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A pharmacological study of the rabbit saphenous artery *in vitro*: a vessel with a large purinergic contractile response to sympathetic nerve stimulation

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1 Mechanical responses to transmural electrical stimulation were recorded in isolated transverse ring preparations of rabbit saphenous artery. Electrical stimulation for a period of 1 s produced a rapid monophasic contraction and, for a period of 1 min, a biphasic contraction consisting of a rapid constriction followed by a slower sustained constriction. All contractions were abolished in the presence of tetrodotoxin (1 $\mu g\,ml^{-1}$) or guanethidine (4 μM).

2 After desensitization of the P_2-purinoceptor with α,β-methylene ATP, contractions to electrical stimulation for 1 s were reduced significantly at all frequencies tested: responses evoked by stimulation at 4 Hz were usually almost completely inhibited, whereas those evoked by stimulation at 64 Hz were only partially inhibited. On the other hand, in the presence of the α-adrenoceptor antagonist, prazosin, neurogenic contractions were only partially reduced: at 4 Hz there was no significant reduction in the neurogenic contractions while at 32 and 64 Hz, contractions were reduced by an average of 20 and 28% respectively. Usually all contractions were abolished by a combination of the two drugs.

3 Prazosin antagonized contractions of the vessel to exogenously applied noradrenaline but not to ATP, whereas desensitization of the P_2-purinoceptor with α,β-methylene ATP blocked responses to ATP but not those to noradrenaline. The concentration-response curve to histamine was not affected by treatment of the vessel with prazosin, or after desensitization of the P_2-purinoceptor with α,β-methylene ATP.

4 These results suggest that noradrenaline and ATP are co-released from sympathetic nerves supplying the rabbit saphenous artery, both substances being involved in the mechanical contractions of this tissue. Further, the ratio of ATP to noradrenaline involved in these mechanical contractions is dependent upon the frequency of stimulation, but at all frequencies tested the purinergic component is greater than the adrenergic component.

Introduction

Non-adrenergic, non-cholinergic nerves have been shown to have a widespread occurrence in the autonomic nervous system of vertebrates (Burnstock, 1969). Burnstock (1972) proposed that adenosine 5'-triphosphate (ATP), or a related nucleotide, is the neurotransmitter released from some of these nerves and that they be termed 'purinergic'. Since then evidence has accumulated that ATP is a cotransmitter, with noradrenaline, in sympathetic nerves supplying a number of smooth muscle preparations (see Burnstock, 1976; 1982; 1985a,b). Studies on the vas deferens and the cat nictitating membrane with the P_2-purinoceptor antagonist arylazidoaminoproprionyl ATP ($ANAPP_3$) or involving desensitization of the P_2-purinoceptor with α,β-methylene ATP, have demonstrated that ATP acts as a contractile cotransmitter with noradrenaline during sympathetic neurotransmission (Fedan *et al.*, 1981; Sneddon *et al.*, 1982; Meldrum & Burnstock, 1983; Westfall *et al.*, 1983; Sneddon & Burnstock, 1984a; Duval *et al.*, 1985). A number of studies on different blood vessels have demonstrated, both mechanically and electrically, neurogenic responses that are not antagonized by α-adrenoceptor antagonists (see Burnstock & Kennedy, 1986). It has been suggested that purines might play a role as cotransmitters with noradrenaline in some vessels (Su, 1975; Head *et al.*, 1977; Katsuragi & Su, 1981; Muramatsu *et al.*, 1981; Sedaa *et al.*, 1986). Recently, with the use of α,β-methylene ATP, there has been direct evidence that ATP acts as a cotransmitter

with noradrenaline in the contractile responses of the rat tail artery (Sneddon & Burnstock, 1984b; Vidal *et al.*, 1986), the mesenteric artery (Ishikawa, 1985; Kügelgen & Starke, 1985; Muramatsu, 1986), and the rabbit ear artery (Kennedy *et al.*, 1986).

The rabbit saphenous artery is a muscular vessel well innervated with noradrenergic nerve fibres, and produces large, rapid contractions to neurogenic stimulation (Gillespie & Rae, 1972). However, neurogenic contractions of this vessel are resistant to α-adrenoceptor antagonists (Holman & Suprenant, 1980). In the present study, the possibility that noradrenaline and ATP act as cotransmitters in the rabbit saphenous artery has been investigated over a range of stimulation frequencies and for short (1 s) and long (1 min) periods of time (see Kennedy *et al.*, 1986). The possibility that the non-adrenergic excitatory neurotransmitter is ATP has been investigated by using α,β-methylene ATP to desensitize the postjunctional P_2-purinoceptors.

