HISTAMINERGIC VASODILATATION IN THE HINDLIMB OF THE DOG

by

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ABSTRACT

Thirty-four dogs were anesthetized with sodium pentothal i.v. and maintained with i.v. alpha-chloralose. Neuro-muscular blockade was accomplished with gallamine triethiodide (Flaxedil). Respiratory P_{CO_2} was monitored continuously while artificial ventilation at a rate of 15 cpm and appropriate tidal volume was adjusted to maintain expiratory P_{CO_2} between 38 and 40 mm Hg. Blood gas analysis (P_{CO_2}, P_{O_2} and pH) allowed maintenance of blood pH between 7.35 and 7.45 by periodic administration of i.v. sodium bicarbonate. Blood volume was maintained with Dextran 75 when necessary. Body temperature was monitored continuously with an esophageal thermister and maintained automatically with heating elements in the operating table.

Arterial vascular isolation of the hindlimbs was accomplished by ligating all major branches of the aorta below the renal arteries except the external iliac arteries. The dog's own blood, taken from a cannula in the abdominal aorta just distal to the renal arteries,

was perfused at constant flow into cannulae in the external iliac arteries through separate pumps. Each external iliac artery pressure was monitored separately (Fig. 1). A bilateral laminectomy allowed access to the $L_{5, 6}$ and $_{7}$ spinal segments for electrical stimulation of their ventral roots after section of the corresponding dorsal root.

In 26 dogs monophasic square wave stimulation (3 to 10 V, 3 msec, 8 to 20 Hz) of the ventral root of L_5, L_6 or L_7 induced 1) a decrease in the perfusion pressure (PP) in the ipsilateral hindlimb (-41.8 ± 2.7 mm Hg; mean $\pm$ SE); 2) a decrease in the PP in the contralateral hindlimb (-32.2 ± 2.7); 3) a fall in the aortic pressure (-15.6 ± 0.7). (Fig. 3). Similar effects were observed on stimulation of the peripheral stump of the ventral root.

The above described vascular effects of ventral root stimulation were resistant to intra-arterial injections of cholinergic and beta-adrenergic blocking agents administered directly into the hindlimb perfusion lines. The effectiveness of the blockades was tested with direct intra-arterial injections of the appropriate agonists. Antihistaminics (diphenhydramine and

mepyramine) similarly administered and tested did abolish the response in doses which did not suppress vascular reactivity to acetylcholine or isoproterenol.

These experiments do not provide a clear explanation of the mechanisms responsible for the contralateral vasodilatation or the fall in aortic pressure. The presence of significant anastomotic channels connecting either the two hindlimbs and/or the hindlimbs with the rest of the body was excluded. Contralateral vasodilatation might perhaps be explained by the presence of nerve fibres crossing the midline in the fused impar ganglion of the dog. The drop in aortic pressure was not due to the activation of afferent fibres coursing in the ventral roots, nor to the peripheral release of a vasodilator substance since the onset of the phenomenom was too fast to be explained on these grounds. The possibility exists that the drop in aortic pressure is due to the activation by the stimulated efferent fibres of some afferent nervous pathways carrying inhibitory impulses to the vasomotor centers. The present experiments, however, do not provide data supporting or

excluding this hypothesis. The experimental results strongly suggest that the described vasodilatation may be mediated by histamine released directly or indirectly by the activation of fibres coursing into the lower ventral roots.

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PART I. INTRODUCTION

LITERATURE REVIEW

1) Active and Passive Vasodilatation

Ever since the experiments of Claude Bernard on the rabbit ear there has been a consensus amoung physiologists that the systemic arterial blood pressure is regulated through the tonic discharge of the sympathetic vasoconstrictor nerves to the resistance vessels (Bernard, 1851; Bayliss, 1923; Beck and Brody, 1961). The mechanism of physiological vasodilatation, on the other hand, has been the subject of considerable dispute over the years, with respect to both its mechanism of occurance and its role in normal blood pressure homeostasis (Beck and Brody, 1961). While Bernard demonstrated the dilatation of the salivary gland vasculature in response to electrical stimulation of the chorda tympani (Bernard, 1858), authorities in the field disagreed on the question of whether dilatation was as active response of the vascular smooth muscle to nerve stimulation or was a passive response occuring on cessation of firing of constrictor fibres to the vessel. The two experiments

of Claude Bernard (Bernard, 1851, 1858) had demonstrated both dilatations, one occuring on nerve section (passive dilatation), and one occuring on stimulation of the nerve supplying the vasculature (active dilatation).

Active and passive vasodilatation have, in more recent years, been classified more exactly according to their causes (Beck, 1958b, 1961, 1963; Beck and Brody, 1961; Brody 1966). Passive vasodilatation has been defined as that occuring on interruption of the secretion of a constrictor substance at the vessel level. This definition, by its nature, makes no implication on the mechanism of action of the dilatation, the transmitter or humoral substance involved, or the precise site of vascular resistance change. It does however include the prerequisite that a certain amount of tone be present in the vascular smooth muscle in order for this dilatation to occur. Active vasodilatation has been defined as that which results from the direct action on the vessel of an agent capable of relaxing vascular smooth muscle. Again, no implication about the mechanism of action in-

volved is included in the definition. While the magnitude of the dilatation must be limited by the pre-existing vascular tone, it is not directly dependant on the existence of such tone as in the case of passive dilatation.

The controversy existing before the turn of the century, stemming largely from Bernard's experiments, was based on two questions (Bayliss, 1923). First, was the tone of the vessels normally maintained by tonic discharge of the constrictor fibres, and second, was the decrease in normal tone due to an active or passive dilatation? In partial answer to this, Bayliss (1923) suggested that there were in the medulla two centers, a vasodilator center and a vasocontrictor center. Essentially, this concept is still held by many as valid today.

During the 1940's and early 1950's the theory was developed that normal neurogenic tone to the vessels arose from the tonic discharge of the vasoconstrictor center of the medulla, that this activity was soely responsible for the maintenance of systemic arterial pressure, and that dilatation of the vessels occurred when the activity

of this center was inhibited by input from the baroreceptors of other inhibitory afferent inputs (Folkow and Uvnas, 1948; Frumin et al, 1953; Heymans and Neil, 1958). This theory was based on severallines of evidence (Folkow and Uvnas, 1948; Lingren and Uvnas, 1954; Beck and Brody, 1961) and was maintained in spite of the discovery of cholinergic vasodilator fibres in the early 1950's by the Scandinavian physiologists (Eliasson, Lingren and Uvnas, 1952, 1954; Folkow et al, 1949; Folkow and Uvnas, 1948; Folkow, Haeger and Uvnas, 1948). Such evidence included the findings that increased aortic arch or carotid sinus pressure led to a total or near-total inhibition of sympathetic discharge (Bronk, Pitts and Larrabee, 1939), and that stimulation of the baroreceptor nerves led to a similar inhibition of discharge (Bronk, Ferguson and Margaria, 1936). The reflex lowering of blood pressure by the pulmonary depressor reflex (chemoreflex) and the coronary shemoreflex (Benzold-Jarisch reflex) have been attributed to this sort of inhibition (Heymans and Neil, 1958). Certainly the fact that in the baroreceptor deafferented animal there is a rise in

systemic blood pressure supports this hypothesis.

2) Evidence Supporting the Existence of Active Vasodilatation.

Notwithstanding the above evidence, there remained the 1958 experiment of Bernard in which stimulation of the chorda tympani caused dilatation of the vessels of the submaxillary gland of the rabbit. Active reflex vasodilatation was shown in 1947 (Binet and Bernstein) using the cross-circulated paw of the dog, and the reflex was shown to be mediated by the baroreceptors and to travel with the sympathetic nerves. The well known cholinergic vasodilator fibres of the Scandinavian authors were discovered about the same time. Clearly, it had to be accepted that passive dilatation was not the only method of lowering blood pressure in the intact animal, even if it were the most important method. Despite the relatively strong evidence in favor of the existence of an active dilator system there remains strong resistance to the concept.

Binet and Bernstein (1947a, 1947b) demonstrated active reflex vasodilatation in an essentially cutaneous preparation, the dog paw. It has been subsequently shown that the reflex occurs in the skinned hindlimb

of the dog as well (Beck, 1961). Just as the inhibition of discharge in the sympathetic nerves in response to increased carotid sinus or aortic arch pressure has been used as evidence in favor of the passive dilatation theory (Heymans and Neil, 1958), increased firing in the rabbit splanchnic nerve has been seen in response to an increase in systemic arterial pressure (Millar and Briscoe, 1965), suggesting possible activation of vasodilator fibres to the mesenteric vascular beds. This was seen in five of sixteen cases studied. More recently, increased firing in the sympathetic nerves of the vetral roots of cats in response to increased systemic blood pressure has been observed (Tuttle, quoted by Brody, 1966).

One of the pieces of evidence most often quoted in favor of an active vasodilator system is that the level to which vascular resistance falls during active reflex dilatation is lower than the sustained level to which it falls following sympathectomy, complete pharmacological ganglionic blockade, or complete alpha-adrenergic blockade (Beck, 1961; Binet and Bernstein, 1947a, 1947b; Sakuma and Beck, 1961; Frumin, Ngai and Wang, 1953; Folkow and Uvnas, 1948; Wyse et al, 1971).

