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ADRENOCEPTOR PROFILE OF BLOOD VESSELS IN THE KNEE JOINT OF THE RABBIT

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SUMMARY

1. An *in vitro* preparation of the rabbit knee joint, perfused with oxygenated Locke solution, was used to assess the nature of adrenoceptors within articular blood vessels.

2. Dose/response relationships were obtained to intra-arterial injection of α- and β-adrenoceptor agonists.

3. Adrenaline and noradrenaline produced a similar pattern of increasing constriction of articular vessels with increasing dose of drug.

4. The α_1-agonist, phenylephrine, also produced dose-dependant constrictor responses, but the α_2-agonist, clonidine, had no effect. The α_2-agonist UK-14304 did, however, produce modest vasoconstriction which was not greatly altered by the α_1-blocker prazosin. The constrictor effect of noradrenaline was abolished by both the $\alpha_{1,2}$-blocker phenoxybenzamine and by prazosin but not by the α_2-blocker rauwalscine.

5. The β-adrenoceptor agonist, isoprenaline, had little effect at a dose of 10^{-6} M or lower, but gave rise to a constrictor effect at higher concentrations. This response was blocked by phenoxybenzamine but not by the $\beta_{1,2}$-blocker propanolol, suggesting that the constrictor effect was mediated via α-adrenoceptors.

6. The results suggest that α_1- and α_2-adrenoceptors are present within articular blood vessels, but that β-receptors are absent. The effects of noradrenaline appear to be mediated principally via α_1-adrenoceptors.

INTRODUCTION

The maintenance of a stable intra-articular environment is critically dependent on synovial fluid formation and the factors determining this have been described in detail by Levick (1984). The perfusion pressure across the synovial vascular bed is known to be an important determinant of trans-synovial flow (Knight & Levick, 1984). However, relatively little is known about the factors influencing articular blood vessel calibre. It has been shown that blood vessels in the dog knee joint are innervated by sympathetic efferent nerve fibres whose action is to constrict these vessels (Cobbold & Lewis, 1956*a*). This finding was confirmed in a more recent study on the cat knee joint (Ferrel & Cant, 1987).

As to the types of adrenoceptors present on articular blood vessels, less is known.

Cobbold & Lewis (1956*b*) found that close intra-arterial injection of adrenaline and noradrenaline both produced vasoconstriction, although noradrenaline produced consistently greater responses. Although not commented upon, this finding could be explained by the presence of β-receptors on these blood vessels in addition to α-

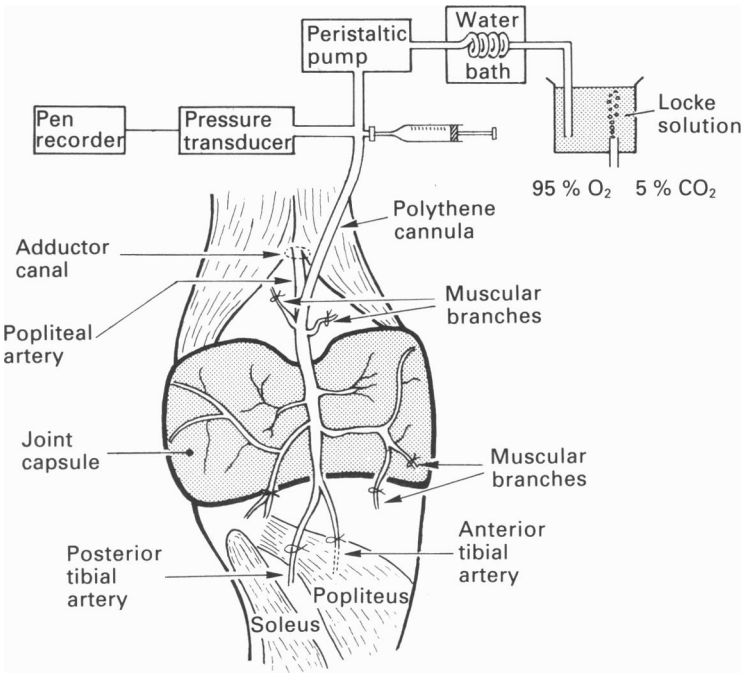

Fig. 1. Diagrammatic representation of the arterial supply of the dorsal aspect of the rabbit knee joint capsule.

receptors. However, these authors do not appear to have systematically investigated the dose/response relationship of these agents, and were therefore unable to establish their relative potencies. The object of the present study was to perform a more extensive and quantitative investigation using selective α- and β-receptor agonists and antagonists in order to characterize the types of adrenoceptors present on knee joint blood vessels in the rabbit.