Methods

Male New Zealand white rabbits (2.8–3.3 kg) were killed by a blow to the head and exsanguination. Two ring segments, 4 mm in length after excision, were removed from the proximal end of each saphenous artery. They were cleaned of excess connective tissue and mounted horizontally, under isometric tension in 15 ml organ baths, by inserting two tungsten wires into the lumen according to the method of Bevan & Osher (1972). The tissues were bathed in Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, $NaHPO_4$ 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52 (Bülbring, 1953). The solutions were aerated with 95% O_2 and 5% CO_2 and maintained at 37°C. Throughout the experiment the Krebs solution was changed every 5–10 min by an overflow method. Preparations were allowed to equilibrate for 1–2 h under a resting tension of 0.75 g. Contractions of the circular smooth muscle were recorded by use of a Grass FTO3C transducer and displayed on a Grass ink-writing oscillograph (model 79).

Electrical stimulation of intramural nerves was delivered to the tissue across two platinum wire electrodes placed parallel to, and on either side of, the vessel using a Grass S11 stimulator. The voltage (40–50 V) and the pulse duration (0.08–0.1 ms) at which the neurogenic responses of the tissue were just maximal were established for each tissue at the start of each experiment and then maintained constant throughout the experiment. With these parameters, the responses were blocked by tetrodotoxin (1 μg ml^{-1}) and were therefore due to nerve stimulation and not to direct stimulation of the muscle. The rabbit saphenous artery was stimulated electrically over a range of frequencies (4–64 Hz) for a period of 1 s or 1 min, with a 4 or 15 min interval between each stimulation, respectively. Stimulations were repeated to ensure consistency of the response. On each preparation, the same range of stimulation frequencies and the periods of stimulation were again repeated either in the presence of the α-adrenoceptor antagonist, prazosin, or after desensitization of the P_2-purinoceptor with α,β-methylene ATP, first at a concentration of 1 μM and then 10 μM and finally in the presence of both drugs, each at 10 μM. If any response remained after treatment with both prazosin and α,β-methylene ATP, stimulations were repeated after a 10 min incubation with tetrodotoxin (1 μg ml^{-1}) to determine whether the residual response was neural or myogenic. The second component of the biphasic contraction induced by electrical stimulation for a period of 1 min was often variable and difficult to assess. Therefore, in order to standardize the method of calculation, the sustained contraction was taken as that response produced after 1 min of continuous stimulation. Desensitization of the P_2-purinoceptor was achieved by three or four exposures of the vessel to α,β-methylene ATP for 5 min at 10 min intervals, until no further contractile response was elicited and tone declined to its resting level. Tissues were incubated for 20 min with prazosin before any prazosin-resistant responses were measured. Noradrenaline and histamine did not cause desensitization of the tissue and therefore could be added cumulatively to the bath. ATP, like α,β-methylene ATP, caused desensitization of the tissue and therefore, it was added at 40 min intervals with repeated washing in between each addition. The responses to cumulative additions of noradrenaline (0.1–30 μM) and non-cumulative additions of ATP (0.1–3 mM) were tested before and after exposure to prazosin (10 μM) and before and after desensitization to α,β-methylene ATP (10 μM). Histamine concentration-response curves (0.1–100 μM) at the start and finish of the experiment served as control responses to ensure that prazosin, α,β-methylene ATP, and nerve stimulations were acting in a specific manner and did not cause a general desensitization of the tissue.

Drugs

Adenosine 5′-triphosphate (sodium salt) (ATP), α,β-methylene adenosine 5′-triphosphate (lithium salt) (α,β-methylene ATP), (−)-noradrenaline bitartrate, tetrodotoxin, and histamine dihydrochloride were all obtained from Sigma Chemical Company. Prazosin hydrochloride was a gift from Pfizer Ltd. Guanethidine sulphate (Ismelin) was obtained from Ciba. All drugs were dissolved in distilled water. Ascorbic acid (100 μM) was added to the noradrenaline solution. All drugs except tetrodotoxin and guanethidine were prepared freshly each day.

Statistical analysis of results

Responses to nerve stimulation were expressed as a percentage of the maximal contraction obtained to histamine. The responses at each stimulus frequency have been expressed as a mean ± standard error of mean (s.e.mean). The pD_2 value for a drug, under a particular set of conditions, was calculated from the mean log (concentration of the drug) ± standard error (s.e.mean) which produced 50% of its maximal response. The slopes of concentration-response curves were calculated from the regression of the percentage responses obtained at each log concentration of the drug. Mean slopes ± s.e.mean, were used for testing parallelism between concentration-response curves in the presence and absence of prazosin and before and after desensitization to α,β-methylene ATP. Results have been analysed using Students *t* test (paired and unpaired as appropriate) and a probability of less than 0.05 was considered significant.

