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Antihistamine-sensitive active vasodilatation in the perfused hindquarters of the rat

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The innervated, constant flow perfused hindquarters of the rat have been used to evaluate post-stimulation vasodilatation, which is a model of active reflex vasodilatation in this species. The vasodilatation resulting from lumbar sympathetic stimulation was dependent on stimulation frequency and duration. Maximal vasodilatation (16 ± 2%) was at 8 Hz for 15 s, while markedly reduced vasodilatation was seen after stimulation for longer than 30 s at all frequencies tested. The vasodilatation was transient. Atropine (2·0 mg kg^{-1} i.v.) failed to attenuate post-stimulation vasodilatation at a time when hindquarter vasodilatation to i.a. acetylcholine had been abolished. The H_1 antihistamine, tripelennamine (2·5 mg kg^{-1} i.v.) significantly reduced (77%) post-stimulation vasodilatation relative to controls at a time when hindquarter vasodilatation due to i.a. histamine was essentially abolished. Reactive hyperaemia is an unlikely cause of vasodilatation since it is not blocked by H_1 antihistamines; 60 s post-occlusion hyperaemia also, was not demonstrable. These data suggest that there is an active component of baroreceptor-mediated vasodilatation in the rat and that histamine, rather than acetylcholine, could be a mediator of this vasodilatation.

Reflexly induced neurogenic vasodilatation mediated via baroreceptors has been demonstrated in the perfused hindlimb or hindquarters of the dog (Beck 1961; Brody 1966), cat (Tuttle 1965, 1967), monkey (Levin et al 1968) and rat (Tobia et al 1969). This vasodilatation is generally believed to consist of two components, one being passive and the other active (Brody 1978). The passive component is defined as the reflex withdrawal of sympathetic tone while active vasodilatation has been defined as that occurring upon release of a local substance that elicits an increase in vascular calibre (Beck & Brody 1961; Brody 1966; Beck et al 1971; Brody 1978, 1980). While the mediator of active vasodilatation remains in question, a large body of evidence (Sakuma & Beck 1961; Beck 1965; Tuttle 1965; Brody 1966; Tobia et al 1969; Beck et al 1971; Brody 1980) supports a role for vascular stored histamine.

Heitz & Brody (1975) described a model for measuring and evaluating active vasodilatation using the autoperfused hindquarters of the dog. In this model, active vasodilatation is defined as being synonymous with post-stimulation dilatation evoked by discontinuous low level, short duration, electrical stimulation of the lumbar sympathetic chains. Although reflex vasodilatation has been observed in auto-perfused hindquarters of the rat (Tobia et al 1969, 1970), systematic evaluation of the active component of neurogenic vasodilatation in this species has not been reported. Since this component could well play a major role in vascular resistance control, and the rat is widely used in hypertension models, there is a need to characterize this vasodilator mechanism in normotensive rats. To this end we have examined active dilatation in the rat using the technique described by Heitz & Brody (1975). We aimed to demonstrate the existence of active reflex vasodilatation and to examine aspects of the nature of its mediation.

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METHODS

Male, Sprague-Dawley rats (275–460 g) (Sasco, Inc., Omaha, NE) were anaesthetized with a 20% solution of urethane (1200 mg kg^{-1} i.p.) in all experiments.

Hindquarter perfusion

This was accomplished according to Brody et al (1963) as modified by Tobia et al (1969) and Merrick & Holcslaw (1981). After tracheal cannulation, the right external jugular vein was cannulated (PE 50).

Following a midline incision, a 2 cm section of the abdominal aorta below the renal arteries was isolated and cannulated (PE 190), in both a proximal and distal direction. Heparin (500 u kg^{-1} i.v.) was administered just before aortic cannulation. An external perfusion circuit between aortic cannulae was completed by 50 cm of Tygon tubing (3/32" i.d. and 5/32" o.d.) and two 3-way T connectors (Cole-Parmer Instrument Co., Chicago, IL), the remaining port of each being connected to a Statham P23A pressure transducer. Both transducers and the external circuit were filled with heparinized saline (2500 u $litre^{-1}$). After the proximal cannulation, the external circuit was allowed to fill with blood before the distal cannulation was made. Systemic and perfusion pressures were recorded on a Grass polygraph (Model 79).

