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RESPONSES OF BLOOD VESSELS IN THE RABBIT KNEE TO ELECTRICAL STIMULATION OF THE JOINT CAPSULE

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SUMMARY

1. An *in vitro* preparation of the rabbit knee joint, perfused with oxygenated Locke's solution, was used to study the response of articular blood vessels to electrical stimulation of the joint capsule.

2. Using trains of stimulus pulses of different durations, frequency–response curves were obtained. Electrical stimulation always produced vasoconstriction of joint blood vessels, which increased as a function of both frequency and pulse width.

3. This vasoconstrictor response was neurally mediated as it was markedly inhibited after addition to both bath and perfusate of tetrodotoxin. In addition, the response to field stimulation of the capsule was virtually abolished in animals pretreated with reserpine which depletes sympathetic nerve endings of noradrenaline.

4. The response to electrical stimulation was substantially reduced by the α-adrenergic antagonist phenoxybenzamine (10^{-5} M), the α_1-blocker prazosin (10^{-6} M), and by guanethidine (10^{-5} M) which inhibits the release of noradrenaline, ATP and neuropeptide Y from sympathetic nerve endings.

5. The attenuation of the vasoconstrictor response to field stimulation by prazosin (10^{-6} M) was little altered by addition of the α_2-adrenoceptor blocker rauwolscine (10^{-6} M) to the perfusate.

6. α,β-Methylene ATP (10^{-6} M), a P_2-purinoceptor desensitizer, had no effect on the vasoconstrictor response to electrical stimulation.

7. These results indicate that the vasoconstrictor response to electrical stimulation of the rabbit knee joint capsule is mediated via noradrenaline acting upon α_1-adrenoceptors.

INTRODUCTION

Although the somatic innervation of joints has received much attention (for review see Gardner, 1950), little is known about the autonomic control of articular blood vessels. Cobbold & Lewis (1956) suggested that these are innervated by sympathetic efferent fibres as they observed that mechanical stimulation of the sympathetic chain resulted in a reduction of blood flow to the dog knee joint. In a recent study on the cat knee, electrical stimulation of the posterior articular nerve to this joint, which is known to contain sympathetic efferent fibres (Langford & Schmidt, 1983), produced an initial vasoconstriction of the blood vessels (Ferrell & Cant, 1987). However, the

nature of the neurotransmitters and receptors which mediate the vasoconstrictor response was not investigated in either of these studies.

Following the work of Ahlquist (1948), catecholamine receptors mediating constriction of arteries have been designated α-adrenoceptors. It was widely assumed that these must be the only postsynaptic receptors despite the observation that many α-receptor antagonists fail to block nerve-induced vasoconstriction (Neild & Zelcer, 1982). Hirst & Neild (1980) suggested that the electrical and mechanical responses of some smooth muscles were resistant to α-adrenoceptor antagonists because neuronally released noradrenaline was acting not only on α-adrenoceptors but also on a new class of adrenoceptors which they designated γ-receptors located near the nerve–muscle junction. However, other studies indicated that the α-blocker-resistant portion of the contractile response to sympathetic nerve stimulation was mediated by ATP, acting either as a co-transmitter with noradrenaline (Nakanishi & Takeda, 1972; Burnstock, 1976; Langer & Pinto, 1976; Westfall, Stitzel & Rowe, 1978; Sneddon & Burnstock, 1984), as a neuromodulator (Su, 1977; Wakade & Wakade, 1978; DeMay, Burnstock & Vanhoutte, 1979; Moylan & Westfall, 1979), or as a neurotransmitter in its own right (Burnstock, Campbell, Statchell & Smythe, 1970).

The suggestion that some nerve cells store and release more than one transmitter was made by Burnstock (1976), largely on the basis of comparative studies of the evolution of the autonomic nervous system (Burnstock, 1969). Su (1975, 1978) used tritium-labelled adenosine and noradrenaline to show that ATP is released together with noradrenaline from sympathetic nerves supplying the rabbit aorta and portal vein. Co-existence of noradrenaline and ATP has also been demonstrated in the rabbit ear artery (Head, Stitzel, Delaland & Johnson, 1977), and in the dog basilar artery (Muramatsu, Fuginara, Muira & Sakakibara, 1981). In the rat tail artery, electrical responses to sympathetic nerve stimulation consist to two components, namely a fast depolarization blocked by α,β-methylene ATP, suggesting mediation by P_2-purinoceptors, and a slow maintained depolarization which was blocked by phentolamine, suggesting that this is mediated by α-adrenoceptors (Burnstock, Griffith & Sneddon, 1984), Since then considerable evidence has accumulated in support of the multiple transmitter concept including electron microscopic evidence of more than one type of vesicle in sympathetic nerve endings (Burnstock, 1986).