Results

Neurogenic contractions of the vessel

Transmural electrical stimulation of the rabbit isolated saphenous artery for a period of 1 s (4–64 Hz) induced a rapid, frequency-dependent contraction. Near maximal contraction was attained at 64 Hz (Figure 1a,b) and this response was approximately 50–60% of the maximal contraction to exogenous histamine. Stimulation for a period of 1 min (4, 8, 16 Hz) produced a response which varied from preparation to preparation (Figure 1c,d). In all preparations there was an initial rapid, transient contraction that reached a maximum within 1 or 2 s. In some preparations this was followed by a sustained contraction that was greater than or equivalent to the initial contraction. This sustained contraction developed in less than 1 min from the start of electrical stimulation and lasted for the duration of the stimulation. In some preparations the initial transient contraction was followed by a much smaller contraction which, in some cases, was sustained for the 1 min of stimulation or in others very gradually decreased towards baseline (Figure 1c,d). In the latter case, this second phase of the contraction was difficult to assess, and, therefore, to standardize the method of calculation of responses to periods of stimulation of 1 min, the sustained contraction was defined as that contractile response produced after 1 min of continuous stimulation. All contractions fell back rapidly to base line at the end of the stimulation period and responses could be repeated every 3–4 min for the 1 s trains, or 10–15 min for the 1 min trains of stimulation without fatigue or desensitization being evident. All responses were abolished by tetrodotoxin (1 μg ml^{-1}) ($n = 5$) and also by guanethidine (4 μM) ($n = 4$).

Effect of prazosin on neurogenic contractions

Neurogenic contractions were compared before (control) and after preincubation of the vessel for 20 min with prazosin (1 μM). In the presence of prazosin, an α_1-adrenoceptor antagonist, there was a small reduction of the neurogenic contractions. This antagonism was directly related to the frequency of stimulation. For the 1 s train of stimulation, at 32 and 64 Hz there was a significant reduction in the contraction by an average of 20 and 28% respectively, whereas at lower frequencies of stimulation any reductions in contractions were not significant (Figures 1a, 2a, 4). Neither

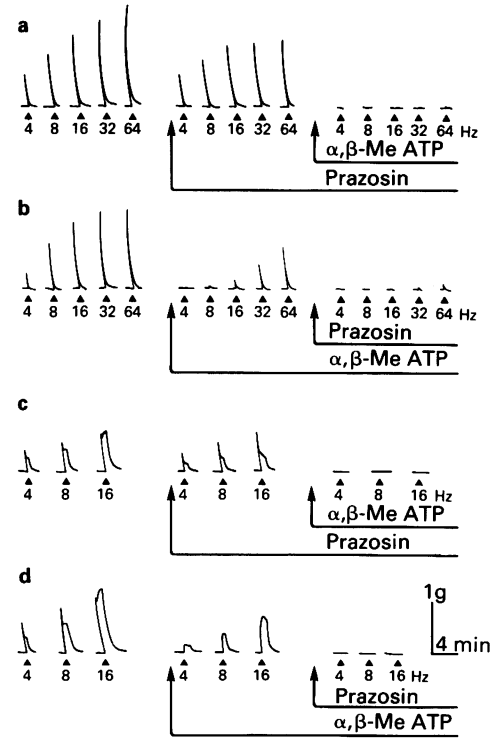

Figure 1 Contractions produced in the isolated saphenous artery of the rabbit on neurogenic transmural stimulation (0.08–0.1 ms; supramaximal voltage) for 1 s (a,b) and 1 min (c,d) at the frequencies (Hz) indicated (▲). Nerve stimulations were repeated in the presence of 10 μM prazosin added before (a,c) or after (b,d) desensitization of the P_2-purinoceptor with 10 μM α,β-methylene ATP (α,β-Me ATP) as indicated on the figure by the arrowed lines. The horizontal bar signifies 4 min and the vertical bar 1 g.

the initial rapid component nor the sustained component of the neurogenic contractions produced by 1 min trains of stimulation at 4, 8 and 16 Hz was significantly altered in the presence of prazosin (Figures 1c, 3a). When, in the absence of prazosin, the sustained contraction was greater than the initial rapid contraction, then this sustained contraction was often reduced in the presence of prazosin. After preincubation for 20 min with a higher concentration of prazosin (10 μM), there was no further change in the neurogenic contractions to 1 s or 1 min trains of stimulations at all frequencies tested (Figures 2a, 3a).

Effect of desensitization of the P_2-purinoceptor on the prazosin-resistant neurogenic contractions

Prazosin-resistant neurogenic contractions were compared before and after desensitization of the P_2-purinoceptor with α,β-methylene ATP (10 μM). Prazosin-resistant contractions to 1 s and 1 min trains of stimulation were usually abolished after desensitization of the P_2-purinoceptor (Figures 1, 2a, 3a). Any residual response was blocked by tetrodotoxin (1 μg ml^{-1}).

