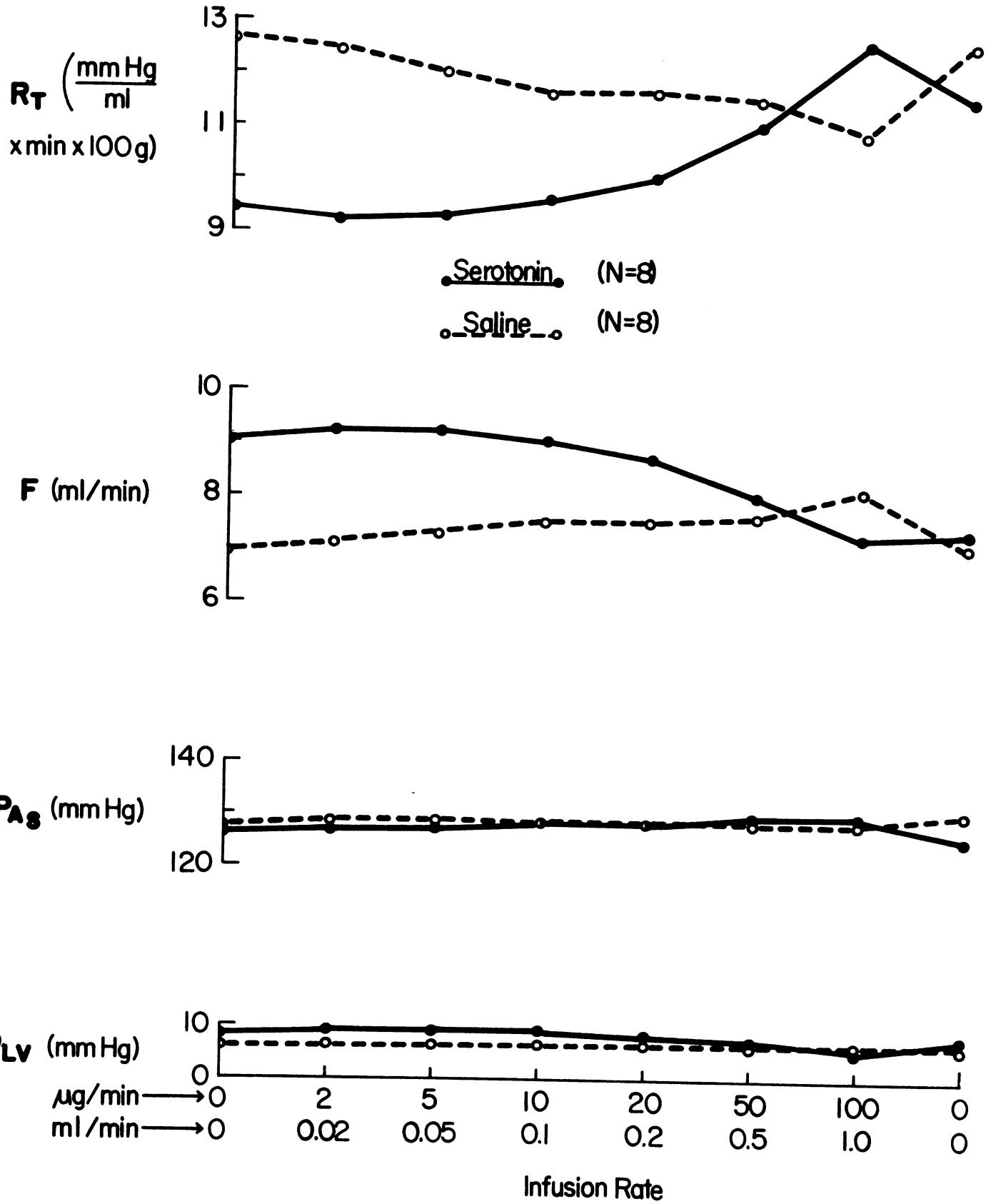

R_T ($\frac{mm\,Hg}{ml}$ x min x 100g)
13
11
9
Serotonin (N=8)
Saline (N=8)
F (ml/min)
10
8
6
P_{AS} (mm Hg)
140
120
P_{LV} (mm Hg)
10
0
μg/min → 0 2 5 10 20 50 100 0
ml/min → 0 0.02 0.05 0.1 0.2 0.5 1.0 0
Infusion Rate

TABLE 2a.

RESISTANCE CHANGES (mmHg/cc/min/100g); CONSTANT FLOW EXPERIMENTS WITH LOW INITIAL RESISTANCE:

Exp. No.	SEROTONIN: CC/MIN (100ug/cc): Control	0.02	0.05	0.10	0.20	0.50	1.00	Rec.
1.	5.2	5.3	5.1	5.0	4.3	4.0	4.0	4.7
2.	9.2	9.8	10.1	9.9	9.9	11.6	12.9	10.3
3.	12.2	12.3	13.8	16.0	17.0	17.8	17.4	13.2
4.	10.7	9.9	9.7	9.6	9.4	8.9	8.7	10.7
5.	12.1	14.7	14.2	14.7	14.7	15.0	13.2	12.1
6.	6.2	5.8	5.4	5.1	4.9	4.8	4.9	5.6
7.	7.6	8.1	8.5	8.4	8.1	8.1	8.0	7.8
8.	7.5	7.8	10.5	12.1	12.2	10.5	12.1	7.5
9.	10.5	11.7	12.5	12.1	12.0	11.4	10.2	11.8
10.	6.5	9.6	11.2	11.3	11.5	11.5	11.3	6.7
11.	6.9	7.5	9.8	9.7	8.9	9.2	9.4	8.0
12.	6.3	6.4	8.4	8.4	8.2	7.7	7.4	5.3
13.	6.1	9.6	10.9	11.6	12.1	12.1	12.3	5.6
14.	12.2	13.8	13.6	14.1	14.4	14.5	14.1	11.6
15.	7.6	7.6	8.3	8.9	9.3	9.4	8.7	6.6
16.	5.7	7.2	7.6	7.8	7.8	7.6	7.2	6.6
17.	10.4	12.7	12.2	13.3	12.3	11.1	10.0	10.1
18.	12.0	12.8	12.8	13.2	12.4	12.5	11.1	12.2
19.	7.9	8.1	8.0	8.0	8.9	9.4	9.6	7.9
Ave.	8.6	9.5	10.1	10.5	10.4	10.4	10.1	8.6
±S.E.	0.56	0.64	0.61	0.70	0.74	0.78	0.75	0.62

TABLE 2b.

