PRE- AND POSTJUNCTIONAL α-ADRENOCEPTORS IN RABBIT ARTICULAR BLOOD VESSELS

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ABSTRACT

Previous in vitro work on rabbit knee joint vessels showed that vasoconstrictor effects of nerve stimulation and administration of α-adrenoceptor agonists were mediated predominantly by α₁-adrenoceptors. The present experiments were performed to assess the nature of α-adrenoceptor subtypes within these blood vessels in vivo. Dose/response relationships for adrenaline and noradrenaline produced a similar pattern of increasing constriction of articular vessels with increasing doses of drug. The α agonist phenylephrine also produced dose-dependent constrictor responses which were diminished by prazosin. Using the α₂ agonists clonidine and UK-14304, responses in vivo differed from those previously observed in vitro. There was virtually no response to clonidine in vitro while responses were obvious in vivo. Although UK-14304 was found to have small effects in vitro, but only at high doses, this agent exerted more potent effects in vivo, significantly greater than those obtained with phenylephrine. Responses to the α₂ agonists were not altered significantly by prazosin but were reduced by rauwolscine. Following injection of UK-14304, the constrictor response to nerve stimulation was reduced.

The results suggest that both α₁ and α₂ adrenoceptors are present postjunctionally within articular blood vessels, and also that prejunctional α₂ receptors are present which presumably regulate neurotransmitter release from sympathetic nerve endings in the joint capsule.

Keywords: Blood flow, Joint, Adrenoceptor, Prejunctional.


INTRODUCTION

The stability of the critically dependent on synovial fluid formation and the factors regulating this have been described in detail by Levick. The perfusion pressure across the synovial vascular bed is known to be an important determinant of trans-synovial flow, but the physiological and pharmacological factors which regulate synovial blood flow are at present uncertain. It has been shown that blood vessels in the dog knee joint are innervated by sympathetic efferent nerve fibers whose action is to constrict these vessels. This finding was confirmed in more recent studies on the cat and rabbit.

As to the subtypes of adrenoceptors present on articular blood vessels, rather less is known. Cobbold and Lewis 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-adrenoceptors on these blood vessels in addition to α-adrenoceptors. In a subsequent study in normal human subjects, evidence was obtained suggesting that α and β-adrenoceptors were functionally active in control of synovial perfusion. However, these authors were unable to systematically investigate the dose/response relationship of the α and β agonists, and were therefore unable to establish their relative potencies and subtypes of adrenoceptors. Using an in vitro rabbit knee joint preparation, a recent study demonstrated the presence of α₂-adrenoceptors on articular blood vessels, but α₁-adrenoceptor agonists were found to have only very weak effects. However, it was observed that the effects of α₂-adrenoceptor agonist administration could be enhanced if blood vessel tone was raised by co-administration of angiotensin II, suggesting that α₂-adrenoceptors might exert greater effects in vivo.

The object of this study was to perform an extensive and quantitative in vivo investigation using selective α₁ and α₂ adrenoceptor agonists and antagonists to characterize the subtypes of α-adrenoceptors present on blood vessels in the anterior region of the rabbit knee joint.

METHODS

White New Zealand rabbits weighing ~3 kg were deeply anesthetized by a mixture of intraperitoneal injection of diazepam (0.5 mg/kg) and intramuscular injection of hypnorm (0.1 mg/kg; Janssen). Anesthesia was maintained by giving 1-2% halothane in O₂ and N₂O which was delivered via a tracheal cannula. The carotid artery was cannulated for the measurement of arterial blood pressure (Elcomatic UK, EM 751). A cannula was inserted into the saphenous artery below the knee joint, advanced in a retrograde direction until its tip was just distal to the branches supplying the knee joint and the artery ligated just below this point. This was necessary as the lumen of the saphenous artery is narrow near the joint and tends to be occluded by the cannula. An additional advantage of retrograde cannulation is that it directs most of the agents to the joint, thereby reducing the administered dose and thus minimizing systemic effects.

The saphenous nerve, which in the rabbit gives branches to the knee joint, was dissected free from the adjacent saphenous artery and vein. Then the thigh was held at a fixed position and a thigh paraffin pool was prepared. After the saphenous nerve was sectioned proximally, it was placed over bipolar silver chloride electrodes connected to an Harvard Advance stimulator.

Blood flow monitoring

To observe relative changes in blood flow, a near infra-

![Graph showing changes in blood flow](https://via.placeholder.com/150)

**Fig. 1.** Changes in blood flow (mean ± SEM) with increasing doses of adrenaline (○) and noradrenaline (●) administered by close intra-arterial injection in vivo. n = 5-8. CTL represents response to saline injection.

![Graph showing responses](https://via.placeholder.com/150)

**Fig. 2.** Responses (mean ± SEM) to close intra-arterial injection of the α₁ agonist phenylephrine (■) and the α₂ agonist UK-14304 (▲) in vivo, n = 5-7.

red (780 nm) laser Doppler flowmeter (MBF3; Moor Instruments Ltd.) was used. The MBF3 probe (0.9 mm diameter) was inserted from the antero-lateral aspect of the knee joint and advanced into the joint cavity to make contact with the internal surface of the fibrous portion of the antero-medial capsule in the infra-patellar region. Previous work has shown that there is good agreement between changes in blood flow assessed with laser Doppler flowmetry and radiolabelled microspheres.

Stimulus parameters

The stimulus parameters were: frequency 10 pulse/sec, voltage 10 volts, and pulse width 1 msec. The stimulus pulse train lasted for 30 seconds.
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**Fig. 3.** A: Responses to close intra-arterial injection of phenylephrine (200 nmol), UK-14304 (20 nmol), and clonidine (200 nmol) before and during infusion of prazosin (10⁻⁵M). n=5-6. B: Responses to phenylephrine (200 nmol), UK-14304 (20 nmol), and clonidine (200 nmol) before and during infusion of rauwolscine (10⁻⁵M). n=5-6. *p<0.05.