However, since myogenic autoregulation will raise vascular resistance after loss of sympathetic tone, it is important to compare the vascular resistance observed during the active dilatation to the immediate transitory levels obtained after denervation and not to the sustained level of post-sympathectomy resistance, which is partly due to the non-neurogenic autoregulatory phenomenon. In accordance with this reasoning, some authors have attempted to explain the active vasodilatations as transient responses of the vessels occurring upon loss of constrictor tone but before autoregulatory compensation (Jones and Berne, 1963; Glick, Wechsler and Epstein, 1968). Wyse et al (1971) disagree with this interpretation of the results seen in most experiments, demonstrating that the transient dilatations seen following sympathectomy are antihistamine sensitive, as are active reflex dilatations, while intentionally induced autoregulatory changes are not.

3) Nervous Pathways Involved in Active Reflex Vasodilatation.

In searching for evidence for a depressor reflex

central relay point, Aoki and Brody (1966) have seen increased activity of the medullary vasodilator centers when blood pressure was suddenly raised by the intravenous injection of pressor amines. Stimulation of these same centers causes a peripheral vasodilatation (Aoki and Brody, 1966). Moreover, it has been shown that a transection of the medulla just cephalad to the obex abolishes all sympathetic constrictor tone but does not abolish active reflex dilatation invoked by intravenous injection of pressor amines (Beck, DuCharme, Gebber, Levin and Pollard, 1968).

In support of the idea that the fibres involved in this reflex are sympathetic is the finding that the reflex can be abolished by the administration of hexamethonium in amounts sufficient to completely block transmission in the sympathetic ganglion (Wyse et al, 1971). It has been known for some time that the ganglionic stimulant DMPP is capable of producing a fall in blood pressure which is greater than that resulting from sympathectomy (Sakuma, 1964). This has been shown true for intravenous veratrine (Brody, DuCharme and

Beck, 1967) and for the dilatations produced by increased carotid sinus pressure (Beck and Brody, 1961) and by low voltage stimulation of the medullary depressor areas (Aoki and Brody, 1966).

4) Evidence Supporting Histamine as a Mediator of Active Reflex Vasodilatation.

Because of the marked effect of antihistamines on active reflex dilatations invoked by several methods, it has been postulated that histamine is involved somewhere in the reflex pathway, very possibly as the final neurotransmitter (Beck, 1958b, 1965). The essential features of the pharmacological evidence in support of such a role for histamine are as follows:

1) Antihistamines of all major classes
   a) abolish active reflex dilatation
   b) abolish the reactivity of the vessels to histamine
   c) do not abolish vessel reactivity to other vasoactive agents (acetylcholine, isoproterenol)

   The above effects do not seem to be related to the central depressant action of antihistamines

2. A correlation can be shown between the progressive reduction of vascular reactivity to exogenous histamine and the progressive reduction of active reflex dilatation by antihistamines
3. The active reflex dilatation is both atropine and propranolol resistant

The above findings (Beck, 1965) are in agreement with those of others from both dogs and cats (Wellans and Wauters, 1966; Tuttle, 1965,1966). An active reflex dilatation that is non-cholinergic, although not necessarily histaminergic, has been seen in rats (Tobia, Miya and Bousquet, 1968). While active dilatation can be demonstrated in primates, its pharmacological characteristics have not been elucidated (Levin, Bartlett and Beck, 1968).

Radioisotope work has shown the release of ^{14}C labelled histamine into the venous effluent of a perfused limb concurrent with the increased blood flow of active reflex dilatation (Brody, 1966). Similarly, stimulation of the sympathetic trunk with parameters that produce active dilatation is followed by the increased

rate of incorporation of labelled histidine into histamine, possibly by the activation of histidine decarboxylase (Schayer, 1960; Tuttle, 1967).

There are numerous reports in the literature of increased blood histamine levels during increased baroreceptor activity (Brody, 1966; Tobia, Adams, Miya and Bousquet, 1969; Tuttle, 1967), while stable histamine levels are reported in chronic buffer nerve deafferented preparations (Went and Varga, 1952). Stimulation of the central nervous system can release histamine into the blood at peripheral sites (Tuttle, 1966; Tuttle and McLeary, 1970).

Examination of the distribution of histamine in the nervous system shows the highest concentrations in the post-ganglionic sympathetic nerves while the lowest is in the spinal cord (Green, 1964; Schayer, 1962; Earle and Palm, 1950). Appreciable concentrations of histamine-N-methyl transferase (responsible for the degradation of histamine to methyl-histamine) have been found in the sciatic nerve in association with sympathetic neurons (Brown, Tomchick and Axelrod, 1959).

5) Evidence Against Histamine as a Mediator of Active Reflex Vasodilatation

Criticism of the hypothesis that histamine is a neurotransmitter at peripheral sites has taken the form that the antihistamines used in such studies interfere with normal pysiological processes of the perfused area. The strongest argument of this type is the claim that certain antihistamines block the reuptake of norepinephrine into the nerve terminals (Isaac and Goth, 1967) and may thus decrease the magnitude of a purely passive dilatation by prolonging the effect of endogenously released amine. On the other hand, it has been claimed that some drugs which inhibit adrenergic transmission also inhibit active reflex dilatation (Glick et al, 1968). However, it has recently been reported that one such drug, phenoxybenzamine, not only blocks alpha receptors, as shown by an increase in blood norepinephrine levels on stimulation of the sympathetic nerves to the area, but also inhibits the release of labelled histamine during active reflex dilatation (Boerth, Ryan and Brody, 1970).

Hence the reduction in magnitude of reflex dilatation following the administration of this drug might possibly be due not to its alpha blocking characteristics but to its ability to prevent release of histamine at the vessel level. Moreover, apart from its antihistaminic properties, the fact that phenoxybenzamine decreases the normal constrictor tone to the vessels would decrease the magnitude of a reflex dilatation.

It has similarly been shown (Boerth et al, 1970) that other pharmacological agents which have been claimed to decrease the magnitude of reflex dilatation have antihistaminic properties. Included amoung these are tripelenamine and cocaine.

A possible alternative explanation for the increased level of labelled histamine shown in the perfusate of a skeletal muscle vascular bed during active reflex dilatation is that the redisribution of blood flow in that region results in an increased washout of histamine without an increased amount of histamine necassarily being released from the nerves in the area (Glick et al, 1967). This argument has been refuted by the ob-

servation that the decrease of the dilatation by blocking agents does not affect the elevation of blood histamine during the reflex (Boerth et al, 1970). If the increase in blood histamine at that time were due to a washout effect then any diminution of the reflex should be accompanied by a diminution of the washout. This was shown to be untrue by Boerth et al (1970).

A complicating factor in the examination of the postulated histaminergic dilator fibres to the extremities has been the action of agents used to block constrictor tone to these regions. Thus bretylium, guanethidine and xylocaine have been shown to inhibit histaminergic vasodilatation as well as adrenergic transmission. Chronic reserpine treatment abolishes both constriction and reflex dilatation, while acute reserpine treatment results in abolution of reflex dilatation befroe abolition of reflex constriction (Sakuma and Beck, 1961). Ergot alkaloids are capable of abolishing histaminergic dilatation at doses that do not affect the constrictor fibres (Beck, 1961; Wellans, 1964). more recently, work by Wyse, Beck, Burks and Spalding (1971) has shown that many drugs

interfere with the release of histamine in the perfused hindlimb of dogs.

Ryan and Brody (1972) have recently challenged the concept of a histaminergic innervation functioning in a classical way, that is, with histamine being directly released by post-ganglionic terminals. They suggest that histamine is probably released from non-neurogenic storage site, possibly the mast cells, under the action of either a collateral branch of a constrictor fibre or of a separate adrenergic neuron.

In a monograph on the subject of smooth muscle, Campbell makes the following statement: "In view of the difficulty in finding drugs which selectively eliminate the vasoconstrictor effects of adrenergic nerves to reveal histaminergic vasodilator responses, it is clear that the most effective way to study the histaminergic vasodilator nerves is by creating a situation in which only these nerves are stimulated, a result which cannot be obtained by stimulation of mixed nerve trunks in the periphery." (Campbell, 1970). While selective stimulation of histaminergic pathways

has now been obtained by medullary stimulation (Tuttle, 1966) there is no report in the literature describing a pure vasodilatation obtained by stimulation of nerves outside the central nervous system.

Donald and Ferguson (1970), in a study of the level of exit of sympathetic constrictor fibres from the spinal cord in dogs, have demonstrated that the lowest level of exit of such fibres is L_3 or L_4 and that stimulation of the ventral roots of L_5, L_6 or L_7 results in a pure hindlimb vasodilatation, uncontaminated by vasoconstrictor responses. They found that this dilatation was resistant to the intravenous injection of atropine, propranolol or Benadryl, an antihistaminic. Since no tests of the effectiveness of any of the blockades were performed by these authors, we decided to investigate this peculiar vasodilatation further, injecting the blocking agents directly into the perfused limbs and checking the extent of the blockades by injecting the appropriate agonists directly into the perfusion lines as well.

METHODS

1) Animals and Anesthesia

Forty-eight adult mongrel dogs weighing from 10.0 to 30.0 kg and eight cats weighing from 2.3 to 4.2 hg were used. Anesthesia was induced with sodium pentothal (20 mg/kg i.v.) in the dogs and with fluothane (Ayerst) in the cats. All animals were maintained at a steady level of anesthesia with alpha-chloralose (60.0 mg/kg i.v. initially followed by 5.0 mg/kg i.v. every 30 min., given as a 1% solution in saline). All i.v. injections were made via a polyethylene cannula inserted in the left saphenous vein and advanced as far as the inferior vena cava.