RESISTANCE CHANGES (mmHg/cc/min/100g); CONSTANT FLOW EXPERIMENTS WITH LOW INITIAL RESISTANCE:								
Exp. No.	SALINE, CC/MIN: Control	0.02	0.05	0.10	0.20	0.50	1.00	Rec.
1.	4.7	4.8	4.5	5.0	5.0	4.6	4.8	5.1
2.	10.3	10.5	10.5	10.7	10.4	10.5	10.3	11.0
3.	18.0	18.0	17.8	17.5	17.5	16.8	15.8	18.0
4.	10.3	10.5	10.5	10.5	10.5	10.2	9.5	10.6
5.	12.6	12.6	12.9	12.6	12.6	11.2	10.1	13.4
6.	6.4	6.6	6.7	6.7	6.7	6.6	6.4	6.7
7.	7.1	7.1	7.1	7.1	7.3	7.3	7.2	7.1
8.	7.2	7.2	7.2	7.2	7.4	7.3	7.1	7.7
9.	13.9	13.6	12.7	12.3	11.8	10.9	10.1	12.3
10.	7.3	7.4	7.4	7.5	7.5	7.4	7.2	8.0
11.	6.6	6.6	6.6	6.3	6.1	6.1	6.6	7.0
12.	5.1	5.1	5.2	5.4	5.5	6.1	5.5	6.2
13.	6.3	6.5	6.4	6.3	6.3	6.3	6.1	6.4
14.	12.4	12.5	13.0	13.2	13.0	12.5	11.5	12.9
15.	7.6	7.6	7.6	7.6	7.4	7.3	7.1	7.6
16.	7.4	7.4	7.4	7.4	6.7	6.4	6.0	6.7
17.	12.6	13.0	13.1	13.1	12.2	11.6	10.4	12.1
18.	12.4	12.4	12.4	12.4	12.4	11.9	11.4	12.5
19.	8.6	8.6	8.6	8.7	8.7	8.7	8.3	9.0
Ave.	9.3	9.4	9.3	9.3	9.2	8.9	8.5	9.5
±S.E.	0.81	0.81	0.80	0.78	0.76	0.70	0.62	0.76

Figure 2. Effects of I.A. serotonin infusion in gracilis muscles with high initial resistance. Flow = natural. R_T = total muscle vascular resistance; F= flow; P_{A_S} = mean systemic arterial blood pressure; P_{LV} = muscle large vein pressure.

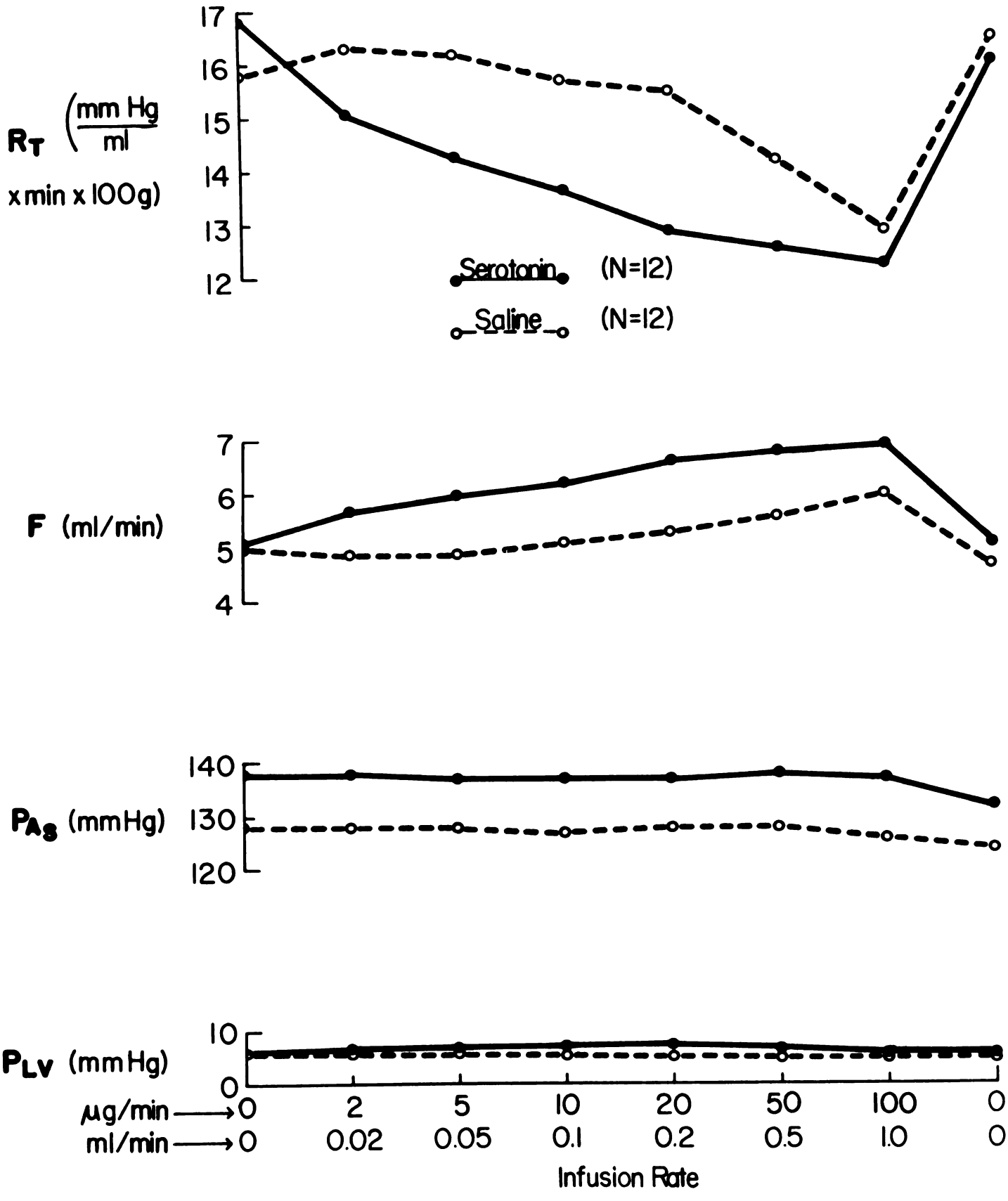

R_T $\left(\frac{mm\ Hg}{ml}\right.$
x min x 100g)
17
16
15
14
13
12
Serotonin (N=12)
Saline (N=12)
F (ml/min)
7
6
5
4
P_{AS} (mmHg)
140
130
120
P_{LV} (mmHg)
10
0
μg/min → 0 2 5 10 20 50 100 0
ml/min → 0 0.02 0.05 0.1 0.2 0.5 1.0 0
Infusion Rate

TABLE 3a.

RESISTANCE CHANGES (mmHg/cc/min/100g); NATURAL FLOW EXPERIMENTS WITH HIGH INITIAL RESISTANCE:								
Exp. No.	SEROTONIN: CC/MIN (100ug/cc): Control	0.02	0.05	0.10	0.20	0.50	1.00	Rec.
1.	16.8	15.8	16.8	15.7	14.0	16.1	15.0	17.5
2.	12.6	11.6	11.7	11.7	11.4	11.5	10.3	14.4
3.	25.8	24.1	21.9	22.0	20.9	20.0	18.8	23.6
4.	19.2	14.7	12.8	11.9	11.2	9.0	8.7	15.2
5.	23.7	22.4	20.3	21.5	20.4	20.3	16.1	20.7
6.	12.8	13.0	13.0	11.9	12.0	11.6	13.5	15.2
7.	15.6	14.8	13.8	12.6	10.8	10.0	11.2	11.5
8.	14.1	9.6	8.8	8.5	7.8	7.2	7.0	13.4
9.	17.9	15.7	14.6	12.8	11.9	11.5	11.6	17.9
10.	16.7	15.0	15.0	13.8	13.2	12.3	13.5	13.4
11.	13.3	12.6	12.6	11.4	10.3	10.0	9.4	14.3
12.	12.8	11.6	10.3	10.9	11.3	11.7	11.9	14.2
Ave.	16.8	15.1	14.3	13.7	12.9	12.6	12.3	16.1
±S.E.	1.25	1.23	1.10	1.18	1.13	1.18	0.96	0.99

TABLE 3b.