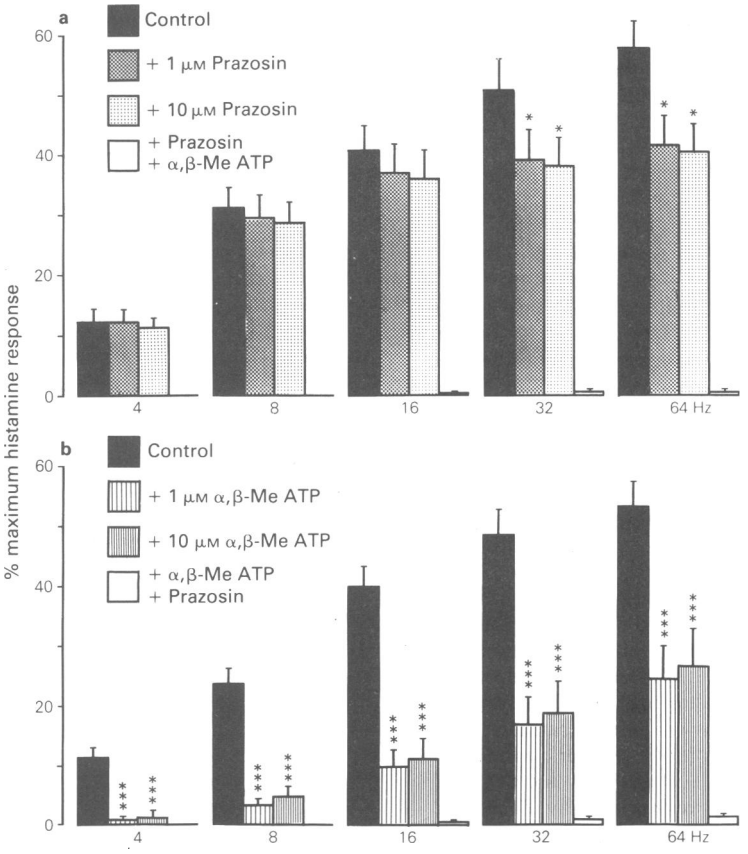

Figure 2 Contractions of the isolated saphenous artery of the rabbit to 1 s periods of perivascular nerve stimulation (0.08–0.1 ms, supramaximal voltage, 4–64 Hz) expressed as a percentage of the maximal contraction to histamine. (a) Contractions produced in the absence of any drug (control) are compared with those obtained in the presence of prazosin, 1 and 10 μM. Note that the prazosin-resistant contractions were abolished (4, 8 Hz) or almost totally inhibited (16, 32, 64 Hz) after desensitization by 10 μM α,β-methylene ATP (α,β-Me ATP) ($P < 0.001$) ($n = 14$). (b) Contractions produced in the absence of drug (control) are compared with those obtained after the P_2-purinoceptors have been desensitized with 1 and then with 10 μM α,β-methylene ATP (α,β-Me ATP). Note that the α,β-methylene ATP-resistant contractions were abolished (4, 8 Hz) or almost totally inhibited (16, 32, 64 Hz) in the presence of 10 μM prazosin ($P < 0.001$) ($n = 15$). Vertical bars denote s.e.mean. Significant differences ($^*P < 0.05$; $^{***}P < 0.001$) between control and experimental contractions were calculated by paired t tests.

Effect of desensitization of the P_2-purinoceptor on the neurogenic contractions

Neurogenic contractions were compared before (control) and after desensitization of the P_2-purinoceptor with α,β-methylene ATP (1 μM). After desensitization of the P_2-purinoceptor there was a significant reduction in the contraction to neurogenic stimulations for periods of 1 s and 1 min at each frequency tested (Figures 1, 2b, 3b). In the case of stimulation for a period of 1 min, there was no longer a biphasic response, only a sustained contraction remained. For

