Sympathetic innervation and α -adrenoceptor profile of blood vessels in the posterior region of the rabbit knee joint

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- 1 Experiments were performed to determine the nature of adrenoceptors mediating neurally-induced vasoconstriction of blood vessels in the posterior region of the rabbit knee joint capsule.
- 2 Electrical stimulation of the posterior articular nerve resulted in frequency-dependent vasoconstriction which was maximal at 10 Hz. This response was mediated predominantly by α_2 -adrenoceptors as it was only slightly reduced by prazosin administration and was not only abolished but converted into a dilator response by the α_2 -adrenoceptor antagonist rauwolscine. Further experiments with another specific α_1 -adrenoceptor antagonist YM-12617 showed that the frequency-response curve in the presence of this antagonist did not differ significantly from control.
- 3 Neurally-induced vasoconstriction did not appear to have a purinergic component as it was unaffected by the P_{2X} -purinoceptor desensitiser α, β methylene ATP.
- 4 The rank-order of potency of α -adrenoceptor agonists given as a bolus by close intra-arterial injection was: adrenaline = UK-14304 > clonidine > phenylephrine, suggesting that the vasoconstrictor effects were mediated predominantly by postjunctional α_2 -adrenoceptors.
- 5 The α_2 -adrenoceptor antagonist rauwolscine converted the constrictor response to close intra-arterial injection of adrenaline into a dilator response. The vasoconstrictor responses to UK-14304, clonidine and phenylephrine were substantially inhibited by rauwolscine. The α_1 -adrenoceptor antagonist prazosin failed to inhibit the vasoconstrictor responses to adrenaline, clonidine and UK-14304 and resulted in enhancement of their constrictor effects.
- 6 The enhancement of the responses to the α_1 and α_2 agonists by prazosin appeared to be specifically related to this agent as administration of YM-12617 did not show such enhancement. The dose-response curves to both clonidine and UK-14304 in the presence of YM-12617 did not differ significantly from control responses. Responses to phenylephrine were significantly reduced by YM-12617, indicating the presence of post-junctional α_1 -adrenoceptors.
- 7 These results show almost complete reversal of the adrenoceptor profile compared to results obtained in an earlier *in vitro* study, where responses were mediated predominantly by α_1 -adrenoceptors with a small population of postjunctional α_2 -adrenoceptors (Ferrell & Khoshbaten, 1989). This suggests that the differing environment *in vitro* may not completely reflect the conditions prevailing *in vivo*.

Keywords: Joint; adrenoceptors; blood flow; neural stimulation

Introduction

The presence of sympathetic nerve fibres innervating articular blood vessels was first demonstrated by Cobbold & Lewis (1956a) who used an electromagnetic flowmeter to measure blood flow of the canine knee joint and observed that traction of the lumbar sympathetic chain reduced knee joint blood flow. In addition, they found that section of the articular nerve supply resulted in increased blood flow, suggesting that these vessels possess sympathetic vasoconstrictor 'tone'. In other experiments Cobbold & Lewis (1956b) found that close intra-arterial injection of noradrenaline produced vasoconstriction, indicating the presence of α -adrenoceptors. In more recent experiments in a rabbit isolated perfused knee joint preparation, it was observed that the α_1 -adrenoceptor agonist phenylephrine elicited potent vasoconstriction but the α_2 agonist clonidine was ineffective and another α_2 agonist UK-14304 exerted only small constrictor effects at high doses (Ferrell & Khoshbaten, 1989). Electrical stimulation of the joint capsule also produced vasoconstriction which was substantially reduced by adding the α_1 -adrenoceptor antagonist prazosin to the perfusate, but inclusion of the α_2 -adrenoceptor antagonist rauwolscine did not produce additional inhibition of the constrictor response (Ferrell & Khoshbaten, 1990a). These results indicate that in this preparation, sympathetic effects are mediated mainly via α_1 -adrenoceptors which predominate in resistance vessels of the rabbit knee joint. Advantages of the *in vitro* preparation