RESISTANCE CHANGES (mmHg/cc/min/100g); NATURAL FLOW EXPERIMENTS WITH HIGH INITIAL RESISTANCE:

Exp. No.	SALINE: Control	CC/MIN: 0.02	0.05	0.10	0.20	0.50	1.00	Rec.
1.	18.9	19.4	17.5	18.2	18.1	17.9	16.1	22.2
2.	13.4	15.2	16.2	15.8	15.7	14.8	12.6	16.0
3.	22.2	24.3	23.1	22.2	21.9	20.8	19.3	21.6
4.	15.2	15.5	16.3	13.7	13.1	12.8	10.8	15.1
5.	20.7	20.2	20.6	21.8	23.4	15.6	13.5	20.7
6.	17.0	16.1	16.0	15.2	14.4	13.0	12.0	16.0
7.	11.1	11.2	10.9	11.0	11.2	11.0	11.0	11.7
8.	13.5	12.9	12.9	11.8	11.8	11.4	11.0	12.8
9.	17.9	18.9	18.9	19.0	19.0	17.1	16.4	19.0
10.	12.5	12.6	14.0	12.7	12.9	11.7	10.5	13.6
11.	13.1	13.9	13.1	13.1	13.1	12.8	11.0	14.3
12.	14.2	15.0	14.4	13.4	11.3	11.4	10.7	14.6
Ave.	15.8	16.3	16.2	15.7	15.5	14.2	12.9	16.5
±S.E.	1.02	1.08	1.00	1.09	1.20	0.89	0.82	1.02

Figure 3. Effects of I.A. serotonin infusion in gracilis muscles with low initial resistance. Flow = constant. R_T = total muscle vascular resistance; F = flow; P_{A_S} = mean systemic arterial blood pressure.

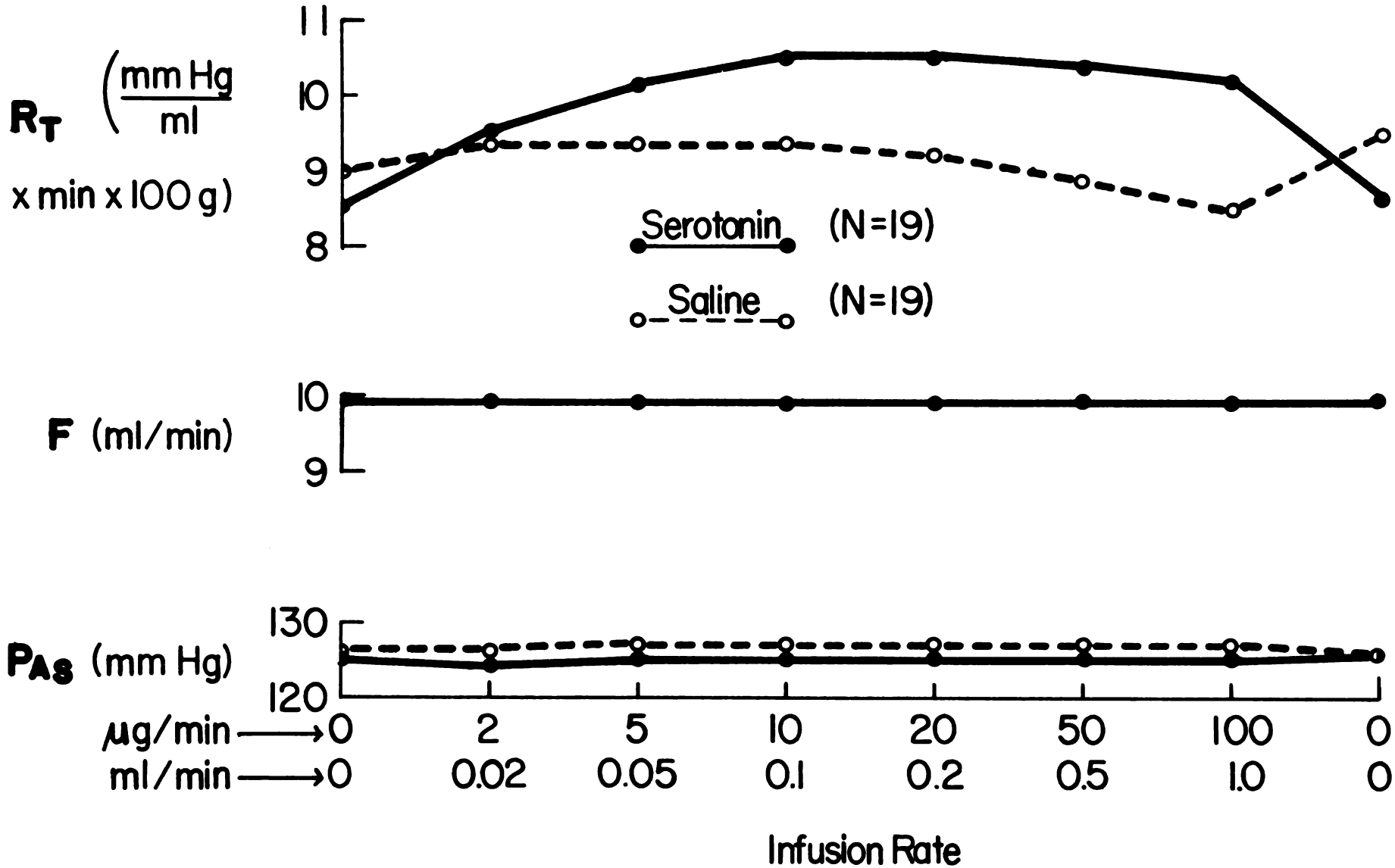

R_T $\left(\frac{mm\ Hg}{ml}\right.$
x min x 100 g)
11
10
9
8
Serotonin (N=19)
Saline (N=19)
F (ml/min)
10
9
P_{AS} (mm Hg)
130
120
µg/min → 0 2 5 10 20 50 100 0
ml/min → 0 0.02 0.05 0.1 0.2 0.5 1.0 0
Infusion Rate

Figure 4 shows that in muscles with high initial resistance ($R_T > 12.3$ units) and perfused at constant flow, serotonin infusion caused a progressive decrease in total muscle vascular resistance ($p < 0.05$). This decrease was again significantly greater than that caused by equivalent volumes of saline.

The left hand portion of figure 5 shows that serotonin caused an average rise in muscle resistance when the initial resistance was low. However, when resistance was increased to a high level by sympathetic nerve stimulation, serotonin then caused a marked fall in total vascular resistance ($p < 0.05$).

The left hand portion of figure 6 shows that serotonin caused a marked drop in total muscle vascular resistance when the initial resistance was high. The right hand portion of figure 6, however, shows that when resistance was decreased to a low steady-state level by exercise dilation, serotonin then caused a caused a rise in total vascular resistance in every case ($p < 0.05$).

The left hand portion of figure 7 shows that I.A. infusion of serotonin (50 ug/min) into 7 gracilis muscles with high initial resistance again resulted in a drop in resistance. The middle portion of figure 7 shows that when these same muscles were electrically caused to contract, thus lowering the resistance levels by exercise dilation, serotonin infusion resulted in a marked increase in total muscle vascular resistance,

which returned to near control level when infusion was stopped. The right hand portion of figure 7 shows that when the resistance levels in these same muscles were subsequently raised by sympathetic nerve stimulation and serotonin was again infused at 50 ug/min, each muscle showed a marked drop in resistance equivalent to the drop caused by serotonin in the first portion of the experiment.

Figure 8 represents the middle 15 experiments when all 52 are arranged according to their initial total resistances. The average effect of serotonin on total muscle vascular resistance is not significantly different in this group than the average effect of saline.