2) Maintenance of the Animal

Respiratory P_{CO_2} was monitered continuously with a Beckman model Lb-1 medical gas analyser. The animals were artificially ventilated with a harvard model 614 respirator. Ventilatory rate was set initially at 15 cpm with appropriate tidal volume to give an end-epiratory P_{CO_2} of 38 to 40 mm Hg and was adjusted as necessary to maintain this value. A 50% O_2, 50% N_2 mixture was administered in open circuit. End expiratory

resistance was set at 3 cm of water. Arterial blood samples were taken periodically and tested for P_{CO_2}, P_{O_2} and pH values with an Instrumentation Laboratory blood gas analyser. Arterial pH was maintained near 7.4 by administration of appropriate amounts of sodium bicarbonate (90 mM% in saline) when necessary. Blood volume was maintained during the experiment by the slow i.v. infusion of Dextran 75. Heparin (3 mg/kg initially followed by 0.75 mg/kg every 30 min. throughout the experiment) was given i.v. just before the cannulation of the hindlimb vessels. Esophageal temperature was monitered continuously with a thermal probe (Yellow Springs Instrument Company) and maintained near 37 degrees C by heating elements in the table and a 0 to 2000 watt variable infra-red lamp above the table.

3) Surgical Methods

A tracheal cannula was inserted and fitted with a side needle for monitering end-expiratory P_{CO_2}. Both right and left carotid arteries were isolated and a loose ligature of umbilical tape was placed around them to allow later periodic occlusion as a test of

baroreceptor respnse. A short stiff polyethylene cannula was placed in the right brachial artery and used for monitoring central arterial pressure and for obtaining arterial blood samples for blood gas and pH determinations.

A bilateral laminectomy was performed between L_4 and L_7 inclusive to allow access to the 5th, 6th and 7th lumbar spinal nerve roots on both sides. After removal of the lamina the dura was left intact until all other surgery was completed in order to minimize trauma to and drying of the spinal cord.

4) Methods of Hindlimb Perfusion

The abdominal aorta was approached through a midline abdominal incision and a polyethylene cannula, as large as could be inserted, was placed in the aorta and advanced to a point just distal to the renal arteries. This cannula was fitted with a "Y" connector attached to the two distal cannulae by two lengths of Tygon tubing. In all cats and some dogs the distal cannulae were inserted into the external iliac arteries. In most dogs the distal cannulae were inserted into the femoral

arteries. To minimize collateral flow between the hindlimbs and other areas, all major branches of the aorta below the renal arteries were ligated and the abdominal aorta was ligated around the central cannula (Fig. 1).

Adequate vascular isolation of a hindlimb was assumed if, on stoping the perfusion pump to that limb, the perfusion pressure fell to about 30 mm Hg, well below both the systemic arterial pressure and the perfusion pressure of the contralateral limb (Fig. 2).

5) Perfusion Pressure Recording

Each hindlimb was perfused with a peparate Watson-Marlowe pump. Both of these roller pumps delivered constant flows over a pressure range of 0 to 300 mm Hg perfusion line pressure. In some experiments a cannulating electromagnetic flow meter probe was placed in the perfusion line distal to the pump and flows were monitored continuously throughout the experiment with a Biotronix flowmeter. The flowmeter was calibrated at the end of the experiment using the same cannulae used during the experiment.

Perfusion pressures were measured distal to the

pumps using Statham P 23 Db transducers and recorded on either Electronics for Medicine or S.E. Laboratories optical recorders. Pump speed was adjusted at the beginning of the experiment to provide a perfusion pressure equal to that in the brachial artery, which was also recorded continuously (Fig. 1).

6) Drugs Used

Drugs were injected into the perfusion line immediately proximal to the pumps (Fig. 1). The following drugs were used:

1. Pentothal sodium (Abott, Montreal) 30 mg/kg
2. alpha-Chloralose (BDH, Toronto) 60 mg/kg initially, 5 mg/kg every 30 min maintenance
3. Heparin sodium (Nutritional Biochemicals, Cleveland) 3 mg/kg initially, 0.75 mg/kg every 30 min maintenance
4. Gallamine triethiodide (Flaxedil, Poulenc, Montreal) 50 mg every hour or more often if required
5. Propranolol (Inderal, Ayerst, Montreal) 1.0 mg/kg
6. Isoproterenol (Isuprel, K & K Laboratories, N.Y.) 1 ug
7. Atropine sulfate (Nutritional Biochemicals, Cleveland) 0.2 mg/kg

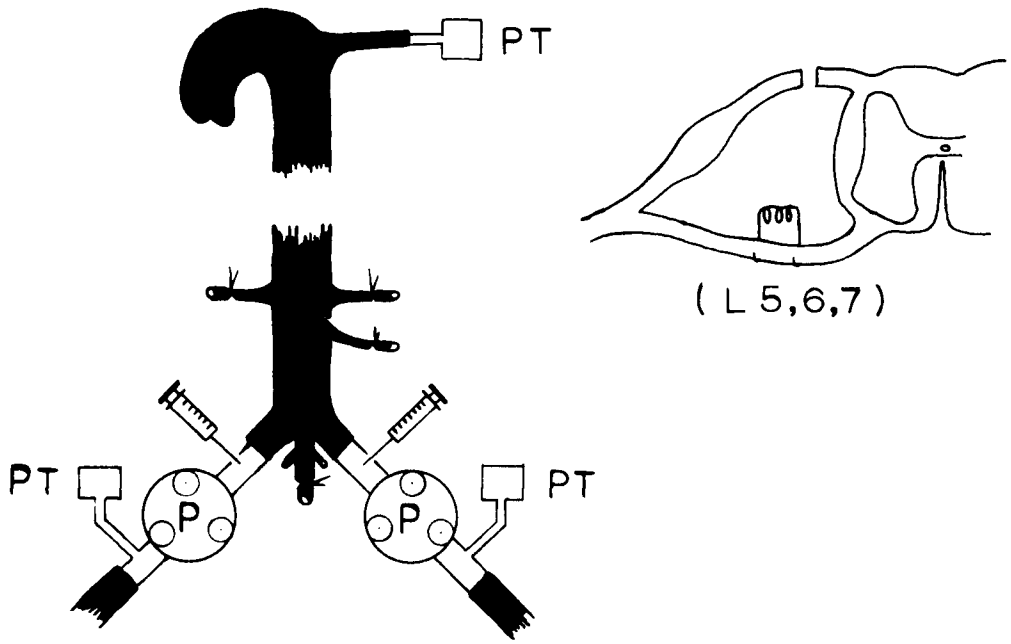

Figure 1.

Schematic representation of the preparation used in most experiments. PT = perfusion pressure; P = perfusion pump; L_5, 6, 7 = lumbar ventral root 5, 6 or 7. Drug administration was into the perfusion line proximal to the perfusion pump.

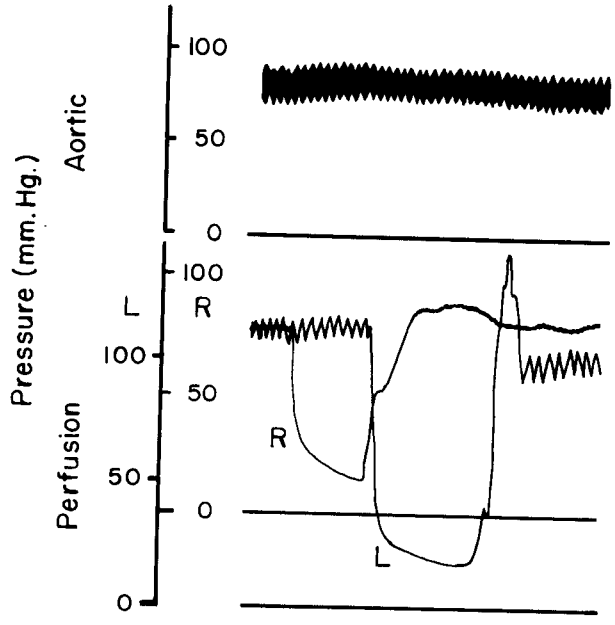

Figure 2.

Effect on perfusion pressures of stopping one perfusion pump at a time. From above: aortic pressure, right hindlimb perfusion pressure, left hindlimb perfusion pressure.

8. Acetylcholine (Calbiochem, Los Angeles) 2ug
9. Diphenhydramine (Benadryl, Parke-Davis, Brockville, Ontario) 1.5 to 3.0 mg/kg
10. Histamine (BDH, Toronto) 1 to 4 ug
11. Mepyramine maleate (Neo-Antergan, Poulenc, Montreal) 5.0 mg/kg
12. 6% Gentran 75 (Dextran 75) (Travenol Laboratories, Morton Grove Ill.)
13. Hexamethonium bromide (K & K Laboratories, N.Y.) 10 mg/kg

7) Experimental Protocol

After administration of Flaxedil (50 mg/kg) the dura was opened and a spinal nerve dorsal root ($L_{5, 6}$ or $_7$) was sectioned and pulled clear of the field. The corresponding ventral root was then placed on platinum wire electrodes and bathed in a pool of warm paraffin oil. Monophasic square wave pulses (10 V, 8-20 Hz, 3 msec) were used for stimulation. At least three minutes were allowed between successive stimulations.