described above are the reduced number of variables which require to be controlled and that this model provides an indication of the overall vascular resistance of the joint capsule. The disadvantage of the *in vitro* technique is that the environment is artificial and lacks many of the vasoactive agents which normally circulate in the blood stream and which could modify the response of adrenoceptors. Recent observations in both *in vitro* and *in vivo* models suggest that in the latter, effects mediated by postjunctional α_2 -adrenoceptors are more readily detectable, whereas *in vitro* these are difficult to detect and vasoconstrictor effects are mediated principally by postjunctional prazosin-sensitive α_1 -adrenoceptors (for review see McGrath *et al.*, 1989). Thus, the present study was performed in anaesthetized rabbits to examine nerve-mediated vascular responses and assess the nature of adrenoceptors which mediate vasoconstriction in the posterior region of the knee joint capsule *in vivo*.

Methods

Experiments were performed on adult New Zealand rabbits (2.2–3.5 kg) which were anaesthetized initially by injection of diazepam (1.5 mg kg⁻¹ i.p.) and Hypnorm (Janssen; 0.15 ml kg⁻¹ i.m.) and thereafter with a 1% halothane/O₂/N₂O mixture during the surgical procedures. On completion of surgery, the gaseous anaesthetic was discontinued and a slow continuous infusion of pentobarbitone (0.25–0.5 mg

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min⁻¹ depending on the weight of the animal) administered throughout the remainder of the experiment via a cannula inserted into the left jugular vein. The trachea was cannulated, with the animals breathing spontaneously throughout the experiments. Deep anaesthesia was maintained throughout as judged by the absence of a flexor withdrawal response to a pinch applied to the forelimb. Arterial blood pressure was recorded throughout the experiment by a cannula inserted into the left carotid artery. A 0.9 mm diameter fibre-optic probe connected to a laser Doppler flowmeter (Moor Instruments MBF3) provided a measure of relative changes in blood flow in the volume of tissue beneath the tip of the probe. Previous experiments have validated the use of this technique in assessing alterations in knee joint blood flow in both cats and rabbits (Ferrell *et al.*, 1990). The probe was positioned just above the surface of the postero-medial aspect of the knee joint capsule. Alterations in blood flow are expressed as percentage change from control (0%) values occurring immediately before each test procedure. The posterior articular nerve (PAN), which arises from the tibial nerve in the popliteal fossa, was dissected free and transected proximally. Bipolar bright silver stimulating electrodes were then placed on PAN and pulse trains delivered. The stimulus parameters were: width 1 ms, voltage 10 V, frequency 1–30 Hz and duration 90 s. In previous experiments we have demonstrated that these stimulus parameters give rise to frequency-dependent vasoconstrictor responses (Ferrell & Najafipour, 1992). As long as the laser Doppler probe remained in the same position, the constrictor responses remained stable and reproducible over several hours. Termination of the experiment was achieved by intravenous injection of 1 M KCl.

Drugs were administered by close intra-arterial injection via a polythene cannula (Portex 2FG) inserted into the caudal femoral artery (Barone *et al.*, 1973) and advanced until the tip was located close to the main branch (popliteal artery) just before the origin of the joint branches. All non-articular branches of the latter were ligated. The α -adrenoceptor antagonists were administered as a continuous infusion (0.25 ml min⁻¹; 10⁻⁴ M) via this cannula, with the duration of infusion being maintained for a minimum of 30 min before α -agonists were administered or nerve stimulation applied. The antagonists were also administered intravenously via the cannula in the external jugular vein as a bolus (1–1.5 mg kg⁻¹ body weight) prior to antagonist infusion. In some animals only one antagonist was administered whilst in other prazosin was administered first followed by rauwolscine. Other drugs were administered as a bolus injection into the caudal femoral intra-arterial cannula in a volume of 0.25 ml. The adrenoceptor agonists were administered in random order. All drugs were dissolved in physiological saline. Control injections of 0.25 ml saline were found to have no effect on blood flow. The following agents were used: adrenaline HCl (Evans); phenylephrine HCl (Sigma); clonidine HCl (Sigma); phenoxybenzamine HCl (Smith Kline & French); prazosin HCl (Pfizer); UK-14304 bitartrate (5-bromo-6[2-imidazolin-2-ylamino]-quinoxaline; Pfizer); rauwolscine HCl (Roth); α,β methylene adenosine 5'-triphosphate (lithium salt; Sigma); YM-12617 ((-)-(R)-5-[2-[[2-(o-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulphonamide hydrochloride; Yamanouchi Pharmaceutical).