METHODS

Experiments were performed on albino New Zealand rabbits of either sex weighing between 2 and 4 kg, which were killed by a blow to the skull and exsanguinated. Immediately thereafter, the posterior aspect of the knee joint was exposed, the popliteal artery cannulated and its muscular branches ligated as shown in Fig. 1. The tissue was perfused with warmed and oxygenated Locke solution by means of a peristaltic pump (Gilson Minipuls). 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 midway through the tibia. The isolated knee joint preparation was then transferred to a thermostatically controlled bath (37 ± 1 °C) containing oxygenated Locke solution. At the start of the experiment the pump was set to provide a perfusion rate of 1–1·5 ml/min which

resulted in a perfusion pressure of about 40–50 mmHg as measured by a pressure transducer connected 'downstream' from the pump. Changes in perfusion pressure thereafter provided an indirect measure of articular blood vessel calibre. Peak response was compared to the control (pre-injection) value and expressed as percentage change from control (or baseline). 'Control' injections of Locke solution were administered periodically and were found to produce little change in pressure, apart from a transient rise during the injection phase.

In order to check that only the joint capsule was perfused and not surrounding structures whose arterial supply might have escaped ligation, in eight experiments Evans Blue dye was injected into the perfusate. After 5 min, tissue samples from the joint capsule, distal femur, proximal tibia and surrounding muscles were excised and the dye content estimated photometrically (620 nm). The dye (mean $\pm$ S.E.M. micrograms Evans Blue/100 mg tissue; $n = 8$) was present in large quantities in the posterior capsule ($48{\cdot}96 \pm 2{\cdot}18$) and to a much smaller extent in the anterior capsule ($3{\cdot}12 \pm 0{\cdot}27$) but little was present in other areas (muscle, $0{\cdot}35 \pm 0{\cdot}1$; femur, $0{\cdot}31 \pm 0{\cdot}11$; tibia, $0{\cdot}23 \pm 0{\cdot}05$). The value for the anterior capsule differed significantly from these other areas ($P < 0{\cdot}001$) and from the posterior capsule ($P < 0{\cdot}001$). These results indicate that the ligatures had successfully isolated the capsular circulation.

The composition of Locke solution per litre 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. $CaCl_2$ was added after oxygenating the solution with a 95% O_2 and 5% CO_2 mixture. Various drugs at different concentrations were administered by 0·2 ml injection into the tubing beyond the pump (Fig. 1). In the case of antagonists and pressor agents (e.g. vasopressin), these were perfused continuously. The following agents were administered: [Arg^8]-vasopressin acetate, adrenaline hydrochloride, noradrenaline hydrochloride, phenylephrine hydrochloride, angiotensin II (acetate salt) and propanolol hydrochloride (Sigma); phenoxybenzamine hydrochloride (Smith, Kline & French); isoprenaline sulphate (Aldrich); UK-14304 bitartarate (5-bromo-6[2-imidazolin-2-ylamino]-quinoxaline) and prazosin hydrochloride (Pfizer); rauwalscine hydrochloride (Roth). All drugs were dissolved in Locke solution.

Statistical data analysis was carried out by either paired or unpaired t test. An F test was used to test the assumption of homogeneity of variances. Where this exceeded tabled F values, modified t values were generated using the formula described by Phillips (1978). All data expressed on graphs are means $\pm$ S.E.M. Differences between means were considered significant if the P values were 5% or less.