Thus, in many tissues noradrenaline and ATP may be co-transmitters but whether this also applies to articular blood vessels is not known. In the present study this possibility was investigated by assessing the effects of α-adrenoceptor antagonists such as phenoxybenzamine which blocks both α_1- and α_2-adrenoceptors (Weiner, 1985) and prazosin which is selective for α_1-receptors (Rang & Dale, 1987), drugs such as guanethidine which inhibit release of noradrenaline, ATP and neuropeptide Y from sympathetic nerve endings (Lundberg, Anggard, Theodorsson-Noheim & Pernow, 1984), and α,β-methylene ATP, a P_2-purinoceptor desensitizer (Kasakov & Burnstock, 1983), on the responses of blood vessels in the rabbit knee to electrical stimulation of the joint capsule.

METHODS

Experiments were performed on albino New Zealand rabbits of either sex weighing between 2 and 3·3 kg, which were killed by stunning and exsanguination. Immediately thereafter, the posterior aspect of the knee joint was exposed, the popliteal artery cannulated and its muscular branches ligated as previously described (Ferrell & Khoshbaten, 1989). The tissue was perfused with

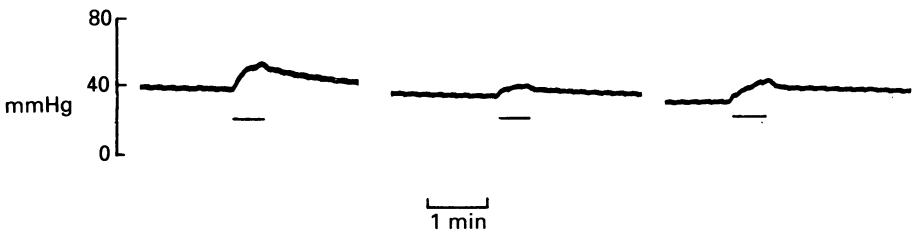

Fig. 1. The effect of field stimulation of the dorsal aspect of the joint capsule (left-hand panel). Twenty minutes after perfusion with TTX (10^{-7} M) and with TTX in the bath (10^{-8} M) the response to stimulation is significantly reduced (middle panel). Twenty minutes after terminating TTX treatment, the response to stimulation returns. The bars under each trace indicate 30 s stimulation periods. Stimulus parameters throughout: 50 V, 10 pulses/s, 1 ms. The ordinate indicates perfusion pressure increase.

warmed and oxygenated Locke's solution by means of a peristaltic pump (Gilson Miniplus). The knee was separated from the animal by sawing through the femur above the point of cannulation. The lower part of the limb was also removed by sawing mid-way through the tibia. The isolated knee joint preparation was then transferred to a thermostatically controlled bath (37 ± 1 °C) containing oxygenated Locke's solution. Three silver chloride wire electrodes were inserted via 21 G hypodermic needles into the synovial cavity which was injected with 1 ml of Locke's solution. The central ends of these wires were connected together to the anodal output of the stimulator (Harvard Advanced Stimulator). The cathodal output was connected to a silver chloride wire electrode shaped to form a zig-zag pattern, which overlayed and made contact with the dorsal aspect of the knee joint capsule. At the start of the experiment the pump was set to provide a perfusion rate of 0·5–0·9 ml/min which resulted in a perfusion pressure of about 40–50 mmHg as measured by a pressure transducer connected 'down-stream' from the pump. After a 30 min equilibration period a steady resting perfusion pressure was achieved. Thereafter, any changes in perfusion pressure as a result of field stimulation provided an indirect measure of articular vasoconstriction.