Figure 9 represents the average results of all 52 experiments (25 with high and 27 with low initial resistances). Again, the average effects of serotonin and saline are very similar. Only when the experiments are divided into low and high initial resistance groups can one see significant differences in the effects of serotonin as compared to the effects of saline.

The average time allowed for total recovery from the effects of serotonin was approximately 15 minutes. The muscles recovered from the effects of saline in an average of approximately 5 minutes.

TABLE 4a.

RESISTANCE CHANGES (mmHg/cc/min/100g); CONSTANT FLOW EXPERIMENTS WITH HIGH INITIAL RESISTANCE:								
Exp. No.	SEROTONIN: CC/MIN (100ug/cc): Control	0.02	0.05	0.10	0.20	0.50	1.00	Rec.
1.	13.4	12.7	9.4	8.6	8.6	7.8	7.4	13.4
2.	12.5	9.8	9.4	9.5	9.3	8.9	8.9	12.5
3.	12.5	13.0	13.4	13.4	13.1	9.0	6.4	15.8
4.	17.1	18.0	17.1	14.5	14.1	13.4	12.0	17.1
5.	12.4	12.1	12.3	12.2	10,4	10.2	10.4	12.3
6.	15.8	14.2	12.5	10.8	10.4	9.5	9.4	14.7
7.	12.5	12.4	12.4	12.4	11.8	11.6	12.5	12.6
8.	11.0	10.9	11.1	11.2	7.2	7.2	6.6	11.1
9.	13.3	10.9	11.1	11.3	11.3	11.3	12.4	13.3
10.	12.3	10.1	9.9	11.1	10.1	9.9	10.1	11.1
11.	25.0	20.9	20.1	19.0	17.8	14.9	12.9	23.2
12.	12.3	12.1	12.1	12.6	15.0	12.6	10.5	12.4
13.	12.3	11.8	12.3	12.6	12.6	12.3	12.1	11.5
Ave.	14.0	13.0	12.6	12.2	11.7	10.7	10.1	13.9
±S.E.	1.04	0.87	0.83	0.71	0.79	0.62	0.61	0.92

TABLE 4b.

RESISTANCE CHANGES (mmHg/cc/min100g); CONSTANT FLOW EXPERIMENTS WITH HIGH INITIAL RESISTANCE:

Exp. No.	SALINE, CC/MIN: Control	0.02	0.05	0.10	0.20	0.50	1.00	Rec.
1.	13.4	13.4	13.1	12.6	12.2	11.9	10.6	14.0
2.	12.1	12.0	12.1	12.0	12.0	11.8	11.4	12.3
3.	12.5	12.5	12.5	12.5	12.3	11.9	9.1	12.5
4.	19.8	18.9	19.1	19.2	17.5	17.1	15.0	20.6
5.	12.0	12.1	12.1	12.0	11.6	11.2	10.9	12.4
6.	16.6	16.7	16.6	16.3	16.1	15.8	15.4	16.3
7.	12.5	12.6	12.8	12.6	12.6	12.6	12.2	13.3
8.	10.9	10.9	10.9	10.9	10.9	10.9	10.6	11.3
9.	13.7	13.7	13.6	13.5	13.4	13.2	13.1	13.5
10.	10.4	10.9	10.7	11.7	11.5	11.5	11.2	10.7
11.	24.4	25.8	25.8	25.8	25.6	22.3	21.3	25.2
12.	15.9	15.9	15.7	15.5	15.3	13.1	11.4	14.4
13.	11.8	12.0	12.3	12.7	13.3	11.3	10.9	11.6
Ave.	14.3	14.4	14.4	14.4	14.2	13.4	12.6	14.5
±S.E.	1.13	1.15	1.15	1.14	1.09	0.89	0.87	0.80

Figure 4. Effects of I.A. serotonin infusion in gracilis muscles with high initial resistance. Flow = constant. R_T = total muscle vascular resistance; F = flow; P_{A_S} - mean systemic arterial blood pressure.

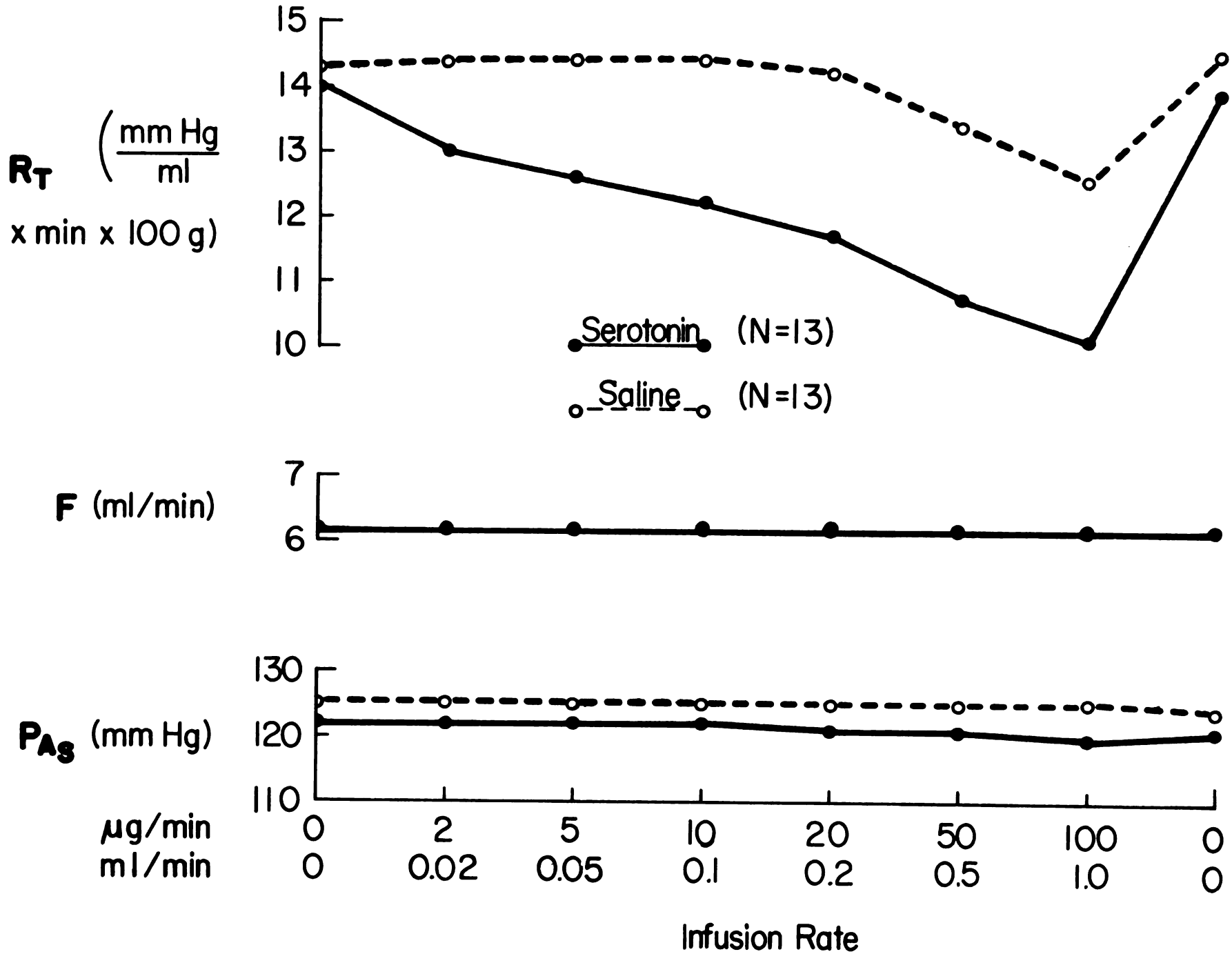

R_T $\left(\frac{mm\ Hg}{ml}\right.$
x min x 100 g)
15
14
13
12
11
10
Serotonin (N=13)
Saline (N=13)
F (ml/min)
7
6
P_{AS} (mm Hg)
130
120
110
µg/min 0 2 5 10 20 50 100 0
ml/min 0 0.02 0.05 0.1 0.2 0.5 1.0 0
Infusion Rate

TABLE 5.