**Drug administration**

The following agonists were administered by bolus injection (0.2 mL) via a polythene cannula which was inserted into the saphenous artery or popliteal artery; adrenaline, noradrenaline, phenylephrine, and clonidine (Sigma; UK); UK-14304 (Pfizer; UK). Antagonists which were infused; prazosin hydrochloride 10⁻⁵M (Pfizer; UK); rauwolscine hydrochloride 10⁻⁵M (Roth; Germany).

**Statistics**

Statistical data analysis was carried out by either paired or unpaired t-test (one-tailed) or ANOVA. 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.¹⁰ All data expressed on graphs are means±S.E.M. Differences between means were considered significant if the P values were 5% or less. In both text and figure legends, n values refer to the number of animals.

**RESULTS**

**Effect of α₁ and α₂ agonists**

Intra-arterial injection of adrenaline and noradrenaline both resulted in dose dependent vasoconstriction of articular blood vessels in vivo (Fig. 1), as previously demonstrated in vitro.³ For both agents the responses at each dose differed significantly from the response evoked by the preceding dose. The responses to adrenaline and noradrenaline were found to be significantly different from control at doses of about 0.002 nmol in vivo (Fig. 1) whereas significant responses to these agents in vitro required doses of about 0.2 nmol.³

To further investigate the nature of adrenoceptors and the differences in response between in vitro and in vivo
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preparations, α, and α2 agonists were administered. In previous in vitro experiments it was found that the selective α1 agonist phenylephrine produced a powerful constrictor effect whilst the selective α2 agonist (UK-14304) produced constrictor responses, but only at high doses. In contrast, when UK-14304 was tested in vivo, it was found to produce a dose dependent vasoconstriction of articular blood vessels in all animals (Fig. 2), but with greater sensitivity compared to the responses previously observed in vitro. Injection of clonidine also resulted in significant constrictor responses in vivo (Fig. 3). Phenylephrine produced vasoconstriction which was significantly less (p<0.01) than that elicited by UK-14304.

Effect of α1 and α2 adrenoceptor antagonists

Figure 3A shows that the response to phenylephrine was reduced by 10−6M prazosin, but responses to UK-14304 (20 nmol) and clonidine (200 nmol) were not significantly changed. Whereas the α2-antagonist rauwolscine did not reduce the constrictor response to phenylephrine, responses to UK-14304 and clonidine were significantly reduced (Fig. 3B). These results suggest that postjunctional α1 and α2-adrenoceptors co-exist on articular blood vessels.

Effect of UK-14304 on response to nerve stimulation

As illustrated in Figure 4A, saphenous nerve stimulation resulted in vasoconstriction of articular blood vessels. Subsequent close intra-arterial injection of UK-14304 induced vasoconstriction and the response to nerve stimulation was reduced after this injection. It was noticeable that the response to nerve stimulation recovered 5 minutes after infusion with normal saline. The reduction in the constrictor response to nerve stimulation following administration of UK-14304 was found to be dose dependent (Fig. 4B).

DISCUSSION

The results of the present experiments clearly demonstrate the presence of postjunctional α1 and α2 adrenoceptors on blood vessels in the rabbit knee joint. There was little systematic difference in the constrictor response to adrenaline and noradrenaline in vitro and this was confirmed in this study, suggesting that these agents were acting mainly on α adrenoceptors. In low doses, the constrictor effect of adrenaline in vivo was found to be (significantly?) greater than that of noradrenaline. This could be the result of α adrenoceptor activation leading to vasodilation in striated muscles around the knee joint which shunted blood flow into these areas and thus away from articular blood vessels. This differs from the findings of Cobbold and Lewis who observed that noradrenaline produced more powerful constrictor effects on dog knee joint blood vessels. However, as they did not quantitate their findings, it is difficult to assess the significance of this effect. Another explanation for this discrepancy may reside in species differences.

Since the arterial branches close to knee blood vessels were left unligated in the in vivo experiments, it is possible that some of the injected drugs enter these branches and thus less drug would reach the articular blood vessels. Despite this, there were greater responses to intra-arterial injection of UK-14304 in vivo compared to the effects of this agent in vitro. The explanation for this might reside in the sensitivity of α receptors, especially α1 adrenoceptors which are more sensitive in vivo than in vitro, since they did not respond to clonidine in vitro but did respond to it in vivo.

Alternatively, synergistic interaction of UK-14304 with circulating substances such as vasopressin and angiotensin II may have occurred in the in vivo preparation. This was supported to some extent by the observation that the response to UK-14304 was greater in vitro when vascular tone was elevated by angiotensin II.

Although in previous studies it was demonstrated that circulating adrenaline, and noradrenaline released from nerve endings, affect articular blood flow by acting mainly through α1 adrenoceptors, the results of the present study suggest that postjunctional α2-adrenoceptors may also play a role in regulating knee joint blood flow.

The vasoconstrictor response to electrical stimulation of the saphenous nerve was significantly attenuated when UK-14304 was injected prior to nerve stimulation. This could have resulted from the activation of prejunctional α1 adrenoceptors on sympathetic nerve endings which inhibited noradrenaline release from these endings.

In conclusion, these results indicate that both α1 and α2 adrenoceptors are present postjunctionally and can mediate vasoconstriction to circulating catecholamines as well as that occurring during electrical stimulation of the nerve supply to the knee joint. In addition, prejunctional α2 adrenoceptors are also present on sympathetic endings innervating articular blood vessels and these are likely to play a role in regulating joint blood flow.

REFERENCES