The peak response was compared to the control (pre-injection) value and expressed as the percentage change from control (or baseline). In most experiments the stimulus pulse width and voltage were varied until the optimal response was obtained and thereafter maintained constant. In most instances the stimulus parameters were: frequency 10 pulses/s, voltage 50 V and pulse width 1 ms. In all instances the stimulus pulse train lasted 30 s. Once suitable stimulus parameters were established, different adrenoceptor and purinoceptor blockers, dissolved in Locke's solution, were perfused continuously for periods of up to 2 h and at different time intervals the electrical stimulus was re-applied. Thereafter, the perfusate was switched back to Locke's solution and the whole organ bath solution was changed several times.

Two animals were treated with reserpine (Sigma) to deplete sympathetic nerve endings of noradrenaline (Burnstock & Sneddon, 1985). The reserpine was administered at a dose of 0·2 mg/kg intraperitoneally for 3 days, with 1 mg/kg being given I.P. 4–5 h before the start of the experiment.

The composition of Locke's solution was (mM): NaCl, 115; KCl, 4·7; $CaCl_2$, 2·5; $MgSO_4.7H_2O$, 1·2; $NaHCO_3$, 24·1; KH_2PO_4, 1·2; and glucose, 5·6. Calcium chloride was added after oxygenating the solution with a 95% O_2 and 5% CO_2 mixture. The following agents were administered: phenoxybenzamine hydrochloride (Smith, Kline & French); prazosin hydrochloride (Pfizer); guanethidine sulphate (Ciba); rauwolscine hydrochloride (Roth); α,β-methylene adenosine 5′-triphosphate (lithium salt); and tetrodotoxin (Sigma).

All data expressed on the graphs are means ± S.E.M.

RESULTS

Responses of articular blood vessels to field stimulation

Electrical stimuli applied to the joint capsule invariably resulted in an elevation in perfusion pressure, indicating vasoconstriction (left-hand panel, Fig. 1), which

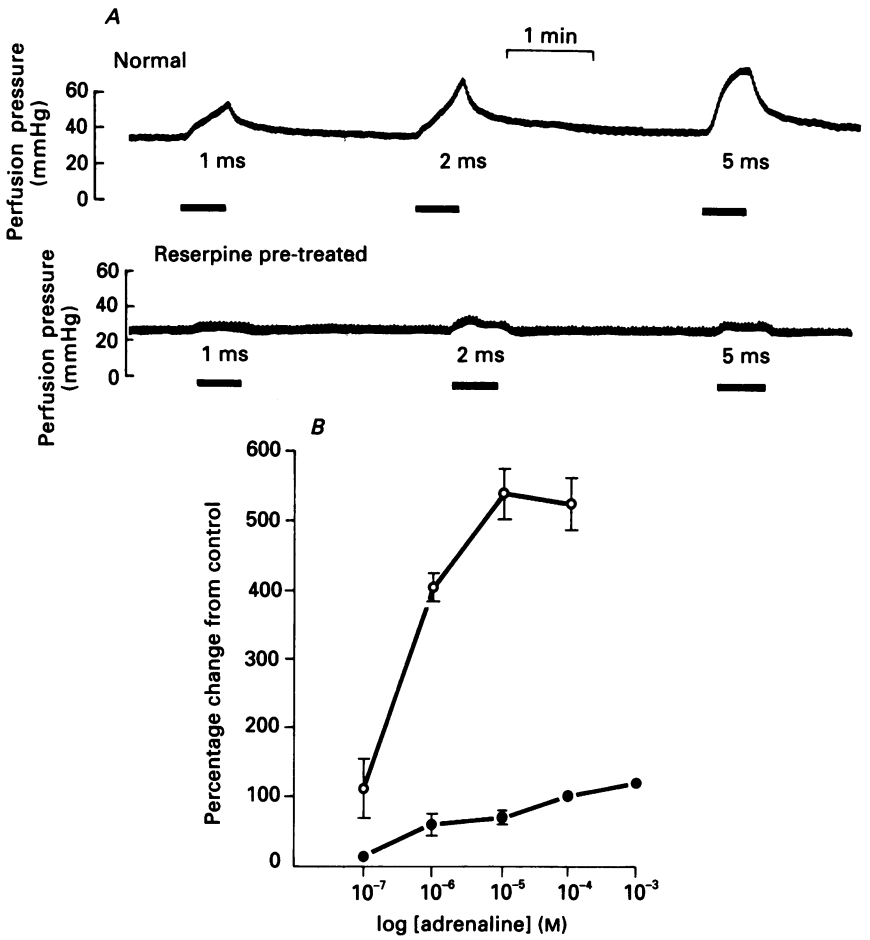

Fig. 2. *A*, the upper three traces show responses to field stimulation at three pulse widths in a normal animal (40 V, 20 Hz). The lower set of traces show the responses at similar stimulus parameters in an animal pre-treated with reserpine. The bars indicate stimulation periods. *B*, dose–response curves to adrenaline injected into the perfusate in normal animals (●) and in reserpinized animals (○), showing the marked hypersensitivity of the α-adrenoceptors in the latter. Normal, $n = 6$–7. Reserpine treatment, $n = 4$.