RESISTANCE CHANGES (mmHg/cc/min/100g); CONSTANT FLOW EXPERIMENTS IN MUSCLES WITH LOW INITIAL RESISTANCE AND WITH RESISTANCE SUBSEQUENTLY INCREASED BY SYMPATHETIC NERVE STIMULATION:

Exp. No.	SEROTONIN, CC/MIN (100ug/cc): Pre-Stim.	Control	0.5cc/min	Recovery	Post-Stim.
1.	----	9.2	8.9	9.2	----
2.	9.6	17.7	13.5	16.8	9.8
3.	12.3	23.3	16.3	28.2	13.2
4.	6.9	13.8	12.4	13.3	7.3
5.	----	25.7	19.9	22.2	----
6.	11.1	18.7	16.6	19.2	12.4
7.	17.7	27.1	25.4	26.7	16.0
8.	11.9	25.7	22.3	23.5	11.9
9.	8.3	16.4	15.0	15.4	8.3
Ave.	11.1	19.7	16.7	19.4	11.3
+S.E.	1.33	2.04	1.70	2.10	1.13

Figure 5. Effects of I.A. serotonin infusion on total vascular resistance in muscles with low initial resistance and with resistance subsequently increased by nerve stimulation. Flow = constant. R_T - total muscle vascular resistance.

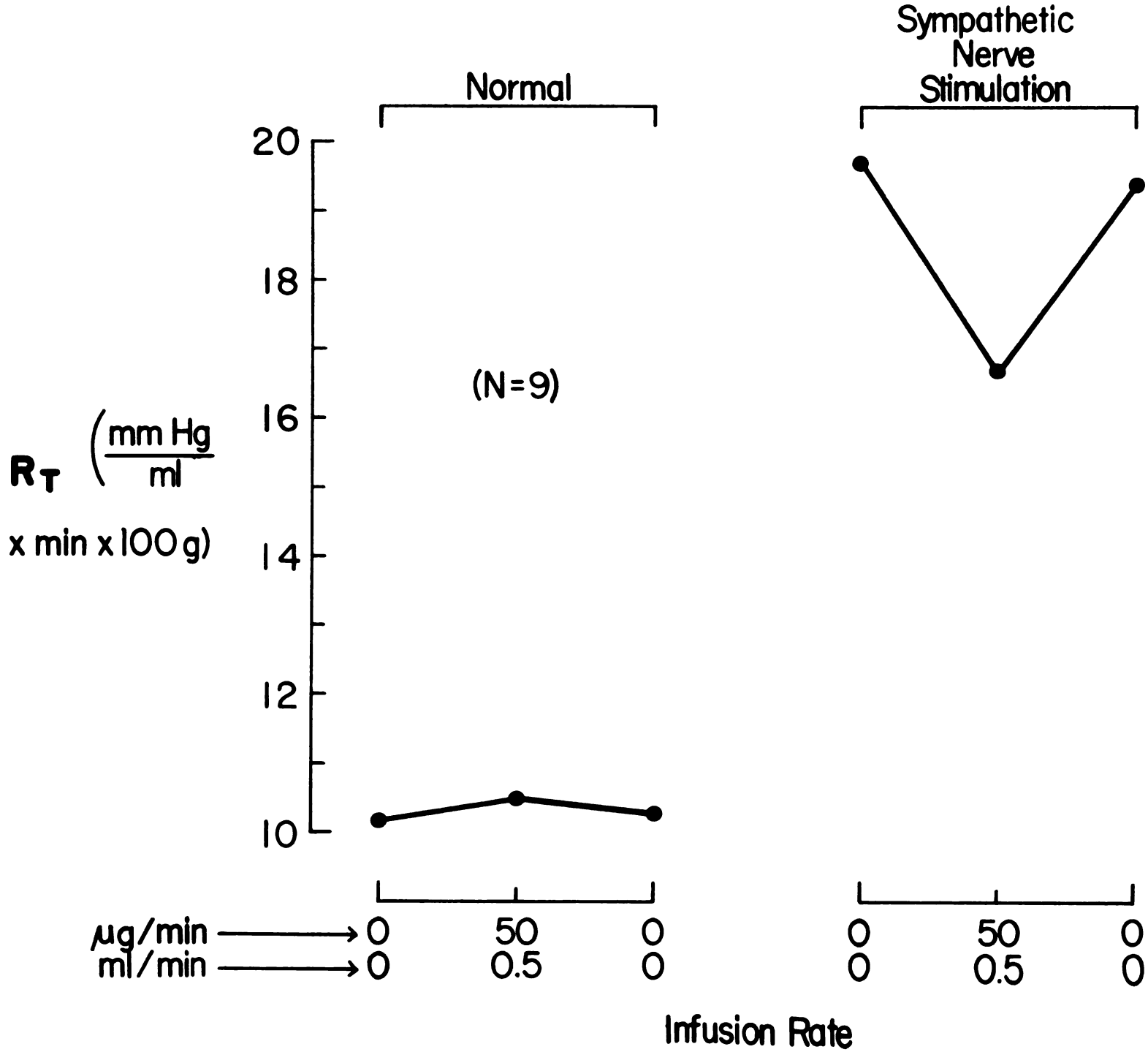

Normal
Sympathetic Nerve Stimulation
20
18
16
14
12
10
(N=9)
R_T $\left(\frac{mm\ Hg}{ml}\right.$
x min x 100 g)
μg/min → 0 50 0 0 50 0
ml/min → 0 0.5 0 0 0.5 0
Infusion Rate

TABLE 6.

RESISTANCE CHANGES (mmHg/cc/min/100g); CONSTANT FLOW EXPERIMENTS IN MUSCLES WITH HIGH INITIAL RESISTANCE AND WITH RESISTANCE SUBSEQUENTLY LOWERED BY MOTOR NERVE STIMULATION:

Exp. No.	Pre-Stim.	Control	0.50cc/min	Recovery	Post-Stim.
1.	----	5.24	7.15	7.15	----
2.	----	5.09	4.72	5.00	----
3.	----	9.70	12.00	10.10	----
4.	----	4.03	5.50	4.07	----
5.	----	3.42	4.92	3.72	----
6.	----	9.26	10.50	8.82	----
7.	----	5.03	5.36	4.95	----
8.	9.67	6.50	10.80	6.18	9.67
9.	8.89	5.29	6.75	5.51	5.51
10.	8.00	4.07	6.30	3.93	6.94
11.	13.30	8.28	11.00	8.06	12.30
12.	12.00	10.10	23.60	9.28	13.30
13.	16.20	9.42	11.10	9.60	11.10
14.	11.90	8.64	11.80	8.56	11.90
15.	8.33	3.53	6.07	3.53	8.33
16.	----	7.42	9.58	7.77	----
Ave.	11.00	6.56	9.20	6.64	9.88
±S.E.	0.95	0.59	1.22	0.57	0.65

Figure 6. Effects of I.A. serotonin infusion on total vascular resistance in muscles with high initial resistance and with resistance subsequently lowered by exercise dilation. Flow = constant. R_T - total muscle vascular resistance.