After aortic cannulation, 1·5–2·5 ml of 6% dextran and/or 0·9% NaCl (saline) was given i.v. to replace blood lost to the external circuit. The animal was allowed to self-perfuse the hindquarters until the systemic pressure stabilized. The external circuit was then placed in a Sage pump (Model 375A), and the perfusion gradually adjusted to 100–120 mm Hg (approximately equal to the systemic pressure) by adjusting the flow rate (3·2–6·0 ml min^{-1}). As constant flow was maintained, any changes in the perfusion pressure were directly proportional to changes in peripheral vascular resistance.

Isolation and stimulation of lumbar sympathetic chains

Isolation of the sympathetic chains was accomplished between the levels of L_1–L_5. Care was taken not to traumatize the chains as a 4·0 silk ligature was placed loosely around them. The chains, bathed in warm mineral oil at all times, were gently placed on the bipolar platinum stimulating electrode which was connected to a Grass stimulator (Model S44). All stimulations were monophasic pulses of 2 ms duration with 0·5 ms delay at 10 V. The frequency of stimulation (Hz) and the duration of stimulation varied with the specific experiment.

Post-stimulation vasodilatation

During stimulation of the sympathetic chains, vasoconstriction occurred in the hindquarters; this was due to release of noradrenaline since phentolamine and reserpine blocked the pressor response. When the stimulation was stopped, the perfusion pressure fell rapidly to a level below that before stimulation (baseline) as described by Heitz & Brody (1975). The difference between the baseline perfusion pressure and the maximum decrease in perfusion pressure immediately after stimulation is post-stimulation vasodilatation (PSV). This is entirely active since, by definition of PSV, there is no withdrawal of vascular tone.

Three minutes were allowed between chain stimulations for stabilization of the perfusion pressure. PSV is represented as a percent change from baseline to normalize differences in perfusion pressure between and within animals.

Experimental procedure

The preparations used showed little loss of blood during surgery, and allowed rapid cannulation, and a perfusion pressure greater than or equal to 100 mm Hg with a normal aortic blood flow (3·0–6·0 ml min^{-1}); there was also good vascular reactivity and reflex-responsiveness with a PSV of at least 10 mm Hg. All studies were with groups of at least 5 animals. Initial experiments determined the parameters of sympathetic chain stimulation (frequency, duration of stimulation) that would elicit the largest magnitude of PSV. In subsequent experiments the magnitude of PSV was determined before and after atropine methylnitrate (2·0 mg kg^{-1} i.v.), and the H_1 antihistamine, tripelennamine dihydrochloride (2·5 mg kg^{-1} i.v.). Close intra-arterial injections of histamine (1μg), acetylcholine (1μg) and (−)-isoprenaline (50 ng) were made before and after the atropine to test for vascular integrity, effectiveness of the blocking agents and to evaluate possible changes in vascular reactivity after blocking drug administration. Intra-arterial injections were made in an injection port in the external circuit in volumes of 1·0–5·0 μl. A 5·0 μl i.a. injection of saline caused no change in perfusion pressure. Drugs given intravenously were washed in with 0·2 ml of saline. All drug solutions were made with or diluted with saline. Doses are expressed as the free base of the drug.

Drugs used were: tripelennamine hydrochloride injection (25 mg ml^{-1}) (Ciba-Geigy Corp., Summit, NJ); heparin sodium injection (1000 u ml^{-1}) (Dexter Corp., Chargin Falls, OH); and urethane, acetylcholine chloride, atropine methylnitrate, histamine dihydrochloride, (−)-isoprenaline, and dextran-70 (Sigma, St. Louis, MO).