increased in magnitude with increase in stimulus voltage. To assess whether field stimulation mediates its vasoconstrictor effect solely via the sympathetic efferent nerve fibres surrounding joint blood vessels, or whether the smooth muscle cells in these vessels are directly affected, tetrodotoxin (TTX) was used which blocks neuronal conduction but not action potentials in smooth muscle. TTX (10^{-7} M) was perfused for 20 min and was also added to the organ bath (10^{-8} M). As shown in Fig. 1

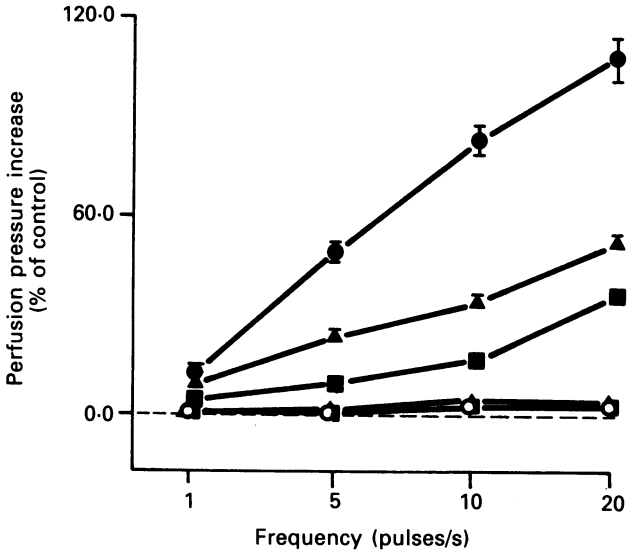

Fig. 3. Responses to field stimulation before (filled symbols) and during perfusion with phenoxybenzamine at 10^{-5} M (open symbols). Three pulse widths were used: 5 ms (●), 2 ms (▲) and 1 ms (■). During perfusion with phenoxybenzamine the responses to electrical stimulation were virtually abolished at all three pulse widths and hence the symbols are superimposed. $n = 6$–8.

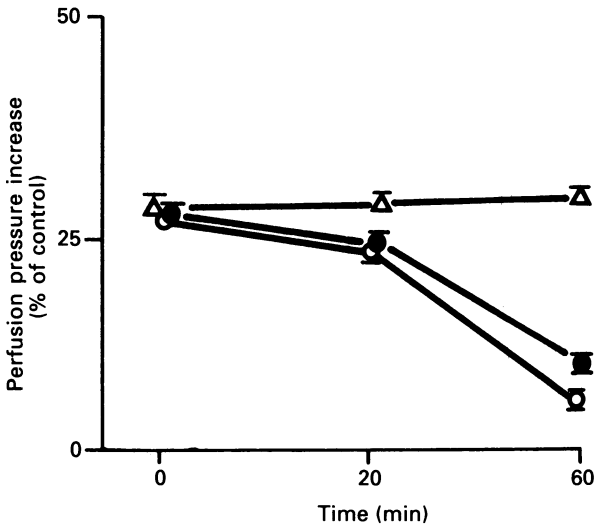

Fig. 4. Comparison of the effect of continuous perfusion of the knee joint with Locke's solution containing phenoxybenzamine at 10^{-5} M (○) or guanethidine at 10^{-5} M (●) on the constrictor response to field stimulation. The effect of field stimulation during perfusion with Locke's solution alone (△) is also shown. $n = 7$–9.