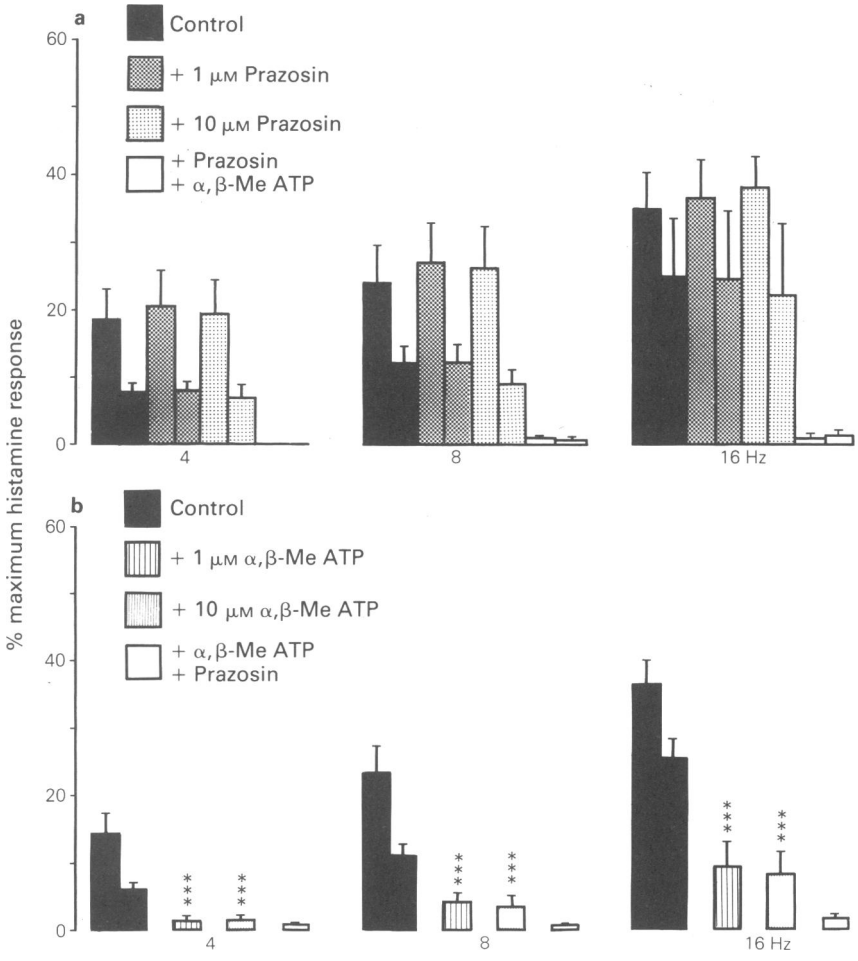

Figure 3 Biphasic contractions of the isolated saphenous artery of the rabbit to 1 min perivascular nerve stimulation (0.08–0.1 ms; supramaximal voltage; 4, 8 and 16 Hz) each expressed as a percentage of the maximal contraction to histamine. The left hand column of each pair of responses under a particular set of conditions represents the initial contraction, and the right the sustained contraction. (a) Contractions produced in the absence of any drug (control) are compared with contractions produced in the presence of prazosin, 1 and 10 μM. Note that prazosin-resistant contractions to perivascular nerve stimulation were abolished (4 Hz) or almost totally inhibited (8, 16 Hz) after desensitization of the P_2-purinoceptor with α,β-methylene ATP, 10 μM ($P < 0.001$) ($n = 10$). (b) Contractions produced in the absence of drugs (control) are compared with those produced after the vessel has been desensitized to α,β-methylene ATP (α, β-Me ATP), 1 and 10 μM. Note that the α,β-methylene ATP-resistant contractions to perivascular nerve stimulation were almost totally inhibited in the presence of prazosin, 10 μM ($P < 0.001$) ($n = 10$). It was not possible to differentiate any component of the rapid contraction from the maintained contraction, hence only the latter was measured. Vertical bars denote s.e.mean. Significant differences (***$P < 0.001$) between control and experimental contractions were calculated by paired t tests.

1 s and 1 min trains of stimulation, the magnitude of the α,β-methylene ATP-resistant contractile component, expressed as a fraction of the whole contraction at a given frequency of stimulation, was directly related to the frequency of stimulation (Figure 4). A further desensitization of the P_2-purinoceptor with 10 μM α,β-methylene ATP caused no further reduction in the neurogenic contraction (Figures 2b, 3b). There was a large variation in the contribution of the α,β-methylene ATP-sensitive component of the neurogenic response and this became more apparent at the higher stimulus frequencies. In some preparations after desensitization of the P_2-purinoceptor with α,β-methylene ATP, the response to stimulation for 1 s was almost totally blocked at 4 Hz, but only partially blocked at 64 Hz. In other cases there was an almost total block at both 4 and 64 Hz. This large variation was also observed during long-term neurogenic stimulation. Generally, if the sustained component were as great or greater than the initial rapid response then this contraction was more resistant to α,β-methylene ATP desensitization than average, whereas if this component were small and not well sustained, it was largely due to an α,β-methylene ATP-sensitive (purinergic) component.