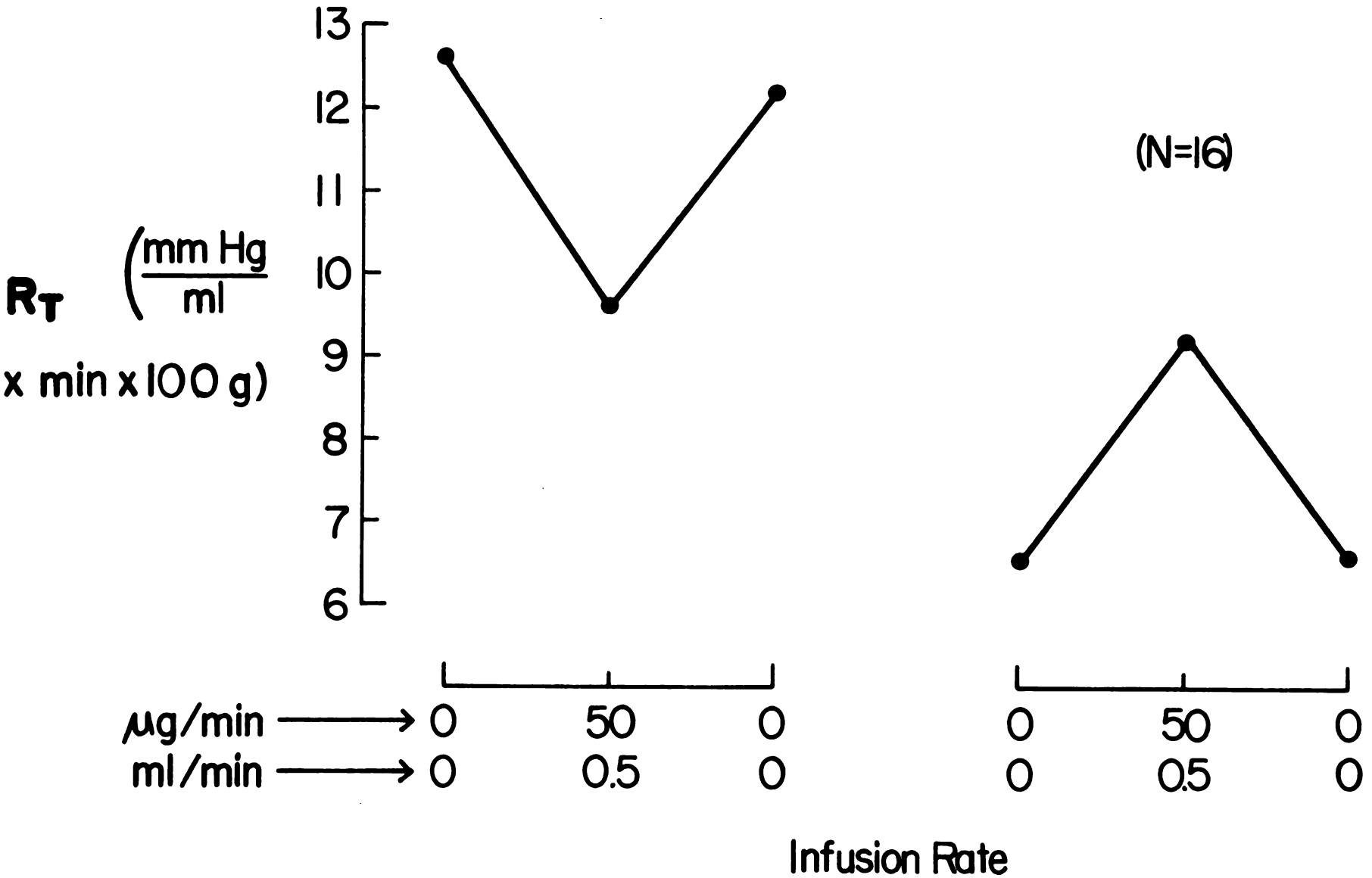

13
12
11
10
9
8
7
6
R_T ($\frac{mm\ Hg}{ml}$ x min x 100 g)
(N=16)
μg/min → 0 50 0
ml/min → 0 0.5 0
0 50 0
0 0.5 0
Infusion Rate

Figure 7. Effects of I.A. serotonin infusion on total vascular resistance in muscles with high initial resistance; in the same muscles after lowering resistance by exercise dilation; and in the same muscles again after subsequently raising resistance to a high level by sympathetic nerve stimulation. Flow = constant. R_T = total muscle vascular resistance. Infusion rates were measured in ug/min (top numbers) and ml/min (bottom numbers).