RESULTS

Intra-arterial injection of adrenaline and noradrenaline resulted in a dose-dependent vasoconstriction of knee joint blood vessels (Fig. 2*A*). Although the dose/response profiles differ at 10^{-6} and 10^{-3} M, these show considerable overlap elsewhere. For both agents the response at each dose differed significantly from the response evoked by the preceding dose except for the highest dose of noradrenaline and the 10^{-5} M dose of adrenaline.

The nature of the α-adrenoceptors mediating the response to noradrenaline was investigated by using selective α-adrenoceptor blockers. Figure 2*B* shows that the responses to increasing doses of noradrenaline were reduced during perfusion with 10^{-6} M-prazosin and abolished by 10^{-5} M-prazosin, whereas perfusion with the α_2-blocker rauwalscine had negligible effect. These results suggest that the constrictor effect of noradrenaline was mediated via α_1-adrenoceptors. To further investigate this, α_1- and α_2-agonists were injected. Comparison of the effects of the selective α_1-agonist phenylephrine with the selective α_2-agonist clonidine (Fig. 3*A*) reveals the contrast between the powerful constrictor effect of phenylephrine, which compares favourably with the effects of adrenaline and noradrenaline, and the lack of response to clonidine. However, when another selective α_2-agonist (UK-14304) was used, it

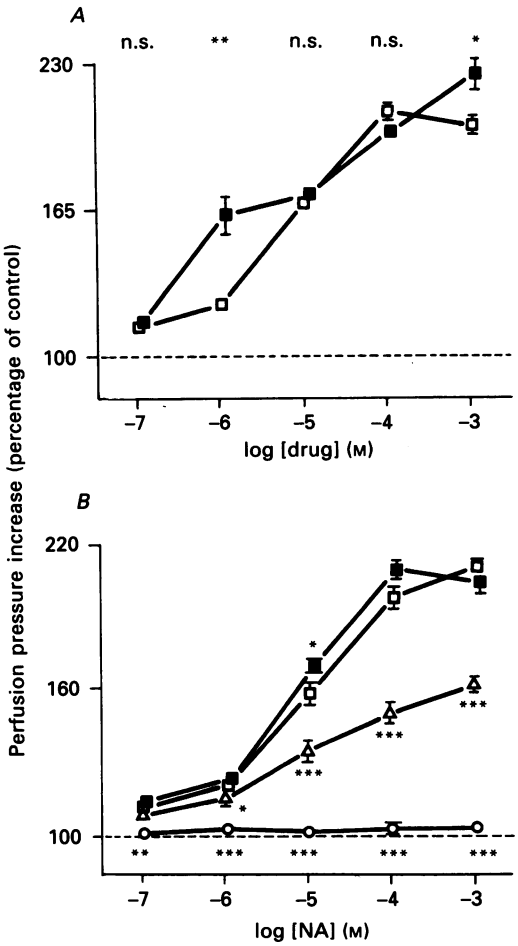

Fig. 2. *A*, change in perfusion pressure (mean ± S.E.M.) with increasing doses of adrenaline (■) and noradrenaline (□); $n = 6$–10. $^{**}P < 0{\cdot}01$; $^{*}P < 0{\cdot}05$; n.s., not significant. *B*, response to increasing doses of noradrenaline (NA) before (■), and during perfusion with 10^{-5} M-rauwalscine (□), 10^{-6} M-prazosin (△) and 10^{-5} M-prazosin (○); $n = 6$–7. $^{*}P < 0{\cdot}05$; $^{***}P < 0{\cdot}001$.

was found that constrictor responses were obtained at higher doses and these were unaffected by prazosin perfusion (Fig. 3*B*), suggesting that this effect was mediated by α_2-adrenoceptors. Raising the tone of blood vessels by the addition of angiotensin II (5×10^{-8} M) enhanced the response to injected UK-14304. Under these conditions prazosin had little effect at low doses of UK-14304, suggesting that these responses were mediated by α_2-adrenoceptors. However, at higher doses (10^{-4} and 10^{-3} M) prazosin produced significant reduction of the constrictor response to UK-14304, suggesting involvement of α_1-adrenoceptors as well. The effect of UK-14304 was tested in eleven preparations and produced responses in nine of these. In two cases no responses were observed even during angiotensin II infusion.