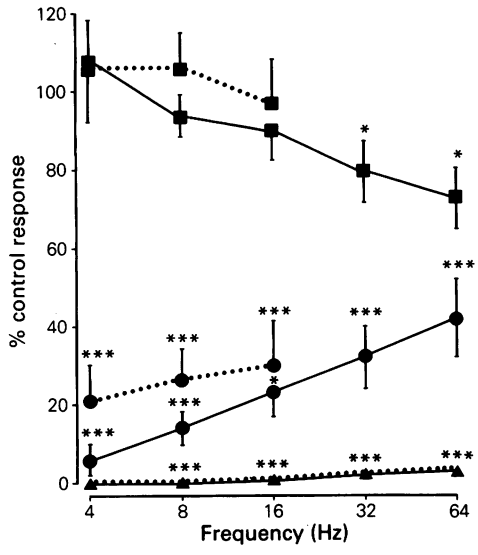

Figure 4 Contractions of isolated saphenous artery of rabbit produced by 1 s and 1 min perivascular nerve stimulation (0.08–0.1 ms, supramaximal voltage) after preincubation with prazosin, 1 μM (■) ($n = 10–14$) or after desensitization by α,β-methylene ATP, 1 μM (●) ($n = 10–15$) or after treatment with both drugs (▲) ($n = 20–29$). Results produced by 1 s and 1 min periods of stimulation (the sustained component only) are represented by points joined by solid and broken lines respectively. Results for each frequency are expressed as a percentage of the neurogenic contraction produced in the absence of drug. Vertical bars denote s.e.mean. Significant differences ($^*P < 0.05$; $^{***}P < 0.001$) between control and experimental contractions were calculated by paired t tests.

Effect of prazosin on neurogenic contractions of vessels in which the P_2-purinoceptors have previously been desensitized

α,β-Methylene ATP-resistant neurogenic contractions were compared before and after incubation of the vessel with prazosin (10 μM) for 20 min. α,β-Methylene ATP-resistant contractions produced by 1 s and 1 min trains of stimulation were usually abolished after incubation with prazosin (Figures 1, 2b, 3b). Any residual response was blocked by tetrodotoxin (1 μg ml^{-1}).

Responses to drugs

Histamine and noradrenaline each produced reproducible, concentration-dependent, sustained contractions of the rabbit saphenous artery. The maximal contractions evoked by the two drugs did not differ significantly; the maximal contraction to histamine was 3.47 ± 0.20 g ($n = 7$) and that to noradrenaline was 3.32 ± 0.23 g ($n = 9$). Although the pD_2 value for each was in a similar concentration range, that for histamine was significantly greater than that for noradrenaline (Table 1). Unlike noradrenaline or histamine, ATP and α,β-methylene ATP produced rapid, transient concentration-dependent contractions that were reproducible after a 30–40 min period of repeated washing. The pD_2 value for contraction of the vessel to α,β-methylene ATP was 5.92 ± 0.08 ($n = 6$). The vessel was far less sensitive to ATP and hence contraction to ATP at the concentrations tested (0.03–3 mM) did not reach maximal response and therefore a pD_2 could not be attained. However, comparison of the pD_2 value of α,β-methylene ATP with the ATP concentration required to produce an equivalent contraction suggests that α,β-methylene ATP is approximately 3500 times more effective than ATP at causing a contraction of the vessel. The concentration-response curve to noradrenaline was significantly shifted to the right after preincubation of the tissue with prazosin (1 μM) for 20 min. On increasing the concentration of prazosin in the bath to 10 μM, there was a further significant shift in the concentration-response curve to noradrenaline (Table 1). The concentration-response curve to noradrenaline was not significantly altered from control values after desensitization of the P_2-purinoceptor with 10 μM α,β-methylene ATP (Table 1). On the other hand, contrac-

tile responses produced by ATP were abolished after P_2-purinoceptor desensitization but were not significantly antagonized in the presence of prazosin (Table 1). After a combined treatment with prazosin and desensitization with α,β-methylene ATP, neither the maximal response nor the pD_2 value of the contraction to histamine was significantly altered from control values (Table 1). From this result it can be concluded that there was no general desensitization of the tissue due to prazosin or α,β-methylene ATP.

Discussion

These results demonstrate that electrical field stimulation of the perivascular nerves of the rabbit isolated saphenous artery produces contractions that are largely mediated by ATP, or a related purine, at P_2-purinoceptors and, to a lesser extent, by noradrenaline at α_1-adrenoceptors. The relative contribution of these two components to the over-all contraction of the vessel is dependent upon the parameters of stimulation applied. Since guanethidine abolishes all neurogenic contractions of this vessel, it is likely that ATP and noradrenaline function as cotransmitters from sympathetic perivascular nerves innervating the arterial smooth muscle, as has been proposed for other blood vessels (Sneddon & Burnstock, 1984b; Ishikawa, 1985; Kügelgen & Starke, 1985; Kennedy *et al.*, 1986; Muramatsu, 1986; Vidal *et al.*, 1986).