In the pharmacological block experiments the following procedure was observed: the vascular response to ventral root stimulation was recorded, the the vascular response to a test dose of the agonist drug was recorded as the agonist was injected into the perfusion line. The

appropriate blocking agent was injected slowly into the perfusion line and its effects tested with another injection of agonist. The degree of block of the vascular response to the agonist was calculated as a percentage of the original response. A second stimulation of the ventral root was made to evaluate the degree of block of neurogenic vasodilatation. The degree of block of the ventral root response was calculated as a percentage of the first (pre-blocking agent) stimulation response.

8) Interpretation of Results

Because both blood flow rate and cannula resistance were constant, a change in vascular resistance in the hindlimb was reflected in a change in perfusion pressure to that hindlimb. Both the absolute magnitude of pressure change and percentage (of total perfusion pressure) change were calculated. results are expressed as the absolute change in perfusion pressure. Responses from each animal were averaged and the resulting value was then used to calculate the mean and standard error presented in the tables. Duration of the stimulus response was measured from the onset of vascular response

to the point at which the perfusion pressure returned to pre-stimulus levels.

Statistical analysis was done using the Triangular Regression Package (TRIP) program on the U.B.C. IBM 360/67 computer.

9) Criteria for a Viable Preparation.

Only preparations which met the following criteria were considered viable: 1) a small blood loss during surgery and an aortic mean pressure above 100 mm Hg; 2) arterial blood gases and pH and body temperature within normal limits; 3) rapid cannulation of the vessels and no evidence of blood clotting in the perfusion lines; 4) satisfactory vascular isolation of the hindlimbs; 5) absence of drying of or trauma to the spinal nerve roots or spinal cord; 6) good vascular reactivity of the hindlimb vessels to carotid clamping or stimulation of the proximal stump of the dorsal root. Thirty-four dogs and eight cats met these requirements.

RESULTS

1) Stimulation of the Intact Ventral Root

In 26 of 34 viable dog preparations, 128 stimulations of the ventral roots of $L_{5, 6 \text{ or } 7}$, after section of the corresponding dorsal roots, produced a fall in ipsilateral hindlimb perfusion pressure of 15 to 60 mm Hg ($\bar{x}$ 42.9 $\pm$ 4.1 SE). The rate of perfusion pressure decrease was in the order of 5 mm Hg/sec, such that the dilatation reached a maximum approximately 10 to 12 seconds after onset. The dilatation showed an "escape" after reaching its peak magnitude and perfusion pressure then returned to control levels despite continued stimulation. In most experiments the stimulation was therefore discontinued when maximum dilatation had been achieved. Recovery time for the dilatation was in the order of 10 to 13 seconds and therefore the entire response had a duration of about 25 seconds (Fig. 3).

Coincidental with the ipsilateral hindlimb response was a fall in contralateral hindlimb perfusion pressure (Fig. 4). While the time of onset and rate of fall were roughly similar to those of the ipsilateral limb, the

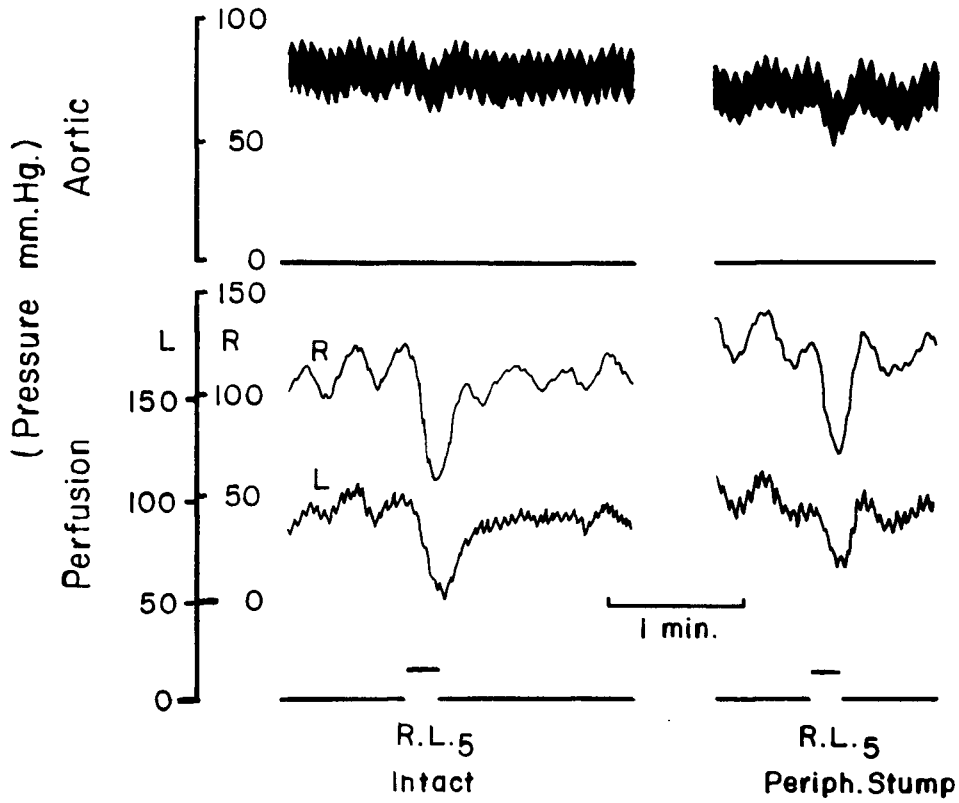

Figure 3.

Effect on hindlimb perfusion pressures of intact and sectioned ventral root stimulation. From above. aortic pressure, right hindlimb perfusion pressure, left hindlimb perfusion pressure.

absolute magnitude of the dilatation was approximately 75 to 80% of that on the ipsilateral side. This was seen in 76 trials in the 17 animals in which the perfusion pressure of the contralateral limb was measured (-36.4 ± 5.1, Table 1). The difference between the ipsilateral and contralateral dilatations was not significant (P 0.2; unpaired t-test). The fibres responsible for these effects appear to have a threshold of 3.5 to 4.0 V in the unsheathed ventral root.

Also coincidental with the onset of the ipsilateral dilatation was a fall in the systemic arterial pressure of 5 to 25 mm Hg (-14.3 ± 1.5), without appreciable changes in heart rate or pulse pressure (Fig. 4, Tabke 1).

The time lag between the start of electrical stimulation and the onset of the above described effects was generally 1 to 2 seconds.

2) Stimulation of the Sectioned Ventral Root

Peripheral stimulation of the sectioned ventral root of $L_{5, 6}$ or $_7$ produced the same effects described above on stimulation of the intact roots. Central stimulation of the sectioned ventral root did not produce any effect. In 5 animals used there was no significant difference

Table I Effect of VR stimulation on the hindlimb PP and Aortic Pressure

ventral root	ipsilateral P	contralateral P	aortic pressure P
intact root	-42.9 ± 4.1 (26)	-36.4 ± 5.1 (17)	-14.3 ± 1.5 (26)
cut root	-37.6 ± 7.2 (5)	-30.4 ± 4.9 (4)	-11.2 ± 1.6 (4)
$\bar{P}$	0.2	0.2	0.5

Table I. P = pressure change. Values are expressed as mean ± S.E. Number in brackets = number of trials. P = paired t-test.

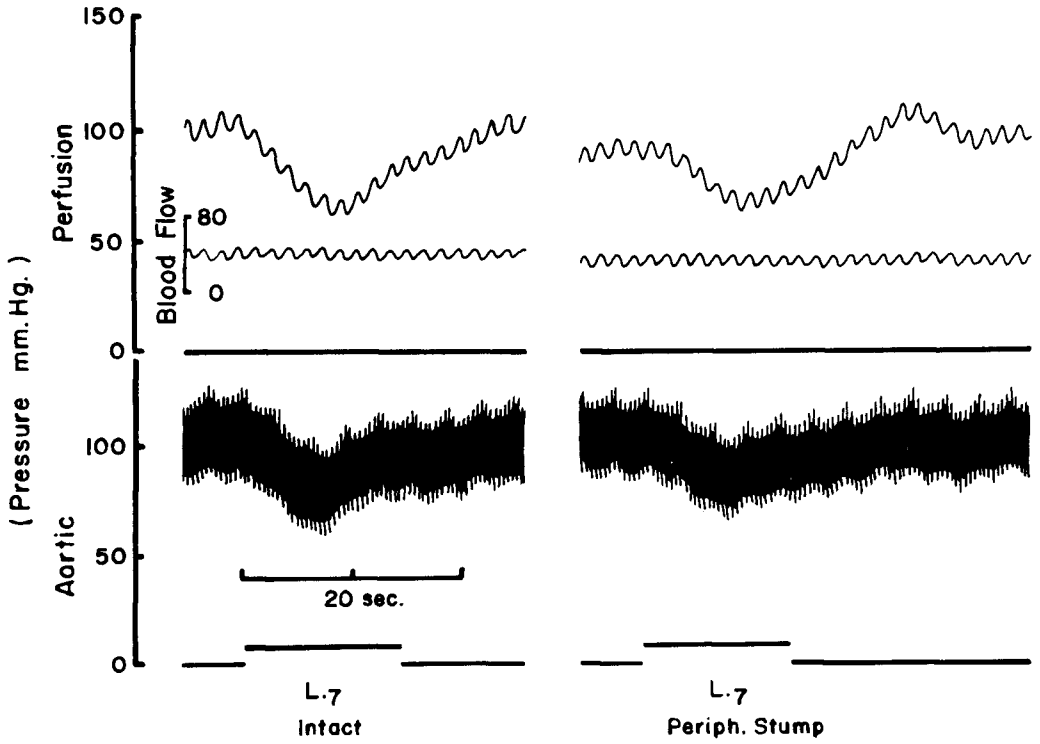

Figure 4.