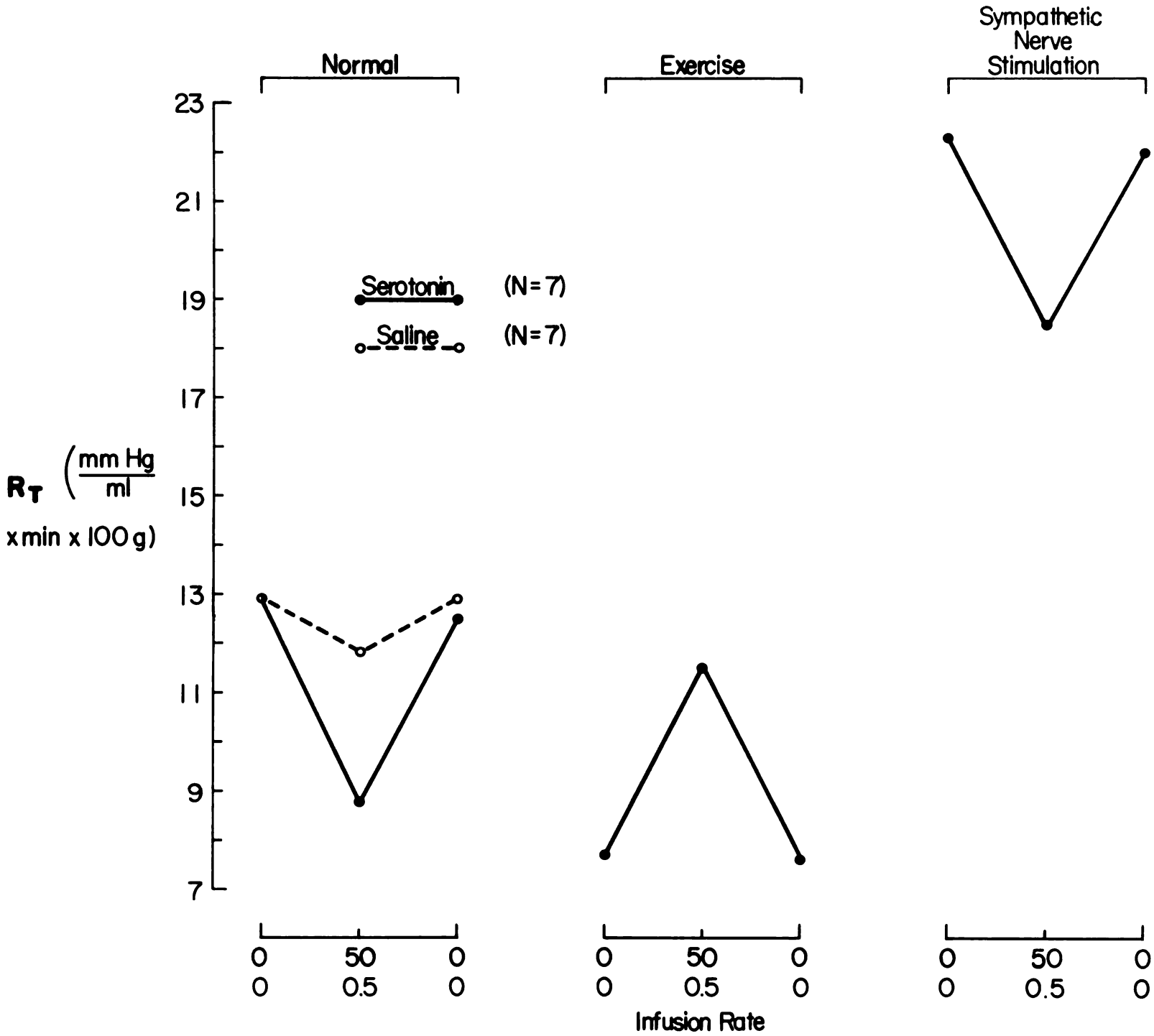

Normal
Exercise
Sympathetic Nerve Stimulation
23
21
19
17
15
13
11
9
7
Serotonin (N=7)
Saline (N=7)
R_T ($\frac{mm\ Hg}{ml}$ x min x 100 g)
0 50 0
0 0.5 0
Infusion Rate

Figure 8. Effects of I.A. serotonin infusion on total muscle vascular resistance in 15 experiments with initial resistance levels near 12.3 units. Natural and constant flow experiments were both used in this group. R_T = total muscle vascular resistance.

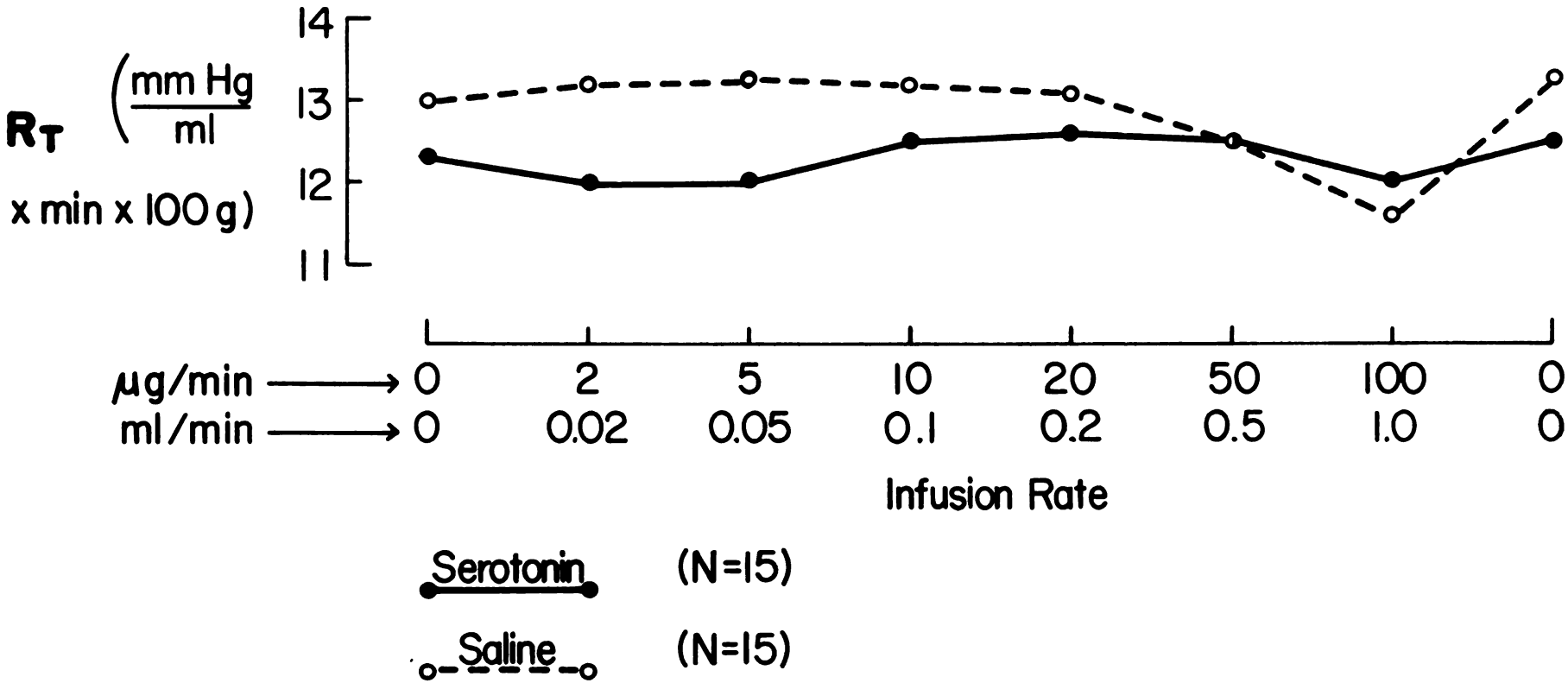
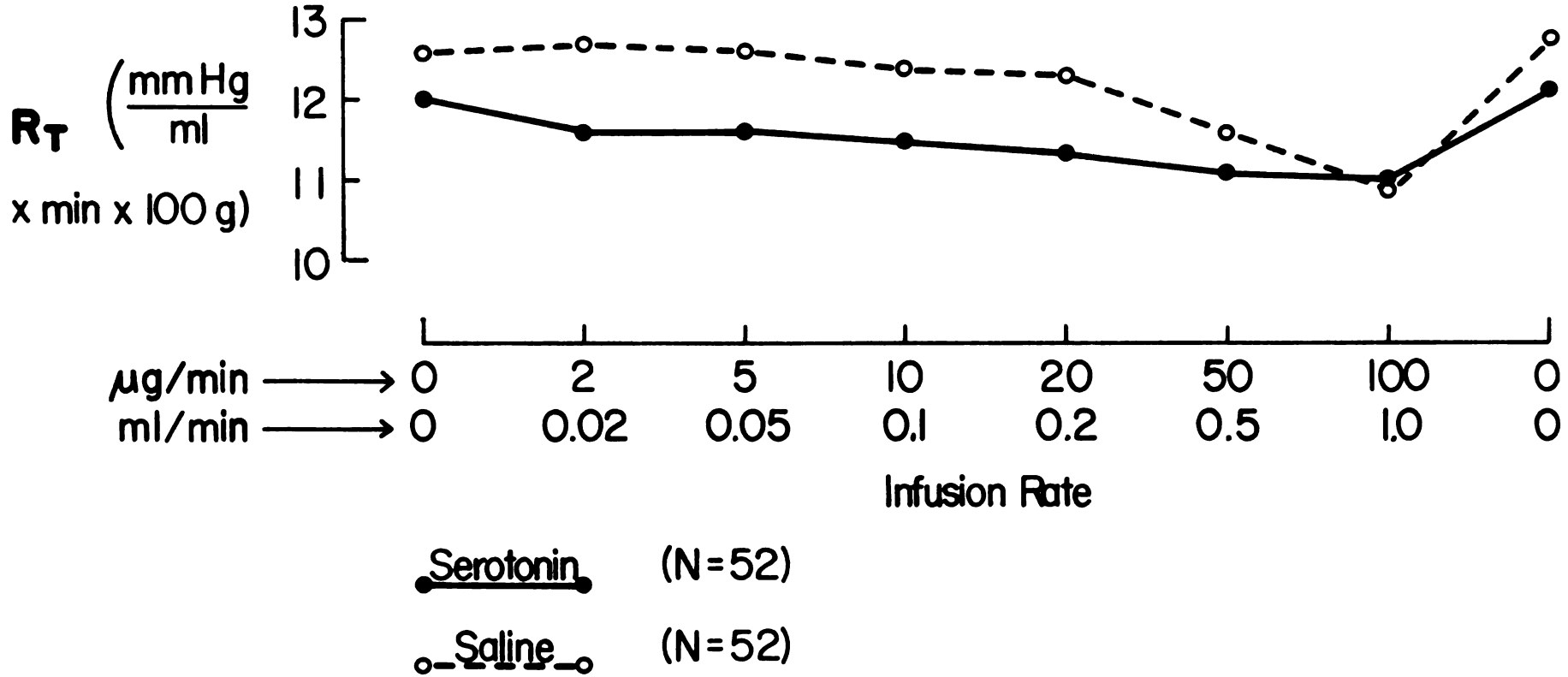