The rabbit saphenous artery contracted well to exogenously applied noradrenaline. Prazosin, a selective postjunctional α_1-adrenoceptor antagonist (Cavero & Roach, 1980), significantly antagonized this response. On the other hand, contractions to electrical stimulations were only partially antagonized

Table 1 Comparison of drug responses in rabbit isolated saphenous artery

(a) *Noradrenaline*

	pD_2	*Slope*	*Relative antagonism*
Control	5.05 ± 0.04(7)	60.30 ± 3.5(7)	
+ 1 μM Prazosin	3.87 ± 0.14(8)***‡	67.75 ± 11.9(8) NS‡	15
+ 10 μM Prazosin	3.52 ± 0.12(6)***‡§	60.60 ± 6.5(6) NS‡	34
+ α,β-Me ATP	4.99 ± 0.06(7) NS†	59.90 ± 3.4(7) NS†	1.1

(b) *Histamine*

	pD_2	*Slope*	*Relative shift*
Start	5.32 ± 0.08(6)	48.35 ± 5.5(6)	
Finish	5.36 ± 0.19(6) NS†	49.90 ± 6.3(6) NS†	0.91

(c) *ATP*

	-log EC_{30}	*Slope*	*Relative antagonism*
Control	2.56 ± 0.08(6)	21.38 ± 1.4(6)	
+ Prazosin	2.64 ± 0.07(6) NS†	27.56 ± 1.8(6)*	0.83
+ α,β-Me ATP	Not reached (6)***†	0	Infinite

All values are given as mean ± s.e.mean with number of observations (n) in parentheses.
(a) pD_2 values and mean slopes for the concentration-response curve to noradrenaline in the absence of any other drug (control), in the presence of 1 and 10 μM prazosin and after desensitization of the tissue to 10 μM α,β-methylene ATP (α,β-Me ATP).
(b) pD_2 values and mean slopes for the concentration-response curve to histamine in rabbit isolated saphenous artery at the start of the experiment and after the tissues have been treated with α,β-methylene ATP and prazosin.
(c) Contractions to ATP expressed as $-\log$ (30% maximal contraction to histamine) ($-\log EC_{30}$) in the absence of any other drug (control), in the presence of 10 μM prazosin, and after desensitization of the tissue to 10 μM α,β-methylene ATP. Note that, at the concentrations of ATP used (0.03–3 mM), pD_2 values were not attained.
Relative antagonism (or shift) in the concentration-response curve between control and experimental conditions (or start and finish of the experiment) were calculated by the antilog (pD_2 control − pD_2 experimental).
Significant differences (*$P<0.05$; ***$P<0.001$; NS = no significant difference) between control and experimental conditions were calculated by paired and unpaired t tests as indicated on the table.

Note:- †: paired statistical analysis; ‡: unpaired statistical analysis; §: there was a significant difference between pD_2 values of noradrenaline in the presence of 1 and 10 μM prazosin, $P<0.05$, unpaired statistical analysis.

by prazosin, and this was significant at the higher frequencies of stimulation. A higher concentration of prazosin caused a further significant rightward shift in the concentration-response curve to exogenous noradrenaline but was no more effective in blocking the neurogenic contraction of the rabbit saphenous artery. These prazosin-resistant mechanical contractions to neurogenic stimulation are probably mediated by the prazosin-resistant excitatory junction potentials described in the rabbit saphenous artery by Holman & Surprenant (1980). Desensitization of the P_2-purinoceptor with α,β-methylene ATP almost totally inhibited these large, prazosin-resistant neurogenic contractions. These results suggest that, although exogenous noradrenaline acts postjunctionally at α_1-adrenoceptors, contractions produced by nerve stimulation do not involve, to a great extent, these receptors and appear largely purinergic in nature. The proportion of the neurogenic response mediated via α_1-adrenoceptors is directly related to the frequency of stimulation. Any residual contraction that might remain after prazosin and α,β-methylene ATP treatment is most likely to be due to an incomplete action of these two drugs. However, one cannot rule out the possibility of a third neurotransmitter, as has been suggested in the guinea-pig vas deferens (Stjärne & Åstrand, 1985).

While it is generally recognised that the excitatory junction potentials recorded on smooth muscle cells of a number of sympathetically innervated vessels are prazosin-resistant (see Burnstock & Kennedy, 1986), it has been noted that a mechanical prazosin-resistant component of the response to sympathetic stimulation is not always evident (see for example the rabbit ear artery; Allcorn *et al.*, 1985). An explanation proposed by Kennedy *et al.* (1986), is that the prazosin-resistant (purinergic) component of the sympathetic nerve-mediated responses is favoured by short (1 s) periods of stimulation, while the noradrenaline component is favoured by longer stimulation periods. In addition it appears that the proportions of noradrenaline and ATP differ in different sympathetic nerves, so that sometimes the mechanical components to ATP and noradrenaline are clearly distinguishable as in the vas deferens (Meldrum & Burnstock, 1983) and the rabbit saphenous artery (present study), while in others such as the rabbit ear artery (Kennedy *et al.*, 1986) and rat tail artery (Vidal *et al.*, 1986) the prazosin-resistant (purinergic) component is less apparent except with short periods of stimulation (Kennedy *et al.*, 1986).