(middle panel), TTX produced a 71% reduction in the response to field stimulation and this could be reversed by perfusion with fresh Locke's solution and repeated changes of the organ bath. (Fig. 1, right-hand panel). This suggests that the stimuli applied are sufficient to affect the nerves supplying the joint but have little direct

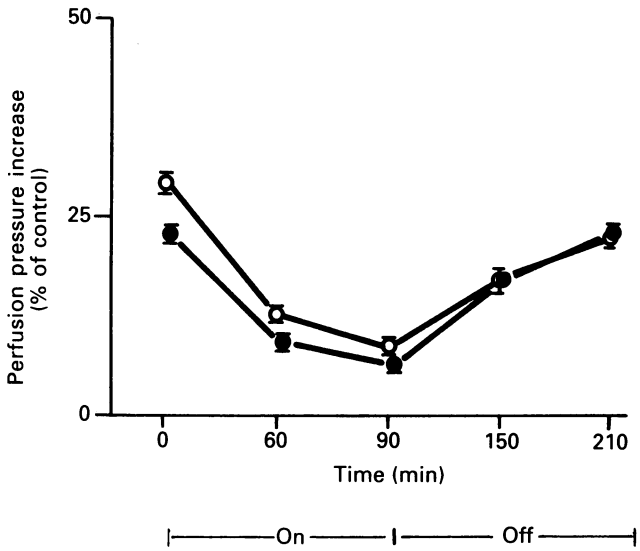

Fig. 5. Comparison of the effect of continuous perfusion of the knee joint with prazosin at 10^{-6} M (●) and another perfusate (○) containing prazosin (10^{-6} M) plus rauwolscine (10^{-6} M). 'On' indicates the period during which the Locke's perfusate contained the adrenoceptor antagonists, whilst 'Off' indicates return to plain Locke's solution. $n = 6$–7.

effect on vascular smooth muscle. Further evidence to support this was obtained by examining the response of articular blood vessels to field stimulation in animals pre-treated with reserpine to deplete sympathetic nerve endings of noradrenaline. As illustrated in Fig. 2*A*, field stimulation elicited very weak contractions in these preparations, even at maximum stimulus parameters. In normal animals, the constrictor responses to field stimulation at 1, 2 and 5 ms pulse width showed a mean rise of $36{\cdot}3 \pm 2$, $52{\cdot}7 \pm 1{\cdot}6$ and $108{\cdot}5 \pm 6{\cdot}7$% ($n = 6$–8) compared to pre-stimulation values, respectively, whereas in reserpinized animals the responses were $9 \pm 2{\cdot}4$, $13{\cdot}8 \pm 2{\cdot}2$ and $16{\cdot}9 \pm 2{\cdot}6$%, respectively ($n = 4$). Figure 2*B* shows that the reserpine treatment did not adversely affect the blood vessels as these still responded to exogenous adrenaline. Indeed, there is clear evidence of denervation hypersensitivity, presumably by up-regulation of α-adrenoceptors.

In normal animals, responses to field stimulation were obtained both by increasing the number of pulses per second and also by increasing the pulse width (Fig. 3). These show considerable enhancement of the constrictor responses which were all inhibited when the joints were perfused with phenoxybenzamine (10^{-5}) for about 1 h before stimulation.

Nature of neurotransmitters mediating the constrictor response

In order to determine the nature of the neurotransmitters mediating the vasoconstrictor response, the effect of electrical stimulation was observed during

perfusion with phenoxybenzamine (10^{-5} M) which blocks both α_1- and α_2-adrenoceptors (Weiner, 1985) and with guanethidine (10^{-5} M) which blocks the release of neurotransmitters such as noradrenaline, ATP and neuropeptide Y. As shown in Fig. 4, the responses to field stimulation gradually decreased during perfusion and were almost abolished after 60 min. Both agents were almost equally effective in blocking the responses and also had similar time courses. Also shown in Fig. 4 is that the constrictor response to capsule stimulation remains constant over a similar time period when perfused with plain Locke's solution.

The effectiveness of phenoxybenzamine in blocking the responses to field stimulation suggests that the neurotransmitter involved may be noradrenaline acting upon α-adrenoceptors. To further investigate which type of α-adrenoceptor was involved, in other preparations the effects of the selective α_1-adrenoceptor blocker prazosin (10^{-6} M) on the response to field stimulation was examined. As illustrated in Fig. 5, 60 min after the start of perfusion with prazosin the constrictor response was substantially reduced, with a further, but smaller, decrease at 90 min. On cessation of the prazosin perfusion the response gradually returned to control values by about 120 min post-perfusion. In another series of experiments the selective α_2-adrenoceptor blocker rauwolscine (10^{-6} M) was added to the prazosin perfusion. However, it is clear from Fig. 5 that adding rauwolscine did not significantly modify the effect of prazosin. Although there were differences in the control responses between the two groups of preparations, by 90 min of perfusion there was little difference in the magnitude of inhibition of the constrictor response.