The presence of β-adrenergic receptors was investigated by injection of the β-agonist isoprenaline. As illustrated in Fig. 4, there was little effect up to a dose of

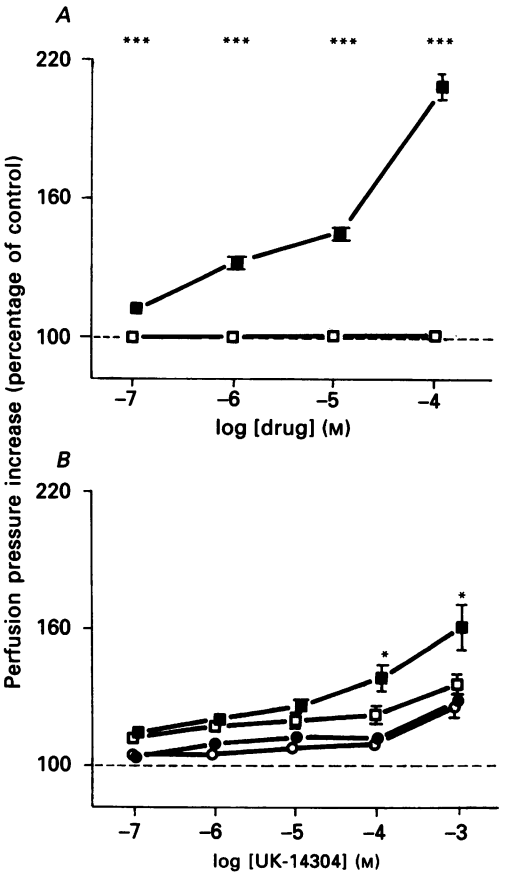

Fig. 3. *A*, responses (mean ± S.E.M.) to α_1-agonist phenylephrine (■), and α_2-agonist clonidine (□); $n = 6$–12. ***$P < 0.001$. *B*, responses to α_2-agonist UK-14304 before (●) and during perfusion with 10^{-6} M-prazosin (○); $n = 6$–13. Upon raising the tone of the blood vessels by perfusion with 5×10^{-8} M-angiotensin II, the response to UK-14304 (■) is enhanced. Addition of 10^{-6}M-prazosin to the perfusate reduces the responses at higher doses (□); $n = 6$–9. *$P < 0.05$.

10^{-6} M, but thereafter a significant constrictor response was obtained. The inability to detect any dilator response could be attributed to a low inherent basal constrictor tone in the absence of tonic sympathetic outflow. Thus, constrictor tone was enhanced by perfusion of the tissue with vasopressin (10^{-8} M), and isoprenaline again injected in increasing doses. However, no significant dilator effect was observed at doses up to 10^{-4} M.

The constrictor effect of isoprenaline illustrated in Fig. 4 was mediated via α-adrenoceptors as it could not be blocked by propanolol (10^{-5} M) but was substantially reduced by phenoxybenzamine (10^{-3} M), as shown in Fig. 5. Also shown is that the constrictor effects of phenylephrine and noradrenaline are blocked by phenoxybenzamine, with only rauwalscine having no antagonistic effect on the actions of noradrenaline.

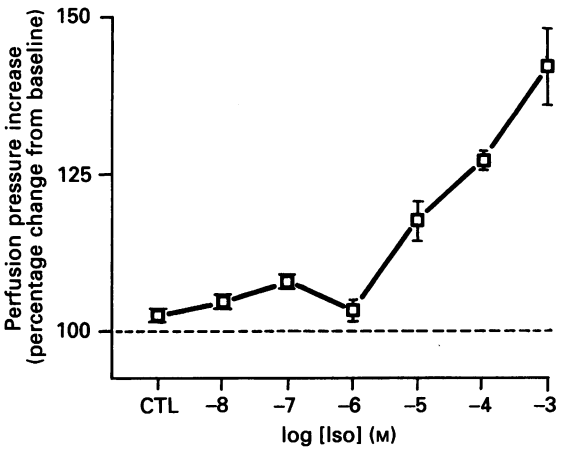

Fig. 4. Responses (mean ± S.E.M.) to increasing doses of isoprenaline (Iso). CTL is the response to injection of Locke solution. Values are compared to the baseline value prior to injection of any agents.