The values expressed in the figures are means $\pm$ s.e.mean and comparisons were performed by use of Student's paired or unpaired *t* test as appropriate.

Results

Nerve stimulation

Electrical stimulation of PAN consistently produced vasoconstriction which was unaffected by pretreatment with α,β methylene ATP (Figure 1a) which is a P_{2X}-purinoceptor

desensitiser (Kasakov & Burnstock, 1983). The results are summarised in Figure 2 and demonstrate that the mean values before and after injection of α,β methylene ATP were not significantly different (unpaired *t* test).

Vasoconstriction elicited by PAN stimulation (Figure 1bii) was, however, abolished by administration of phenoxybenzamine and converted into a dilator response (Figures 1bii and 2). Adrenaline (2.5 nmol), given by close intra-arterial injection, produced vasoconstriction (Figure 1bi) which was similarly altered by phenoxybenzamine (Figures 1bii and 2). In both cases the change in response was significant ($P < 0.001$; unpaired *t* test).

Vasoconstriction elicited by PAN stimulation was frequency-dependent over the range 1 to 10 Hz (Figure 3). Administration of the α_1 -adrenoceptor antagonist prazosin produced a significant reduction in this constrictor response at all frequencies except 30 Hz. In contrast, the α_2 -adrenoceptor antagonist rauwolscine completely reversed the response and produced significant vasodilatation during nerve stimulation.

As prazosin appeared to give a smaller reduction than rauwolscine, suggesting that the constrictor response to PAN stimulation might be partially mediated by α_1 -adrenoceptors, to clarify further the role of α_1 -adrenoceptors, another selective α_1 -adrenoceptor antagonist, YM-12617 (Honda *et al.*,

Figure 1 (a) Constrictor response to electrical stimulation of the rabbit posterior articular nerve (PAN) (10 V, 10 Hz, 1 ms width; 90 s train; denoted by black bar over trace) is unaffected by pretreatment with the P_{2X}-purinoceptor desensitiser α,β methylene ATP (each dose = 2.5 nmol in a volume of 0.25 ml injected at the arrows). (b) (i) PAN stimulation (as in a) and close intra-arterial injection of adrenaline (2.5 nmol at the arrow) produce constrictor responses; (ii) 45 min after onset of phenoxybenzamine administration, these responses are abolished and replaced by dilations. (c) Responses to PAN stimulation (black bar; parameters as in (a)), and close intra-arterial injection of phenylephrine (PE), adrenaline (Ad), clonidine (Clon) and UK-14304 (UK) before prazosin treatment. Dose for each agent = 2.5 nmol. (d) As in (c) but 45 min after onset of prazosin administration. The responses are little affected. (e) Responses to PAN stimulation and the same agents as in (c) before the administration of rauwolscine. (f) As in (e) but 45 min after onset of rauwolscine administration. Responses to agents and nerve stimulation are substantially modified. See methods for details of administration of agonists and antagonists.