Statistics

Statistical analysis was performed on data expressed as percent change in baseline perfusion pressure (the perfusion pressure immediately preceding the test).

Mean values and standard errors of the mean were computed for each group of data. Statistical significance between different means was determined by paired test or with one way analysis of variance followed by Duncan's multiple range test (Snedecor 1956). The criterion for significance was $P < 0.05$ in all experiments.

RESULTS

Maximal post-stimulation vasodilatation

Determination of maximal post-stimulation vasodilatation (Fig. 1) was accomplished by stimulating the sympathetic chains at frequencies of 2, 4, 8 and 16 Hz for 15, 30 and 45 s. As seen in Fig. 2, maximal PSV (16·1%) occurred after 8 Hz for 15 s. Stimulation for longer than 30 s did not increase the magnitude, rather there was a sharp reduction in response (Figs 1, 2). Increasing the frequency beyond 8 Hz also caused a sharp reduction which was most apparent at 45 s. The magnitude of PSV in most preparations was equivalent to approximately 80% of noradrenaline-induced reflex vasodilatation seen in this and in other experiments from this laboratory. Additionally, we found that lumbar chain stimulation at 0·05 ms, while eliminating hindquarter vasoconstriction, unmasked a vasodilatation that began as stimulation started (unpublished data).

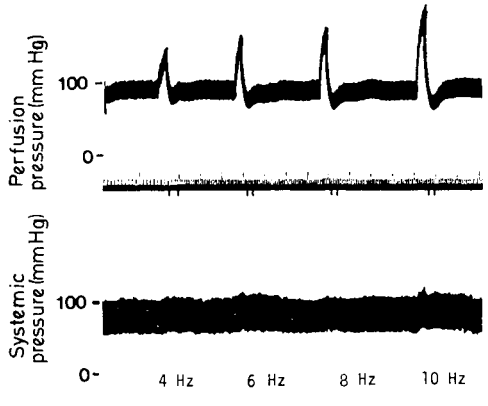

FIG. 1. Demonstration of post-stimulation vasodilatation (active vasodilatation) in the perfused hindquarters of the normotensive rat. The hindquarters were perfused at constant flow and the lumbar sympathetic chains at L_2–L_4 stimulated at varying frequencies for 15 s at 10 V, 2 ms duration with repetitive square wave pulses. After 15 s the stimulator was turned off and the vasodilatation measured. Post-stimulation vasodilatation is the difference between maximal decrease in perfusion pressure and prestimulation perfusion pressure.

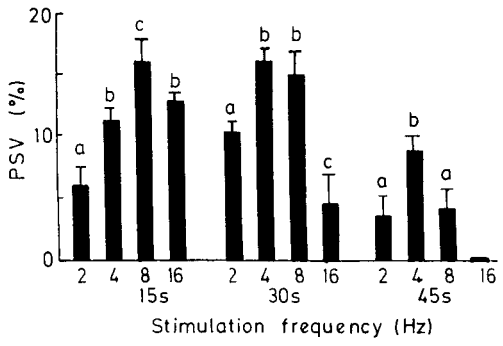

FIG. 2. Post-stimulation vasodilatation (PSV) at varying frequencies of lumbar sympathetic chain stimulation and duration of stimulation in the perfused hindquarters of rats. Hindquarters of 11 male, Sprague-Dawley rats were perfused at constant flow rates. The magnitude of PSV was measured in each rat at varying frequencies and periods of lumbar sympathetic chain stimulation. Analysis of variance indicated significant differences in magnitude of PSV at all periods of stimulation. Different letter superscripts denote significant differences between stimulation frequencies within a given period of stimulation ($P < 0.05$).

Effect of atropine on post-stimulation vasodilatation

Atropine did not modify the magnitude of PSV 10 min after its administration, a time at which vasodilatation by i.a. ACh was decreased significantly from 23·2 ± 3·5% to 2·1 ± 1·0% (Table 1, Fig. 3). Atropine did not alter either perfusion or arterial pressure.