ATP and α,β-methylene ATP both produce concentration-dependent, rapid, transient vasoconstrictor responses in the rabbit saphenous artery. α,β-Methylene ATP is approximately 3500 times more effective than ATP at causing contraction of the vessel. On repeated administration of the drug, α,β-methylene ATP caused desensitization of its contractile response. After total desensitization to α,β-methylene ATP, contractions to ATP up to a concentration of 3 mM were totally abolished and in some cases a relaxation was observed, whereas there was no change in the sensitivity of the vessel to noradrenaline. It has been reported in other vessels and other tissues that α,β-methylene ATP is far more potent than ATP in causing a contraction and that it also selectively desensitizes the P_2-purinoceptor (see Burnstock & Kennedy, 1985). The results from the present study demonstrate that in the rabbit saphenous artery there are also postjunctional P_2-purinoceptors and that the desensitizing action of α,β-methylene ATP was selective for these receptors. Evidence suggests that α,β-methylene ATP acts only postjunctionally and any purine-mediated prejunctional inhibitory actions of perivascular nerve activity appear to be mediated by P_1-purinoceptors rather than P_2-purinoceptors (Burnstock & Brown, 1981). Also it has been shown that α,β-methylene ATP has little or no effect on stimulation-induced release of [^{3}H]-noradrenaline from sympathetic nerves of guinea-pig and mouse vas deferens (Stjärne & Åstrand, 1985; Westfall *et al.*, 1986), rabbit mesenteric artery (Kügelgen & Starke, 1985) or rat tail artery (Vidal *et al.*, 1986). In the present study, in support of this view, after pretreatment of the rabbit saphenous artery with prazosin, the neurogenic contraction was partially reduced, but only significantly at the higher frequencies, whereas exogenous noradrenaline was significantly antagonized at all concentrations. This suggests that only a small adrenergic component, but a large non-adrenergic component, was being stimulated to evoke the neurogenic contraction, and that any prejunctional inhibition of noradrenaline release would account for only a small reduction in the contractile response. The inhibitory effect of α,β-methylene ATP on neurogenic contractions, therefore, appears to be due to specific desensitization of postjunctional P_2-purinoceptors, although some prejunctional action has not been wholly discounted. Neurogenic contractions, in the absence of prazosin, were substantially reduced after desensitization of the P_2-purinoceptor with α,β-methylene ATP. The relative contribution of the α,β-methylene ATP-sensitive (purinergic) component to the overall contraction for a given frequency was inversely related to the frequency of stimulation. This was true both for the rapid contractions produced by a 1 s stimulation and for the sustained contraction produced by a 1 min stimulation. On stimulation for 1 s, $94 \pm 4\%$ of the response at 4 Hz, but only $58 \pm 10\%$ of the response at 64 Hz was purinergic. A similar frequency-dependent relationship has been demonstrated for ATP as a cotransmitter in the rabbit ear artery (Kennedy *et al.*, 1986) and the urinary bladder of various species (Moss & Burnstock, 1985). Desensitizing the P_2-purinoceptor of the rabbit saphenous artery with a higher

concentration of α,β-methylene ATP did not alter the contractions. However, this α,β-methylene ATP-resistant response was almost completely abolished after incubation of the tissue with prazosin. Also, the whole neurogenic contraction was abolished by guanethidine. Hence, on looking at neurogenic responses either in terms of prazosin-resistant contractions or in terms of α,β-methylene ATP-sensitive contractions, from each approach it can be concluded that a large component of the contraction of the rabbit saphenous artery in response to sympathetic nerve stimulation is purinergic in nature, but that noradrenaline also plays a role, especially at the higher frequencies of stimulation.

Electrical stimulation of the rabbit saphenous artery for 1 min produces a biphasic contraction consisting of an initial rapid contraction followed by a sustained contraction. Biphasic contractions are also seen in the guinea-pig vas deferens, and it has been proposed that in this tissue the first phase is mediated mainly by ATP and the second mainly by noradrenaline (Sneddon *et al.*, 1982; Burnstock & Sneddon, 1985). On the other hand Stjärne & Astrand (1985) have presented evidence that noradrenaline and ATP each contribute to both phases of the contractile response of the vas deferens, and that the ability to produce a biphasic response is a built-in property of the muscle. Likewise, in the rabbit ear artery, each of the components of the biphasic contraction are probably mediated by both noradrenaline and ATP (Kennedy *et al.*, 1986). In the rabbit saphenous artery, the rapid contraction produced by a 1 s train of stimulation was mostly mediated by ATP but also, to a lesser extent, by noradrenaline. Likewise, the sustained contraction of the biphasic response was mediated largely by ATP, but also by noradrenaline. Hence, considering both these results, it is concluded that in the rabbit saphenous artery ATP and noradrenaline each contribute to both components of the biphasic response, as is the case for the rabbit ear artery. The contribution of the two transmitters to each component of the biphasic contraction is dependent upon the frequency of stimulation, but unlike the rabbit ear artery, ATP plays the predominant role at each of the frequencies tested.

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