Figure 2 Vascular responses to stimulation of the posterior articular nerve (PAN) before treatment with α, β methylene ATP (NS-CTL) and after four injections of this agent (NS + α, β mATP). PAN stimulation before treatment with phenoxybenzamine (NS-CTL) produced vasoconstriction but during administration of phenoxybenzamine (NS + Pbz) this converts to vasodilatation. Close intra-arterial injection of 2.5 nmol adrenaline also produces constriction prior to phenoxybenzamine (Ad-CTL) and this also becomes a dilator response during treatment with this agent (Ad + Pbz). *** $P < 0.001$.

Figure 3 Frequency-response curves to electrical stimulation of the posterior articular nerve (PAN) (10 V, 1 ms, width, 1 min train) under control conditions (●; $n = 15-17$), during prazosin administration (○; $n = 4$), and during perfusion with rauwolsine (□; $n = 5$). Data shown are means and vertical lines indicate s.e.mean.

1985) was used in a smaller series of experiments. This showed that the frequency-response curve in the presence of YM-12617 only differed significantly from the control curve at the lowest frequency (Figure 4), again highlighting the importance of α_2 -adrenoceptors in nerve-mediated vasoconstriction.

α -Adrenoceptor agonists and antagonists

In order to assess the nature of the adrenoceptors mediating these constrictor responses, selective α -adrenoceptor antagonists and agonists were employed. The α_1 agonist phenylephrine failed to show any effects at doses below 2.5 nmol (Figure 5d) whereas the α_2 agonists UK-14304 and clonidine both gave rise to constrictor responses at lower doses (Figure 5b and c). The dose-response curves of these agents suggest that adrenaline and UK-14304 are equipotent (Figure 5a and b), with clonidine giving rise to smaller responses which in general showed the same trend as those of UK-14304 (Figure 5c).

The effect of the α_1 -adrenoceptor antagonist prazosin was investigated as illustrated in Figure 1c and d, which demonstrates that the constrictor responses to close intra-arterial injection of phenylephrine, adrenaline, UK-14304 and clonidine (all 2.5 nmol) were not attenuated by prazosin administration. These results are detailed in Figure 5 for different doses of each of the agonists, which show that in the presence of prazosin the constrictor responses to clonidine, phenylephrine and UK-14304 across a range of doses were actually significantly greater than control values. This effect appeared to be specific to prazosin, as repeating these experiments with YM-12617 at similar intravenous doses and perfusate concentrations (10^{-4} M) neither potentiated nor inhibited the actions of clonidine and UK-14304 (Figure 5b and c). The constrictor response to phenylephrine was, however, significantly reduced by YM-12617 (Figure 5d) suggesting the presence of α_1 -adrenoceptors. The responses to adrenaline at doses below 2.5 nmol were reduced by both prazosin and YM-12617, again indicating the involvement of α_1 -adrenoceptors at these lower doses.

The α_2 -adrenoceptor antagonist rauwolsine gave rise to pronounced inhibition of the responses to all the agonists. As illustrated in Figure 1e and f the responses to PAN stimulation and close intra-arterial injection (2.5 nmol in all cases) of phenylephrine, clonidine, adrenaline and UK-14304 were

Figure 4 Frequency-response curves to electrical stimulation of the posterior articular nerve (PAN) (10 V, 1 ms, width, 1 min train) under control conditions (●; $n = 4-6$) and during administration of YM-12617 (Δ; $n = 4-6$). Data shown are means and vertical lines indicate s.e.mean.

Figure 5 Dose-response curves for close intra-arterial injection (0.25 ml volume) of (a) adrenaline, (b) UK-14304, (c) clonidine and (d) phenylephrine under control conditions (●; $n = 5-10$), during administration of either prazosin (○; $n = 4$), YM-12617 (△; $n = 4-6$) or rauwolscine (□; $n = 5$). Data shown are means and vertical lines indicate s.e.mean. p = pico. n = nano.

substantially inhibited by rauwolscine. Figure 5 shows that rauwolscine shifted the dose-response curves to the right and that adrenaline now gave rise to dilator responses. In all cases rauwolscine produced significant alteration in the responses.