Effect on hindlimb perfusion pressures of intact and sectioned ventral root stimulation. From above: hindlimb perfusion pressure, hindlimb blood flow, aortic pressure.

between the pre and post section results (Table 1); ipsilateral vasodilatation -37.6 ± 7.2, contralateral -30.4 ± 4.9, aortic pressure change -11.2 ± 1.6

3) Cholinergic Blockade

Atropine was injected i.a. in 6 animals and tested with acetylcholine as described in the Methods. The resulting cholinergic blocks were between 70 and 100%. However, there was no significant decrease in the ipsilateral (P 0.10), contralateral (P 0.10) or aortic (P 0.90) responses to ventral root stimulation (Fig. 5). In 3 dogs the intravenous injection of atropine was tested and not found to alter ventral root stimulation effects.

4) Beta-adrenergic Blockade

In 4 animals the i.a. injection of propranolol (1 mg/kg) was effective in producing an 80% blockade of i.a. isoproterenol (1 ug). Again, ventral root stimulation effects on the ipsilateral (P 0.10, cantralateral (P 0.20) or aortic (P 0.40) pressures were not significantly altered. The intravenous injection of the same dose of propranolol in 2 dogs was without effect in blocking the ventral root responses (Fig. 6).

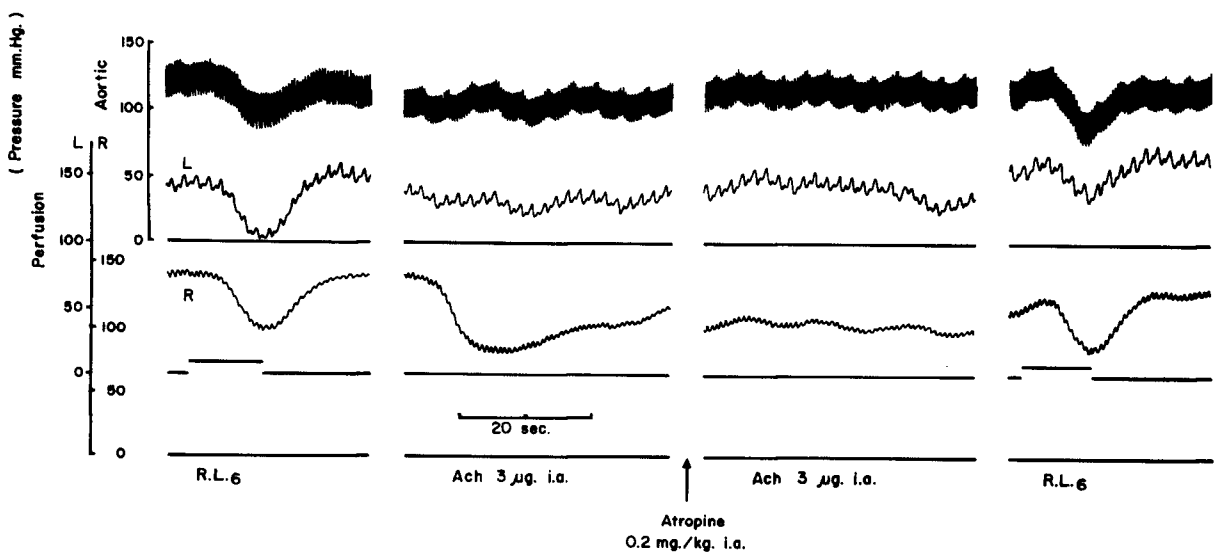

Figure 5.

Effect of ventral root stimulation and intra-arterial injection of acetylcholine on hindlimb perfusion pressure before and after intra-arterial atropine. From above: aortic pressure, left hindlimb perfusion pressure, right hindlimb perfusion pressure.

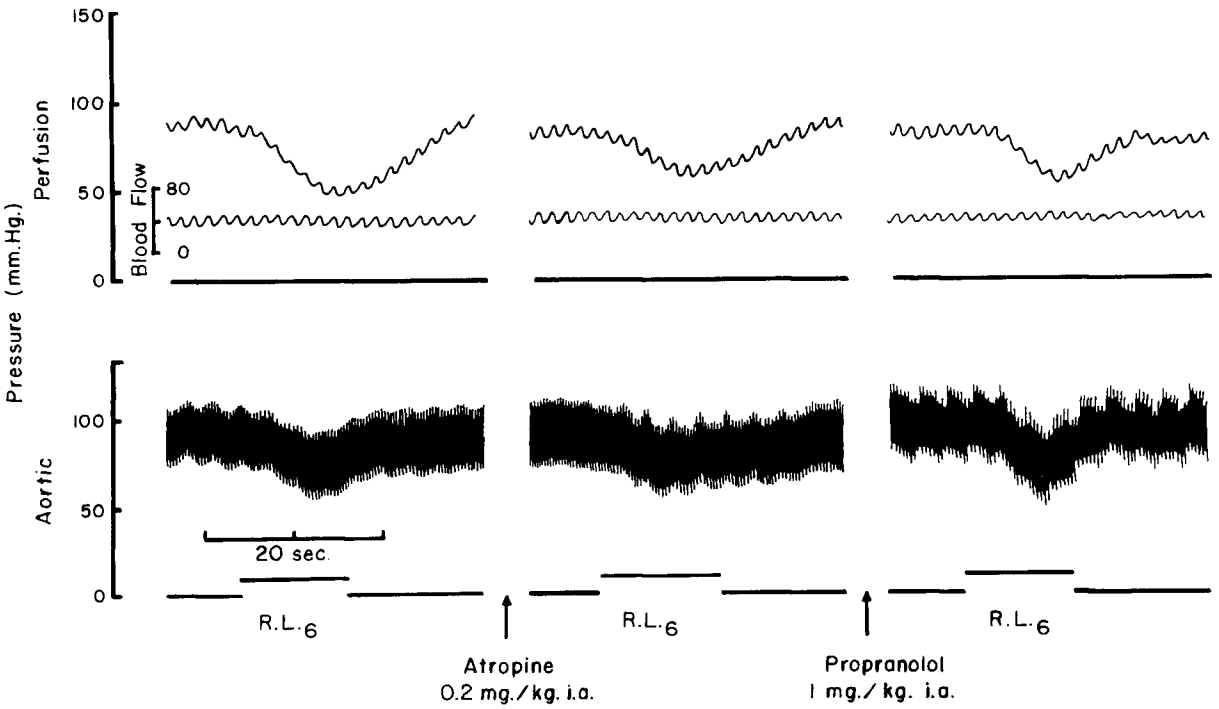

Figure 6.

Effect of ventral root stimulation on hindlimb perfusion pressure and blood flow before and after administration of atropine or propranolol. From above: hindlimb perfusion pressure, hindlimb blood flow, aortic pressure.

5) Histaminergic Blockade

Intravenous administration of Benadryl (2.5 mg/kg) in 3 animals did not alter the vascular responses to ventral root stimulation. When, however, Benadryl (2 animals, 2.5 mg/kg) or mepyramine maleate (4 animals, 5.0 mg/kg) were injected i.a. blockades of between 65 and 100% were obtained to test doses of histamine, and the ipsilateral ventral root response was either drastically reduced or eliminated. Contralateral response was reduced after a time lag of several minutes. The aortic effect was sometimes reduced in the face of large doses of antihistamine but at times could not be abolished at all (Fig. 7, Table 2).

In 5 experiments smaller doses of antihistamines were injected i.a. in a stepwise fashion until the total dose had been injected. A progressive and parallel reduction of the vascular responses to ventral root stimulation and to test doses of i.a. histamine (1 to 2 ug) was observed (Fig. 7). Figure 8 shows that there is a direct relationship between the percent reduction of the vascular response to ventral root stimulation and to i.a. histamine. The regression coefficient for this set

Table II Effect of Anti-Histamines on the Vascular Effects of VR Stimulation

Group	Ipsi Lateral (Δ P)	Contra Lateral (ΔP)	Aortic Pressure (ΔP)
Control	-54.8 ± 9.6 (6)	- 50.3 ± 14.0 (4)	- 18.9 ± 3.8 (6)
Antihistamines	- 6.46 ± 3.1	- 27.4 ± 17.0	- 9.0 ± 4.2
P<	0.005	0.05	0.10

Table II. Abbreviations as in Table I. Values are expressed as mean ± S.E. Number in brackets = paired t-test.