R_T ($\frac{mm\ Hg}{ml}$ x min x 100 g)
14
13
12
11
μg/min → 0 2 5 10 20 50 100 0
ml/min → 0 0.02 0.05 0.1 0.2 0.5 1.0 0
Infusion Rate
Serotonin (N=15)
Saline (N=15)

Figure 9. Effects of I.A. serotonin infusion on total vascular resistance in all experiments combined. R_T = total muscle vascular resistance.


R_T $\left(\frac{mm\,Hg}{ml}\right.$
x min x 100 g)
13
12
11
10
µg/min → 0 2 5 10 20 50 100 0
ml/min → 0 0.02 0.05 0.1 0.2 0.5 1.0 0
Infusion Rate
Serotonin (N=52)
Saline (N=52)

Since the serotonin was dissolved in normal saline, the effects of normal saline alone were of great importance. In every group of experiments, saline had very little, if any, effect on resistance at 0.02, 0.05, 0.1, and 0.2 ml/min levels of infusion, but at 0.5 and 1.0 ml/min there were small but consistent decreases in resistance. Since the average initial flow through the muscles was about 7.5 ml/min, an infusion of 1.0 ml/min of saline would make up approximately 13% of the total blood volume entering the muscle. The hematocrit would obviously be lower than normal, so it seems probable that this factor could be responsible for part of the decrease in resistance at the higher levels of infusion.

This study shows the direct effects of serotonin on the muscle vasculature, which may be different from the indirect effects. Serotonin was infused into the gracilis artery at rates that would not result in systemic effects, but would cause only local effects. The direct effect of serotonin on muscles with low initial resistance was an increase in resistance. Muscles with high initial resistances consistently showed drops in resistance when serotonin was infused.

Takacs and Vajda (1963) reported an increase in blood flow in skeletal muscles following the intraperitoneal injection of 10 mg/kg of serotonin. Goodman (1965) has also stated that vasodilation is the usual response of the skeletal muscle vasculature to serotonin. Daugherty and colleagues (1968) recently made a comparison of the effects of intrabrachial and intravenous administration of serotonin on forelimb blood flow in the dog. Almost no change in total flow from the limb was observed during intrabrachial or intravenous administration of serotonin. However, a shift in flow from the cephalic (skin) vein to the brachial (muscle) vein occurred with no net change in total outflow (during intrabrachial infusion at flow = K), suggesting that serotonin affects the two parallel beds in opposite directions, increasing vascular resistance in the skin and possibly decreasing it in the muscle (Daugherty et al., 1968). From these earlier reports

one would think that infusion of serotonin and vasodilation of the muscle vasculature would go hand in hand. However, more recent research, including the author's study, indicate that this is not the case. Emerson and associates (1968) investigated the local effects of serotonin on resistance to blood flow in 20 isolated innervated gracilis muscles of dogs; six of the muscles were perfused at constant flow and 14 at natural flow. Intra-arterial infusion of serotonin at 2-100 ug/min had variable effects on perfusion pressure and no effect on small vein pressure in the constant flow experiments. In the natural flow experiments, infusion of 2-100 ug/min of serotonin increased the blood flow in the ten experiments in which the initial total vascular resistance per 100 grams was above 10 mmHg/ml X min X 100g. In the four experiments with initial resistances below 10, blood flow fell in two and did not change in the other two. It was concluded that "the response of the innervated gracilis muscle was irregular and perhaps in part related to the initial level of resistance (Emerson et al., 1968)."

Others have related serotonin's effects, in part, to the initial level of resistance. Serotonin has been observed to lower blood pressure in animals with neurogenic hypertension and elevate pressure in animals with neurogenic hypotension (Page and McCubbin, 1956; Haddy, 1960; Garattini and Valzelli, 1965; Haddy and Scott, 1966). After completing a series of

experiments on the dog foreleg, Haddy described this response by stating, "Serotonin antagonizes extremes of vascular tone induced by neurogenic means. It produces net dilation when the bed is constricted and net constriction when the bed is dilated (Haddy, Gordon and Emanuel, 1959)."

The author's own experiments show quite clearly that the effect of serotonin upon total vascular resistance in the dog gracilis muscle is dependent upon the initial level of resistance in that muscle. In 50 out of 52 experiments (constant and natural flow), muscles with an initial total resistance (R_T) greater than 12.3 mmHg/ml X min X 100g showed a drop in resistance and those with an initial R_T lower than 12.3 showed a rise in resistance during serotonin infusion. Several muscles with initial resistances in the "twilight zone" (near 12.3) showed drops in resistance at some levels of serotonin infusion and rises at others. In two experiments serotonin initially dropped resistance, but later during the same experiments, resistance dropped spontaneously; serotonin now caused a marked increase in resistance.

This information would seem to back previous statements calling the degree of autonomic nervous activity the most important mechanism controlling vascular reactivity to serotonin (Page, 1953; McCubbin, Kaneko and Page, 1962). However, the decrease in resistance caused by serotonin in 25 muscles with high initial resistance could be reversed to an increase in resistance when the resistance was

subsequently lowered by metabolically induced vasodilation. Thus the muscle's response to serotonin does not appear to depend upon neurogenic tone as such, but rather upon the level of resistance at the time serotonin is infused.

It should be emphasized that the average effects of serotonin and saline in all 52 experiments combined are quite similar, i.e., if all experiments are simply evaluated together, serotonin appears to have no significant effect on muscle resistance. In order to show the real effect of serotonin, the experiments must be divided into groups according to the initial level of vascular resistance based on a uniform muscle weight. More insight could be obtained if it were known how the distribution of total resistance (large versus small vessels) affects the response to serotonin.

SUMMARY AND CONCLUSIONS

1. The direct effect of serotonin on muscles with low initial resistances was an increase in resistance.

2. Muscles with high initial resistances consistently showed drops in resistance when serotonin was infused.

3. The decrease in resistance caused by serotonin in muscles with high initial resistance could be reversed to an increase in resistance when the resistance was subsequently lowered by metabolically induced vasodilation. This implies that the muscle's response to serotonin does not depend upon neurogenic tone, but rather upon the level of total muscle vascular resistance at the time serotonin is infused.

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