Effect of H_1 antihistamine on post-stimulation vasodilatation

The effect of tripelennamine, on PSV was separately determined to establish the role histamine may have

Table 1. Effect of atropine on the magnitude of post-stimulation vasodilatation (PSV) in the perfused hindquarters of the rat[a].

Vasodilator agent[b]	Control	Atropine
PSV	11·8 ± 1·7 (102 ± 4)[c]	8·8 ± 3·0 (108 ± 3)
Acetylcholine	23·2 ± 3·5 (98 ± 4)	*2·1 ± 1·0 (100 ± 5)

[a] The hindquarters of 5 rats were perfused at constant flow (3·0–4·0 ml min^{-1}). Vasodilator responses to stimulation of lumbar sympathetic chains (6–8 Hz at 10 V, 2 ms, for 15 s) or drug administration were elicited before and 5 min after atropine methylnitrate (2·0 mg kg^{-1} i.v.). Data reported are mean percent reduction in perfusion pressure relative to baseline perfusion pressure ± s.e.

[b] Acetylcholine (1 μg) was administered intra-arterially directly into the hindquarters via an injection port in the external perfusion circuit. Maximal PSV was determined in each preparation in the control period by varying the frequency of stimulation of sympathetic chains.

[c] Actual mean perfusion pressure (mmHg) just before initiating a response ± s.e.

* Significantly different from controls; $P < 0.01$.

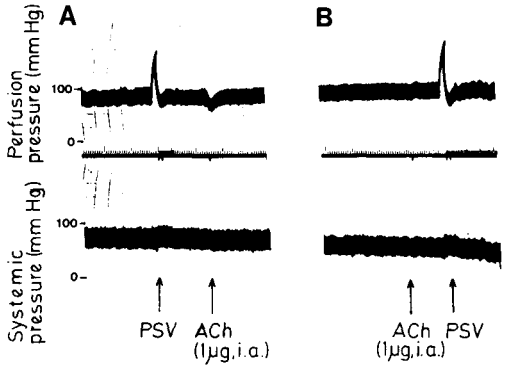

FIG. 3. Effect of atropine, 2 mg kg^{-1} i.v., on post-stimulation vasodilatation (PSV) in the perfused rat hindquarters. Hindquarters of male, Sprague-Dawley rats were perfused at constant flow. Post-stimulation vasodilatation was measured before (Panel A) and 5 min after (Panel B) atropine methylnitrate (2·0 mg kg^{-1} i.v.). PSV was evoked by stimulating the lumbar sympathetic chains at 6–8 Hz for 15 s at 10 V. Acetylcholine (1 μg, i.a.) was administered before and after atropine.

Table 2. Effect of tripelennamine on the magnitude of post-stimulation vasodilatation (PSV) in the perfused hindquarters of the rat[a].

Vasodilator agent[b]	Control	Tripelennamine
PSV	18·1 ± 1·6 (106 ± 3)[c]	2·3 ± 1·0* (105 ± 4)
Histamine	31·7 ± 2·1 (102 ± 3)	7·3 ± 1·8* (103 ± 3)
Acetylcholine	40·0 ± 1·9 (110 ± 4)	32·9 ± 2·5 (108 ± 4)
(−)-Isoprenaline	35·1 ± 3·3 (107 ± 4)	26·2 ± 3·5* (105 ± 3)

[a] The hindquarters of 7 rats were perfused at constant flow (3·2 − 4·8 ml min^{-1}). Vasodilator responses to stimulation of lumbar sympathetic chains (6–10 Hz at 10 V, 2 ms for 15 s) or drug administration were elicited before and 10 min after tripelennamine (2·5 mg kg^{-1} i.v.) injection. Data reported are mean percent reduction in perfusion pressure relative to baseline perfusion pressure ± s.e.

[b] Acetylcholine (1 μg), histamine (1 μg), (−)-isoprenaline (50–200 ng) were administered intra-arterially directly into the hindquarters via an injection port in the external perfusion circuit.