In order to assess whether any component of the constrictor response was mediated via purinergic receptors, α,β-methylene ATP (10^{-6} M), a P_2-purinoceptor desensitizer, was perfused and the effect of field stimulation was examined during the period of perfusion. In none of the preparations was this agent found to influence the constrictor response even after 90 min of perfusion.

DISCUSSION

The results of the present experiments indicate that the vasoconstrictor response to electrical stimulation of the dorsal aspect of the joint capsule is neurally mediated as it was substantially reduced by tetrodotoxin. Complete blockage was not obtained which may be due to the relatively large mass of tissue involved or the doses employed. It has been shown that even with doses of tetrodotoxin as large as 10^{-6} M it is not possible to completely block sensory C fibres, although such doses do block myelinated nerve fibres (Kirchoff, Reeh & Waddell, 1989). The substantially attenuated vasoconstrictor responses to electrical stimulation of the capsule in reserpine-pre-treated animals also indicates that these responses are neurally mediated. Additional supporting evidence is that the α-blocker phenoxybenzamine completely abolished the response to electrical stimulation, even when a pulse width of 5 ms was used (Fig. 3). None of these procedures would have been expected to prove successful had the vasoconstrictor response resulted from direct electrical stimulation of vascular smooth muscle.

The vasoconstrictor response to electrical stimulation was blocked almost equally well by the α_1,α_2-blocker phenoxybenzamine and by guanethidine which inhibits

release of various neurotransmitters, suggesting that vasoconstriction is principally mediated by noradrenaline. If any other neurotransmitters are co-localized within sympathetic efferent fibres innervating knee joint blood vessels, these do not appear to contribute significantly to the constrictor response. This was borne out in the reserpinized animals where the constrictor responses were markedly reduced. These were not completely abolished, possibly due to incomplete depletion of noradrenaline from sympathetic nerve endings, coupled with the marked hypersensitivity of α-adrenoceptors in the treated animals. Noradrenaline appears to be acting upon α_1-adrenoceptors as vasoconstriction was blocked by the α_1-antagonist prazosin, but there was little additional effect either on the magnitude or the time course by the addition of the α_2-antagonist rauwolscine to the perfusate. The control experiment involving perfusion without antagonists (Fig. 3) demonstrates that these effects can be attributed to these agents and not to any deterioration of the tissue. The length of time taken by antagonists to reduce the response to electrical stimulation of the capsule (e.g. Figs 3 and 4) suggests that these agents do not themselves interact directly with the receptors. Haloalkylamines such as phenoxybenzamine are known to have a slow onset of action due the time required for formation of reactive intermediates which bind with receptors (Weiner, 1985). Similarly, prazosin is known to have a slow onset of action (Bowman & Rand, 1980). Additional factors may be the time taken for the agents to reach effective concentrations at sympathetic nerve endings in the joint capsule due to the relatively large mass of tissue involved (anterior and posterior capsule) coupled with a relatively slow flow (< 1 ml/min).

These results are consistent with previous observations that α-adrenoceptors are present on rabbit knee joint blood vessels and that vasoconstriction elicited by noradrenaline injection is mediated principally via α_1-adrenoceptors (Ferrell & Khoshbaten, 1989).

Although it has recently been shown that P_1- and P_2-purinoceptors are present within articular blood vessels, and that the P_2-receptor mediates vasoconstriction (Ferrell & Khoshbaten, 1990), in the present experiments no evidence was obtained to indicate that ATP is released from nerve endings and contributes towards the vasoconstriction elicited by electrical stimulation of the joint capsule. Desensitization of P_2-purinoceptors by α,β-methylene ATP perfusion had no effect on the magnitude of the constrictor response, and reserpine pre-treatment, which specifically depletes noradrenaline but not adenine nucleotide from storage vesicles (Burack, Weiner & Hagen, 1960), resulted in negligible responses to capsule stimulation. Whether purinoceptors play a role in regulating articular blood vessel calibre could not be verified in the present experiments.

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