Administration of the α -adrenoceptor antagonists produced some reduction in arterial blood pressure and consequently of synovial blood flow. Before administration of antagonists mean arterial blood pressure ($\pm$ s.e.mean) was 71 ± 7 mmHg ($n = 8$). The bolus injection of antagonist produced an initial reduction of blood pressure and joint blood flow, but these partially recovered and levelled out by 40 min at which time the test procedures were once again performed. Mean arterial blood pressure at this time for phenoxybenzamine, prazosin and rauwolscine was 54.3 ± 4.4 ($n = 8$), 60.8 ± 3.7 ($n = 7$) and 62 ± 3 ($n = 7$) mmHg, respectively, and these values did not differ significantly from each other although they did differ significantly ($P < 0.0004$) from control (unpaired t test). Administration of YM-12617 decreased

blood pressure from 82.8 ± 5.6 to 77.7 ± 5.9 mmHg ($P < 0.05$; $n = 6$; paired t test)

Discussion

The results of this study have shown that electrical stimulation of the nerve supply to the posterior region of rabbit knee joint resulted in vasoconstriction which was mediated predominantly via α_2 -adrenoceptors. Although purinergic co-transmission has been described in other vascular beds such as the urinary bladder (Kasakov & Burnstock, 1983), vas deferens (Sneddon & Burnstock, 1984) and colon (Hedlund *et al.*, 1983), no evidence was obtained in the present study to indicate that ATP has a role to play in mediating the actions of sympathetic vasoconstrictor fibres innervating the joint. The pronounced constrictor effect of the first injection of α , β methylene ATP (Figure 1a) suggests the presence of P_{2X} -purinoceptors in these knee joint blood vessels, although this

could also have resulted from a direct depolarizing effect of α, β methylene ATP on smooth muscle cells (Komori *et al.*, 1988). However, previous experiments in an isolated knee joint preparation showed both P_1 - and P_{2x} -purinoceptors to be present, although relatively high doses of purines were required (Ferrell & Khoshbaten, 1990b). If these purinoceptors are indeed present, their functional role remains unclear.

Although phenoxybenzamine is not specific to α -adrenoceptors, the finding that rauwolscine gave rise to pronounced inhibition suggests that phenoxybenzamine was acting at these receptors. It was clear that the constrictor responses to nerve stimulation and the α_2 -adrenoceptor agonists were mediated predominantly via α_2 -adrenoceptors as these were substantially altered by rauwolscine but unaffected by YM-12617. The effects of prazosin are difficult to interpret as it potentiated the responses to UK-14304, clonidine and phenylephrine but not to the catecholamine, adrenaline. The mechanism underlying the potentiation is unclear at present but was specifically related to prazosin, as the structurally different α_1 -adrenoceptor antagonist YM-1267 did not produce this effect. The potentiating effect of prazosin on the above α_1 and α_2 agonists meant that its expected inhibitory effect on phenylephrine could not be detected. However, the nerve-mediated vasoconstriction (Figure 3) was significantly reduced by prazosin which could be explained by its lack of potentiation of the effect of neurally-released catecholamine, noradrenaline, leaving the inhibitory effects of prazosin on α_1 -adrenoceptors unopposed. The vasoconstriction induced by phenylephrine at higher doses was reduced both with YM-12617 and rauwolscine (Figure 5d), suggesting that phenylephrine may act at both α_1 and α_2 -adrenoceptors at such high doses. The conversion of the constrictor responses to nerve stimulation and adrenaline into vasodilator ones suggests that the latter are normally masked by the more pronounced constrictor responses. In the case of adrenaline, the dilator response during blockade with phenoxybenzamine (and rauwolscine) could be explained by adrenaline acting on β -adrenoceptors. This would be consistent with the finding of Cobbold & Lewis (1956a) that close intra-arterial injection of noradrenaline produced greater vasoconstriction of dog knee joint blood vessels than adrenaline, suggesting the presence of β -adrenoceptors, although this was not discussed in their paper. Although vasoconstriction induced by adrenaline was not antagonized by prazosin or YM-12617 at doses ≈ 2.5 nmol, both of these agents produced inhibition at lower doses, with rauwolscine being effective at all doses (Figure 5a). This could be interpreted as adrenaline acting at both α_1 - and α_2 -adrenoceptors, with the α_1 subtype having a lower activation threshold.