[c] Actual mean perfusion pressures (mmHg) just before initiating a response ± s.e.

* Significantly different from control, $P < 0.05$.

in mediating the PSV. Data presented in Fig. 4 and Table 2 demonstrate that tripelennamine caused a significant reduction in the magnitude of PSV and in vasodilatation caused by i.a. histamine. Data in Table 2 also indicate that while the vasodilator response to histamine was reduced 77% by tripelennamine, the responses to acetylcholine and (−)-isoprenaline were reduced by 18 and 25%, respectively, from the control vasodilatations. Tripelennamine reduced the baseline perfusion pressure in all preparations in this series by less than 10%.

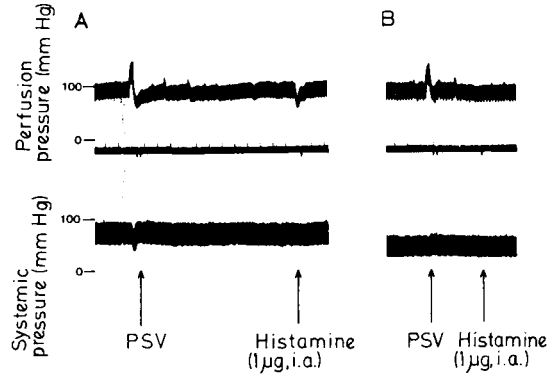

FIG. 4. Effects of tripelennamine, 2·5 mg kg^{-1} i.v., on post-stimulation vasodilatation (PSV) in the rat perfused hindquarters. Hindquarters of male, Sprague-Dawley rats were perfused at constant flow. PSV was measured before (Panel A) and 10–15 min after (Panel B) tripelennamine hydrochloride (2·5 mg kg^{-1} i.v.). PSV was evoked by stimulating lumbar sympathetic chains at 6–10 Hz for 15 s at 10 V. Acetylcholine (1 μg i.a.) and histamine (1–2 μg i.a.) were administered before and after tripelennamine and vasodilatation measured.

DISCUSSION

Reflexly-induced neurogenic vasodilatation mediated via the baroreceptors was first reported in the perfused hindquarters of the rat by Tobia et al (1969, 1970). We have since confirmed (Merrick & Holcslaw 1981) and recently extended this finding (Holcslaw & Lassiter 1984). The studies of Tobia et al implied active vasodilatation but did not directly demonstrate the existence and quantify the magnitude of this active response. The results of the present study, which used post-stimulation vasodilatation as reported by Heitz & Brody (1975), demonstrate a transient hindquarter dilator response which is considered to be active and neurogenic. This response was related to stimulation frequency with maximal vasodilatation occurring between 6–10 Hz. While no significant differences were noted between 15–30 s durations of sympathetic chain stimulation,

the size of the reduction in perfusion pressure was diminished at 45 s, particularly at 16 Hz. This latter effect may be due to high concentrations of noradrenaline present in the synaptic gap precluding vasodilatation. The existence of post- stimulation vasodilatation in the rat thus agrees with that seen in the perfused gracilis muscle (Heitz & Brody 1975) and hindquarters (Beck 1963) of the dog.

Several of our observations support this dilator response being active and neurogenic. Prior vasoconstriction eliminated by adrenoceptor blocking drugs or by stimuli of short duration, is not needed to observe post-stimulation vasodilatation, thus indicating that passive withdrawal of sympathetic tone is not involved, a fact further supported by our finding (Holcslaw & Lassiter 1984) that the sustained reduction in perfusion pressure upon sectioning the sympathetic chains (maximal withdrawal) is smaller than the post-stimulation vasodilatation observed in the present studies. Lastly, if passive withdrawal were involved, a similar transient vasodilatation following vasoconstrictor responses to intra-arterially administered noradrenaline would be expected, but this has not been found so far.

While the use of intact sympathetic nerves could favour vasodilatation mediated primarily by afferent stimulation, this seems unlikely since our pilot studies produced virtually no vasodilatation in hindquarters following stimulation of the central cut ends of sympathetic chains.