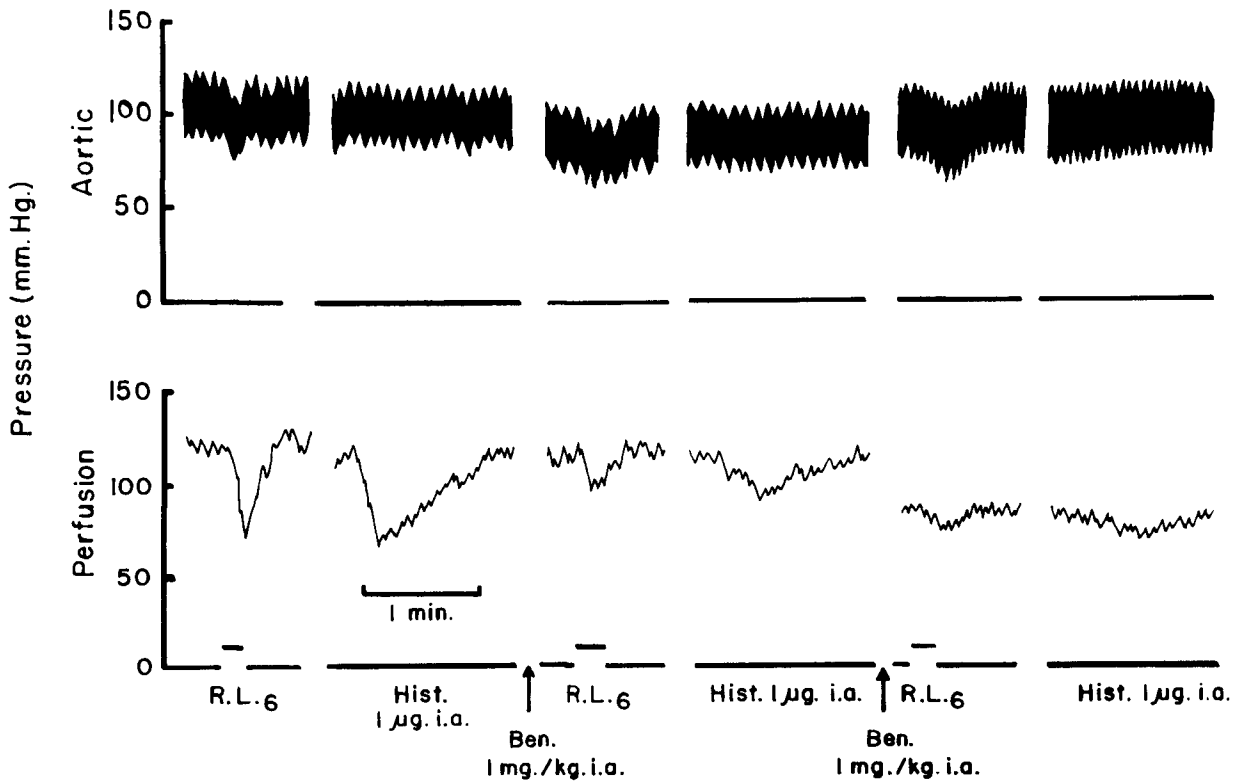

Figure 7.

Progressive blockade of hindlimb vascular response to ventral root stimulation and to intra- arterial histamine by repeated Benadryl administration.

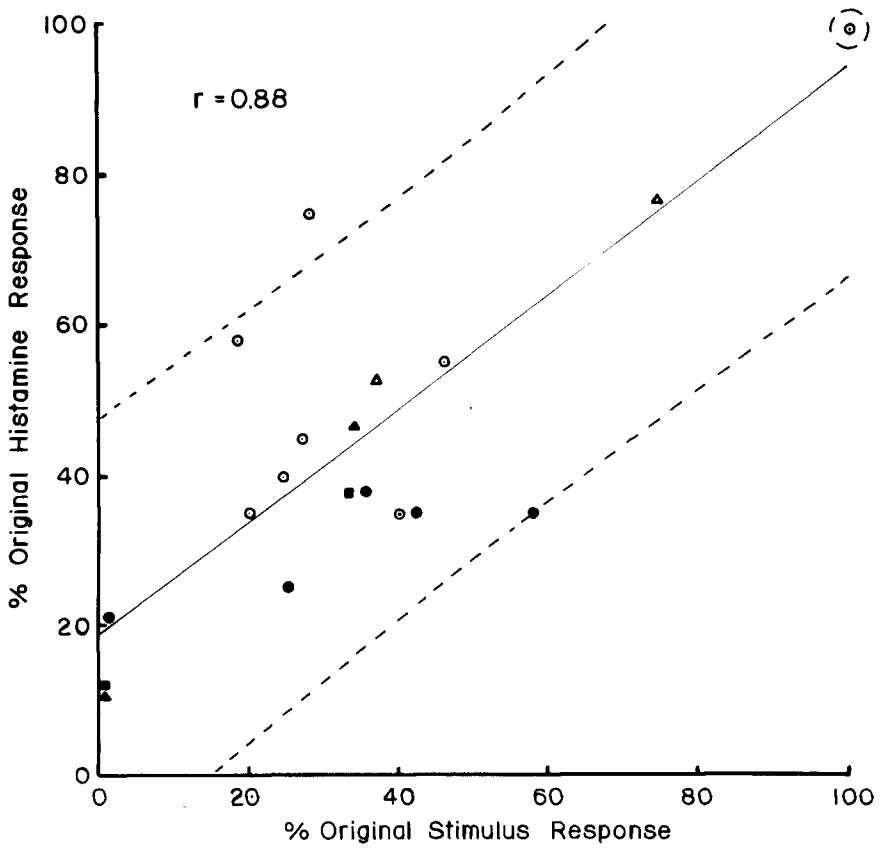

Figure 8.

Regression line plot of the relationship between reduction of vascular response to ventral root stimulation and to intra-arterial histamine. Dotted lines represent 95% confidence limits, r = regression coefficient.

of data is 0.88.

During complete blockade of vascular response to ventral root stimulation the vessels were still responsive to i.a. acetylcholine (2 to 3 ug) or isoproterenol (1 ug) (Fig. 9), and vasoconstriction was still present following clamping of the carotid arteries (Fig. 10).

6) Hexamthonium Blockade

In 3 experiments the intravenous administration of hexamthonium (5-10 mg/kg) caused a fall in both aortic and perfusion pressures and the complete abolition of of all the vascular effects induced by ventral root stimulation. The completeness of ganglionic blockade was demonstrated by the disappearance of the vascular responses to carotid clamping or to dorsal root central stimulation. However, the vessels could still respond to test doses of i.a. acetylcholine, isoproterenol or histamine (Fig. 10).

In only one of the 8 cats used, stimulation of the ventral root induced the above described effects. Also, in this animal the dilatation was atropine and proranolol resistant but antihistamine sensitive. The results of this single experiment were not pooled with those obtained in dogs.

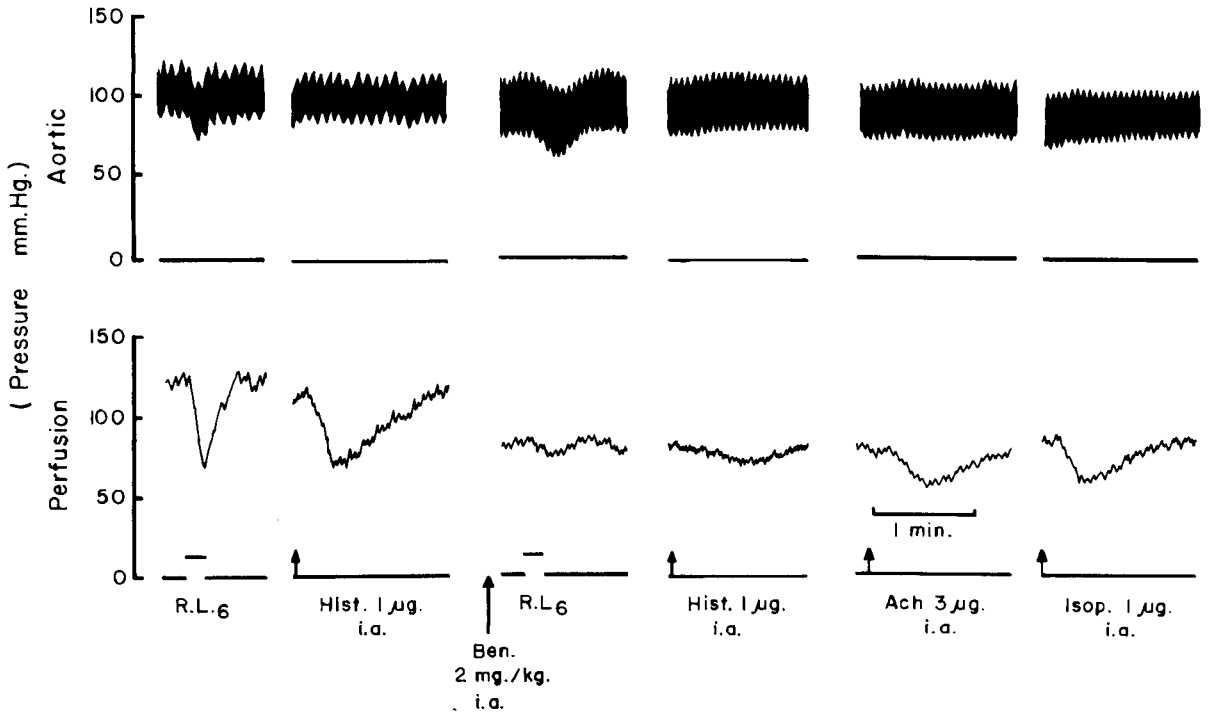

Figure 9.

Effect of ventral root stimulation, intra-arterial histamine and Benadryl on hindlimb vasodilatation. Hist. = histamine, Ben. = Benadryl.