The mechanisms underlying the dilator response which occurred with PAN stimulation during α -adrenoceptor blockade were not investigated in the present study. However, it is possible that this effect was mediated by substance P released from nerve endings of C afferent fibres present in PAN as in previous experiments on cat knee joint blood vessels it was shown that dilator responses following the initial constrictor responses to PAN stimulation were reduced by a substance P antagonist (Khoshbaten & Ferrell, 1990).

Although the administration of all the α -adrenoceptor antagonists resulted in similar decrease in arterial blood pressure and thus altered vascular resistance in the knee joint, the

strikingly different responses to PAN stimulation and α -adrenoceptor agonists occurring during rauwolscine treatment contrasts with the lack of inhibitory effect of prazosin. This suggests that the differences between these agents reflects their differing effects on α -adrenoceptors in these vessels and is not secondary to altered vascular resistance.

In conclusion, the most striking finding of this study was that α_2 -adrenoceptors play a major role in mediating the vasoconstrictor responses to α -agonists and nerve stimulation, whereas in previous *in vitro* studies these responses were predominantly mediated by α_1 -adrenoceptors (Ferrell & Khoshbaten, 1989; 1990a). The mechanisms underlying this change are unclear, but may reflect the differences in the experimental conditions in terms of the artificiality of the *in vitro* preparation. It has been demonstrated in the rabbit isolated distal saphenous artery that contractile responses to sympathetic nerve stimulation are substantially reduced by prazosin but a small residual component remains which is potentiated in the presence of angiotensin II (Dunn *et al.*, 1991). Thus, it is possible that circulating vasoactive substances which are present *in vivo* modify the response to nerve stimulation. However, in the present experiments the virtual absence of inhibition by YM-12617 coupled with the powerful effect of rauwolscine suggests that in this tissue the nerve-mediated constrictor responses are mediated predominantly by α_2 -adrenoceptors. Evidence for α_2 -adrenoceptor involvement in blood vessel contractions evoked by nerve stimulation has proved difficult to obtain in animal tissues, and when demonstrated, the effects were predominantly mediated by α_1 -adrenoceptors (for review see Docherty, 1989). Only in the human saphenous vein have contractions evoked by nerve stimulation been shown to be mediated predominantly by α_2 -adrenoceptors, although even in this preparation some inhibition by prazosin was observed at concentrations above 10^{-7} M (Docherty & Hyland, 1985).

It could be argued that the difference in the results obtained in the *in vitro* and *in vivo* preparations may be related to different methodologies. The isolated knee joint preparation reflects changes of the resistance vessels in the whole capsule, whereas Laser Doppler flowmetry samples a small volume of tissue under the probe and therefore is more regionally specific. In addition, laser Doppler flowmeters are designed to sample flow in the microcirculation where it has been shown that α_2 -adrenoceptors predominate, whereas α_1 -adrenoceptors are more common on the larger resistance arteries (Faber *et al.*, 1991). However, recent *in vivo* experiments using the laser Doppler flowmeter on the anterior knee joint capsule in anaesthetized rabbits have shown that vasoconstrictor effects to nerve stimulation are mediated predominantly via α_1 -adrenoceptors, even though postjunctional α_2 -adrenoceptors are also present (Khoshbaten & Ferrell, unpublished observations). This implies that there are differences in α -adrenoceptor subtypes even within different regions of the same joint. The functional significance of this finding is unclear at present. The present investigation indicates that studies using isolated tissue preparations require careful interpretation and may not accurately reflect the conditions prevailing *in vivo*.

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