As in dogs (Beck 1963), the maximal active portion of reflex vasodilatation in the perfused hindquarters of the rat also represents approximately 80 percent of the maximal vasodilatation mediated by the baroreceptors. We have routinely observed a 25–28 mm Hg reflex dilator response in the perfused hindquarters of normotensive rats after intravenous noradrenaline (Holcslaw & Lassiter 1984) which is consistent with the suggestion that the active portion of reflex-induced neurogenic vasodilatation plays a relatively greater role than the passive component in this type of vasodilatation in the rat. Furthermore, we have shown that when the sympathetic chains are severed at L_1–L_4 the hindquarter perfusion pressure falls to a sustained level approximately 10–12 mm Hg below controls. This occurs in preparations where the total noradrenaline-induced reflex vasodilatation corresponds to 25–28 mm Hg (Holcslaw & Lassiter 1984). These findings further support the contention that, in this vascular bed, the active component plays a greater role in the vasodilatation than does the reduction in tonic release of noradrenaline.

The possibility that the post-stimulation vasodilatation observed is due to reactive hyperaemia secondary to vasoconstriction exists but seems unlikely because tripelennamine significantly attenuated the dilatation and Powell & Brody (1976) have shown that post-occlusion reactive hyperaemia is not susceptible to either H_1 or H_2 type antihistamines; also, we have recently found that post-stimulation vasodilatation does not require prior vasoconstriction.

Atropine, in a dose sufficient to abolish the vasodilatation induced by intra-arterial acetylcholine, failed to alter significantly the magnitude of post-stimulation vasodilatation. This indicates that acetylcholine release plays an insignificant role in mediating this dilator response and confirms a similar observation in the dog (Heitz & Brody 1975).

Abundant evidence suggests vascular stored histamine to represent the active dilator substance released during baroreceptor-mediated neurogenic vasodilatation in perfused skeletal muscle beds of several species (Brody 1978, 1980; Holcslaw & Imhoff 1978). Tobia et al (1969) have provided supportive evidence implicating histamine in a mediating capacity in noradrenaline-evoked reflex vasodilatation in the rat. Our present findings are consistent with a role for histamine in mediating active reflex vasodilatation in this predominantly skeletal muscle vascular bed since tripelennamine significantly attenuated (89%) the maximal post-stimulation vasodilatation at a time when the vasodilator response to intra-arterial histamine was essentially abolished. Although after tripelennamine there was a slight reduction in the dilator responses to acetylcholine and isoprenaline compared with the control, this was not enough to account for the magnitude of the blockade by tripelennamine. Furthermore, tripelennamine in previous and later experiments was not found to alter the non-specific vasodilator effect of nitroglycerin given intra-arterially into the perfused hindquarters. The existence of an H_1 antihistamine sensitive post-stimulation dilatation in rat hindquarters is in agreement with the findings of Heitz & Brody (1975) for the gracilis muscle of the dog.

That the reduction in post-stimulation vasodilatation produced by tripelennamine is related to a cocaine-like action in neuronal reuptake seems unlikely, since vascoconstrictor responses to nerve stimulation and to low doses of intra-arterial noradrenaline are unaltered after the antihistamine (Holcslaw & Lassiter 1984). We have recently confirmed this with mepyramine, an H_1 antihistamine devoid of neuronal reuptake blocking ability. Additionally, β-blockade by tripelennamine seems unlikely since

the response to isoprenaline after antihistamine was not reduced to the magnitude seen for post-stimulation vasodilatation. Previous studies have demonstrated a weak β-blocking effect with H_1 antihistamines at high doses (Altura & Altura 1974).

In conclusion, our results support the existence of a significant active component or baroreceptor-mediated reflex vasodilatation in the rat hindquarter vascular bed. The pharmacological evidence is consistent with a mediator role for vascular stored histamine in this regulatory response rather than acetylcholine.

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