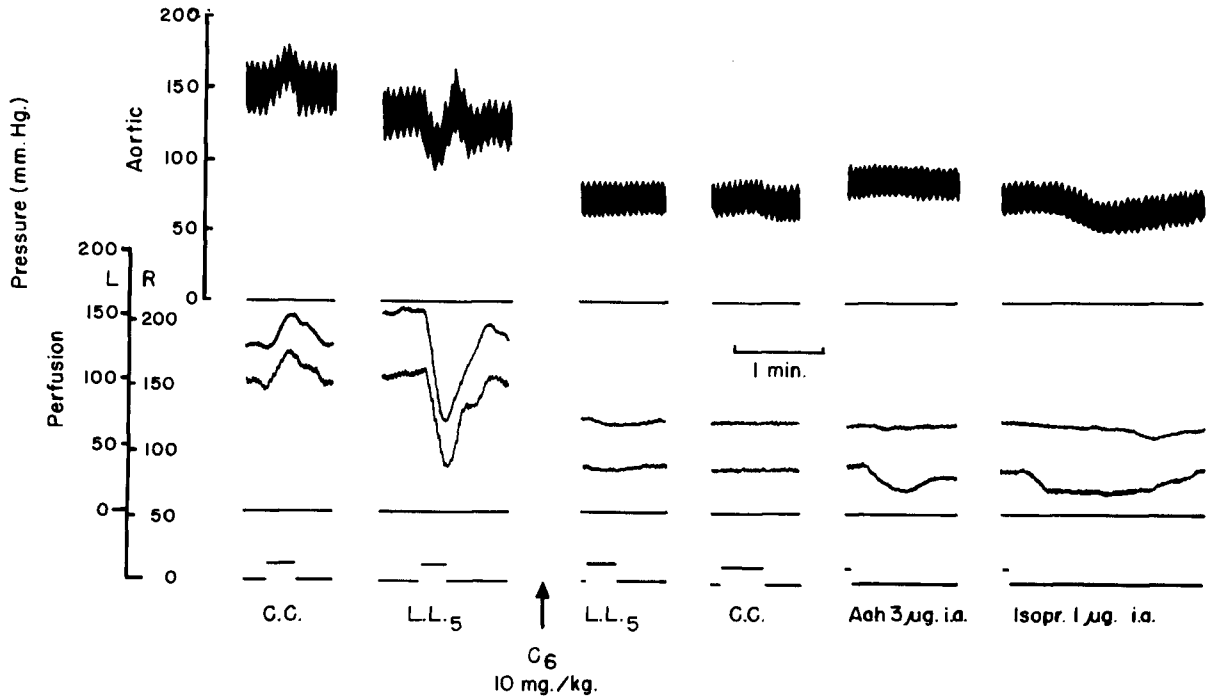

Figure 10.

Effect of carotid clamping, ventral root stimulation and hexamethonium on hindlimb perfusion pressures. From above: aortic pressure, right hindlimb perfusion pressure, left hindlimb perfusion pressure. C.C. = carotid clamping, C_6 = hexamethonium, Ach, = acetylcholine, isopr. = isoproterenol.

1) Negative cases

Of the 34 dogs which satisfied the criteria for a viable preparation outlined in the Methods, 8 showed no dilatation of the hindlimb vasculature on stimulation of the ventral roots of L_5, $_6$ or $_7$, and no substantial explanation can be given for these cases, although several possibilities might be considered. In the dog the two paravertebral sympathetic chains fuse in the region of the sacrum or as high as L_7 (Mehler et al, 1952). It is conceivable that during the cannulation procedure this ganglionic chain or perhaps the perivascular nerve plexuses were damaged, destroying the vasodilatory innervation of the hindlimb vasculature. Indeed, a higher frequency of failures was observed in those experiments in which distal cannulation was at the level of the iliac arteries rather than the femoral arteries. Alternatively, ischemia of the sympathetic ganglia in the lumbar region as a result of ligation of the 5th, 6th or 7th lumbar arteries could have contributed to the negative results. Finally, it is possible that some damage of the ventral roots may have occurred in spite of the efforts to maintain them in O_2 saturated warm paraffin oil, with the result that

peripheral (efferent) conduction was impaired.

2) Donald and Ferguson Experiments

In our experiments the result of unilateral ventral root stimulation (L_5, 6, 7) was, in the majority of cases, a bilateral vasodilatation in the hindlimbs and a fall in aortic pressure within 1 or 2 seconds from the onset of the stimulation, and lasting no more than 25 or 30 seconds despite maintained stimulation (Fig. 4, Fig. 10).

Donald and Ferguson (1970) observed ipsilateral hindlimb vasodilatation in dogs induced by stimulation of the ventral roots of L_5 to L_7. The major difference with the present experiments was that in their preparations the spinal cord had been sectioned and the spinal cord segments corresponding to the stimulated roots removed. In our experiments the spinal cord was left intact, although in some cases the ventral root was isolated from the CNS by section.

Perhaps as a consequence of these differences in preparation, several major differences appear between the results of those workers and those reported here:

i) the duration of the response reported here was in the order of 25 seconds while that of Donald and Ferguson was in the order of several minutes; ii) the contralateral vasodilatation seen in these experiments was not observed by Donald and Ferguson; iii) the systemic arterial pressure change seen here was not a part of the response reported by Donald and Ferguson; iv) while those workers found no abolition of the response by atropine, propranolol or Benadryl when administered i.v., our experiments show that Benadryl, when administered intra-arterially does abolish the response, as does mepyramine administered by the same route; v) although sympathectomy from L_2 through L_7 does not abolish the vasodilatation observed by Donald and Ferguson, these experiments demonstrate the abolition of the vasodilatation by hexamethonium.

In short, these two studies differ in preparation by the presence or absence of an intact spinal cord and the route of administration and dosage of the drugs given. The results differ in duration of the response, involvement of the systemic arterial pressure, involve-

ment of the contralateral vasculature, effects of antihistaminics, and effect of sympathectomy (whether surgical or chemical). In view of these differences, most particularly the systemic arterial pressure changes, the hindlimb contralateral vasodilatation and the duration of the response, the question of whether these two phenomenon are perhaps different must be considered.

Certainly, the responses seen in the two studies were both elicited by the stimulation of $L_{5, 6 \text{ or } 7}$ ventral roots, the greatest magnitude of response being elicited from L_6, and both occurred within 2 seconds of onset of stimulation, suggesting a neurogenic vasodilatation. However, in almost all other parameters the phenomenon differed. The most convincing evidence that these two phenomenon are indeed the same would be the abolition of the central and contralateral effects and an increase in the duration of the response seen in our experiments after section of the spinal cord. The persistance of the Donald and Ferguson dilatation after sympathectomy and the abolition of our dilatation after sympathectomy and the abolition of our dilatation after hexa-

methonium administration could perhaps be explained since it is possible that the fibres responsible for these effects synapse in the parasympathetic ganglia which would be blocked by the hexamethonium used in our experiments but would not be removed by the sympathectomy carried out by Donald and Ferguson. On the basis of the presently available evidence the assumption that these two dilatations are mediated by the same pathway may only be made with considerable caution.

3) Unanswered Questions

The observations reported here leave several important questions to be answered. First, is the hindlimb vasodilatation active or passive as those terms have been defined by Beck and Brody (1961)? Second, is the hindlimb dilatation the cause or the result of the central pressure change, or is there any association between the two? Third, what is the relationship between the ipsilateral and contralateral vasodilatations, And fourth, what is the mechanism responsible for the effect on aortic pressure? These questions will be considered seperately below.

i) Active vasodilatation as defined here is that

which results directly from the increased concentration at the vessel level of a dilator substance of either neural of exogenous origin (Beck and Brody, 1961). This study indicates that the dilatation is abolished by antihistamines, an abolition that parallels the progressive loss of vascular reactivity to histamine, which is consistent with the findings of Beck (1965), Tuttle (1965, 1966) and Wellans and Wauters (1966), all of whom describe active reflex dilatations. While the speed of onset of the dilatation following stimulation in these experiments supports the assumption that the dilatation is neurogenic, and while the sensitivity to antihistamines supports the possibility that it is analagous to the antihistamine sensitive active reflex dilatations described by others, a definite conclusion to this effect must await the demonstration of neurogenic release at the vessel level of histamine.

ii) The possibility of the ipsilateral vasodilatation being secondary to a drop in central arterial pressure is unlikely for several reasons. The constant flow perfusion pumps isolate the hindlimbs from changes in central

arterial pressure of the magnitudes seen here. Moreover, the difference in magnitude of the dilatations of the two hindlimbs would be difficult to explain on the basis of a common central cause. And finally, the different sensitivities of the peripheral pressure changes and the aortic pressure change to antihistamines weigh against a common cause.

iii) Contralateral vasodilatation is observed here in response to stimulation of the ventral roots of L_5, $_6$ and $_7$. It is possible that this is due to the perfusion by both pumps of a common vascular bed despite all efforts to isolate the hindlimbs surgically. However, the experiments included in their protocol the stoppage of one pump at a time (Fig. 2) to ensure that perfusion pressure in that limb fell to less than 30 mm Hg indicating that the limb was adequately isolated from the contralateral limb and from the central arterial pressure. Persistance of a perfusion pressure closer to that of the other limb or the central arterial pressure could have indicated a colateral arterial perfusion to the hindlimb.