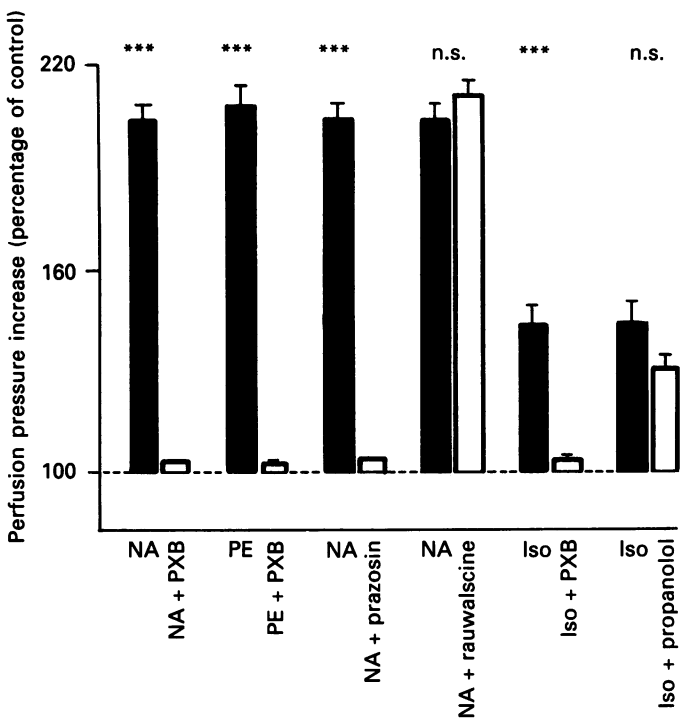

Fig. 5. Comparison of the effect of phenoxybenzamine (PXB, 10^{-3} M) on noradrenaline (NA, 10^{-3} M), phenylephrine (PE, 10^{-4} M) and isoprenaline (Iso, 10^{-3} M). PXB blocks the response to NA and PE. The constrictor effect of Iso is not blocked by propanolol (10^{-5} M) but by PXB. The response to NA is blocked by prazosin (10^{-5} M) but not by rauwalscine (10^{-5} M). ***$P < 0.001$; n.s., not significant.

DISCUSSION

The results of the present experiments clearly demonstrate the presence of α_1-adrenoceptors on articular blood vessels. There was little systematic difference in the constrictor response to adrenaline and noradrenaline across a range of doses suggesting that these agents were acting solely on α-adrenoceptors. This differs from the findings of Cobbold & Lewis (1956*b*) who observed that noradrenaline produced more powerful constrictor effects on dog knee joint blood vessels. However, as they did not quantify their findings, it is difficult to assess the significance of this effect. Another explanation for this discrepancy may reside in species differences.

There was no evidence to indicate that β-adrenoceptors are present on blood vessels in the rabbit knee. The constrictor effect of isoprenaline was mediated by α-adrenoceptors as it could not be blocked by propanolol but was blocked by phenoxybenzamine. Even with enhanced constrictor tone induced by perfusion with vasopressin, elevated doses of isoprenaline failed to evoke significant vasodilatation.

The results of these experiments suggest that the dose-dependant constriction of articular blood vessels by noradrenaline is mediated principally via α_1-adrenoceptors. Although post-junctional α_2-adrenoceptors could be demonstrated, these would appear to contribute relatively little to this constrictor response.

An interesting feature of the present results is that although the popliteal artery divides to give muscular and articular branches in close proximity to each other, the adrenoceptor profile of articular blood vessels is closer to that of superficial tissues such as skin than that of blood vessels supplying muscle (Weiner & Taylor, 1985). Thus, although both skeletal muscle and joints are considered 'deep' structures, there is no homogeneity in the distribution of adrenoceptor types which may reflect the differing function of these two vascular beds.

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