A bilateral vasomotor response to a unilateral preganglionic nerve stimulation is not described in the literature. However, apart from the description by Donald

and Ferguson (1970), neither is there in the literature a description of vasomotor fibres leaving the spinal cord below the level of L_3 - L_4 of dogs and therefore cross-innervation should not be ruled out as the possible cause of bilateral vasomotor response in these experiments. On the contrary, Mehler et al (1952) demonstrated that in at least 35% of a series of 100 dissections in dogs there was a preganglionic outflow from the spinal cord below the L_4 level and that these fibres coursed diagonally caudad to join the L_6 or L_7 sympathetic ganglion. Moreover, the L7 ganglion could often be shown to be a fused ganglion composed of both right and left counterparts, and that the fibres did cross the midline as shown by osmium staining. However, these workers were not able to show contralateral vasomotor responses by electrical stimulation of the ganglia just rorstral to the point of fusion, but it should be noted that their criteria for vasomotor activity was a vascular response in the dog paw, which is an almost purely cutaneous vascular territory. The abolition of the responses seen in our experiments by hexamethonium supports the possibility of a preganglionic pathway that

could well synapse in the fused L_7 sympathetic ganglion and cross the midline to innervate some contralateral vascular beds.

iv) Most puzzling is that the stimulation of a supposedly purely efferent pathway (the ventral root) innervating a vascularly isolated territory should cause a central arterial pressure drop. Sherrington (1894) described afferent fibres within the ventral roots of cats and monkeys as have others more recently in cats and rats (Kato and Hirata, 1965; Dimsdale and Kemp, 1966). The "central effect" on aortic pressure in our experiments occurs also on stimulation lf the peripheral stump of the sectioned ventral root while it was absent when the central stump of the ventral root was stimulated. These findings should rule out the possibility of these fibres contributing to the central effect. Previous section of the dorsal roots rules out the possibility of a reflex arc at this spinal level causing afferent stimulation of CNS centers. Bilateral section of the dorsal roots from L_5 through L_7 in three experiments did not abolish

the central effect so presumably it is not mediated by a reflex arc within one or two spinal segments of the peripherally stimulated ventral root.

It would be possible to produce the central effect by ventral root stimulation if there were in the root vasodilator fibres which were innervating a large vascular bed not controlled by the pumps. This is not likely the case in these experiments however for two reasons. The major branches of the aorta below the renal arteries (including the inferior mesenteric artery) had all been ligated in these experiments. Furthermore, the innervation of the splanchnic vascular beds is derived from much higher spinal segments (Miller, 1967). It is unlikely that venous dilatation in the hindlimb in response to ventral root stimulation with consequent trapping of blood in the periphery resulting in decreased venous return is responsible for the fall in aortic pressure. Not only does the central effect occur too soon after the onset of stimulation, but the lack of change in pulse

pressure or heart rate suggests a change in peripheral resistance rather than a change in cardiac output as the cause of the aortic pressure change.

Anastomotic channels between central arterial supply and the hindlimb vascular beds could theoretically allow a reflection of massive peripheral vasodilatation in the central arterial pressure. However, in these experiments great care was taken to eliminate such channels by ligating all arteries below the renal arteries and above the point of cannulation. The success of these precautions is reflected in the lack of sustained arterial pressure in either hindlimb during the stoppage of its respective perfusion pump (Fig. 2).

Another possibility is that the efferent fibres which are being stimulated in the ventral roots travel cephalad within the sympathetic ganglia from which afferent fibres then reach the CNS through some as yet undefined pathway. Absence of the central effect (and the contralateral effect) in the Donald and Ferguson

experiments, which included bilateral sympathectomy supports this possibility, since, if such were the case, no central effect would appear in their experiments in which segments of the spinal cord had been removed. Abolition of the central pressure changes in our experiments by the administration of hexamethonium is consistent with this hypothesis.

The central effect cannot be explained by postulating the release of a vasodilator substance in the hindlimbs, which would then reach more central vascular beds through the venous return from the hindlimbs, causing a more generalized vasodilatation because the time course of the effect is too short. The immediate onset of the central effect suggests a neurogenic mediation, either by active dilatation or by central inhibition of vascular tone such as is seen in the vaso-vagal and baroreceptor reflexes.

4) Evidence Supporting Histamine as the Mediator of Vasodilatation

Evidence presented here supports histamine as the

dilator substance responsible for at least the peripheral effects. The time course of the vasodilatation and the observation that the histamine content of post-ganglionic sympathetic neurons is the highest found anywhere in the nervous system (Green, 1964; Schayer, 1962; Werle and Palm, 1950) make it likely that these dilatations are mediated by histamine released from post-ganglionic sympathetic neurons at the vessel level. The hexamethonium block of the response to ventral root stimulation suggests that preganglionic fibres are being stimulated within the ventral root. It can be seen (Fig. 6) that the antihistamine block of vasodilatation does not block the vessel response to acetylcholine or isoproterenol. Also, it is seen that the progressive loss of vessel reactivity to exogenous histamine following repeated additions of antihistamine (Benadryl) is accompanied by progressive and parallel loss of vasodilator response to ventral root stimulation (Fig. 7, Fig. 11). A similar abolition

of vasodilatation can be demonstrated following intra-arterial injection of mepyramine.

While these observations support the possibility that histamine is responsible for the dilatations they do not provide complete proof. It remains to be demonstrated that histamine is released in response to nerve stimulation. Others have demonstrated that labelled histamine is released in vascular beds undergoing active vasodilatation (Brody, 1966; Tuttle, 1967), and the concomitant increase in the rate of conversion of labelled histidine to histamine (Schayer, 1967). Stimulation of the CNS or the sympathetic trunk can cause the release of histamine peripherally (Tuttle, 1966; Tuttle and McLeary, 1970). Significant levels of the degrading enzyme necessary for the removal of active histamine from the region of the nerve terminal, histamine-N-methyl transferase, has also been demonstrated in post-ganglionic sympathetic nerves (Brown, Tomchick and Axelrod, 1959), as has histamine itself (Green, 1964).

The accumulated evidence cited here would appear

to fulfill the requirements generally recognized as necessary for acceptance of a substance as a neurotransmitter (see McLennan, 1969). However, there remain at least three objections to such a conclusion, most of which have been discussed in the literature review (see Introduction above). The ubiquity of histamine in the vascular system and the perivascular tissues allows postulations of histamine release from tissues other than nerve terminals (Ryan and Brody, 1972). The increased tissue perfusion resulting from vasodilatation suggests the possibility that histamine appearing in the perfusate may merely represent histamine that is being washed out of previously unperfused areas, although this possibility has been largely eliminated by the use of labelled histidine as already discussed (see Introduction). Finally, the necessity in most experimental preparations of providing cholinergic and adrenergic blockade in order to unmask the histaminergic vasodilatation introduces the complication of interference of these agents with the release or action of histamine on the vascular

smooth muscle. Antihistamines themselves have been known to interfere with the reuptake of norepinephrine into the preganglionic nerve terminals (Isaac and Goth, 1967). Wyse et al (1971) have shown that many drugs used in this field have some histamine releasing activity.

5) Advantages of this Preparation

An advantage of the preparation used in this study is the ability to elicit an antihistamine sensitive vasodilatation in the absence of cholinergic and adrenergic blockade, thus avoiding some of the above mentioned pharmacological complications. The possibility of interference with the reuptake of norepinephrine by antihistaminics, which would decrease the magnitude of a dilatation by prolonging the presence of a constrictor substance (i.e. norepinephrine) in the vicinity of the vessels applies to the vasodilatations reported here only if they are passive in nature. Without demonstrating histamine release during ventral root stimulation this dilatation cannot be differentiated as either

active or passive, although the striking similarity of these findings with those of Beck, Tuttle and others make it reasonably safe to assume that it is an active histaminergic dilatation.

Beck (1965, 1966) has outlined the conditions which should be satisfied in order to demonstrate an active histaminergic vasodilatation (see Introduction above). The results described here satisfy all those conditions as they apply; i) the vascular responses to nerve stimulation are abolished by antihistamines of the two major classes that were used; ii) the reactivity of the vessels to other vasoactive agents is maintained during histamine blockade; iii) a correlation is shown between the reduction of vascular response to exogenously released histamine and to neurogenically induced dilatation; iv) the dilatation is both atropine and propranolol resistant.

In view of these similarities is seems possible that the efferent nervous pathway responsible for the

active reflex dilatation described by Beck and that responsible for this dilatation are the same. The pathway responsible for the active reflex dilatation of Beck apparently synapses in the sympathetic ganglion since the reflex is abolished by doses of hexamethonium sufficient to block transmossion in the sympathetic ganglion (Wyse et al, 1971). The same dose of hexamethonium is found to block the vasodilatation reported here.

6) Conclusions

The ipsilateral, neurogenically mediated vasodilatation seen here is sensitive to antihistamines but resistant to cholinergic and adrenergic blocking agents. It appears, in most parameters, similar to the active reflex vasodilatation induced by sudden increases in arterial pressure as demonstrated by Beck and his coworkers (Beck, 1958). There are several points remaining unexplained in these observations. No explanation can be offered for the contralateral vasodilatation, nor for the concomitant fall in central arterial pressure. The

ipsilateral vasodilatation is abolished by antihistamines of two major classes but there has been no demonstration in these experiments of histamine release from the area of vasodilatation. The cause of "escape" of the vasodilatation despite continued stimulation seen in these experiments has not been explained, although depletion of the pools of available histamine is a possibility. These experiments demonstrate a neural output to the vessels via ventral roots which were not known to have one before the Donald and Ferguson work, and further, they demonstrate an efferent preganglionic pathway of "pure" vasodilatory fibres when none was previously known.

If this vasodilatation is confirmed to be histamine mediated, either through demonstration of labelled histamine release, or any other method, this preparation would offer a distinct advantage over other preparations in use for the study of histaminergic vasodilator fibres since no adrenergic or cholinergic blockade is required

before the histaminergic component of the dilatation is seen, thus avoiding the uncertainty caused by the use of multiple drugs.

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