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Effects of reserpine and 6-hydroxydopamine on the adrenergic and purinergic components of sympathetic nerve responses of the rabbit saphenous artery

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1 The effects of reserpine and of 6-hydroxydopamine on the contractions of the rabbit isolated saphenous artery produced by stimulation of the sympathetic nerves were studied.

2 In vessels exposed to reserpine, substantial contractions to nerve stimulation were recorded despite a 95.7% reduction in the noradrenaline content of the tissue. These responses of the vessel were not significantly affected by the α_1-antagonist, prazosin, whereas after desensitization of the P_2-purinoceptor with α,β-methylene ATP, no response to nerve stimulation remained.

3 In vessels exposed to 6-hydroxydopamine, no nerve-mediated responses were observed.

4 Noradrenaline-containing nerves were observed by fluorescence histochemistry in control tissues, but were not observed in tissues treated with reserpine or 6-hydroxydopamine.

5 The potencies of ATP and histamine were not significantly affected by reserpine or 6-hydroxydopamine treatment. However, there was a slight supersensitivity to noradrenaline in reserpine-treated and 6-hydroxydopamine-treated vessels compared with that of control vessels. Prazosin was selective for α-adrenoceptors, while α,β-methylene ATP was selective for P_2-purinoceptors.

6 These results substantiate the finding that ATP and noradrenaline are sympathetic cotransmitters in the rabbit isolated saphenous artery, and demonstrate that ATP can act as a transmitter independently of noradrenaline in this vessel.

Introduction

In many blood vessels, perivascular nerve stimulation produces nerve-mediated contractions which are blocked by α-adrenoceptor antagonists. However, there is evidence that the sympathetic neurogenic responses in some arteries are not blocked by such antagonists (see Burnstock & Kennedy, 1986). Perivascular nerve stimulation of the isolated saphenous artery of the rabbit produces mechanical contractions and excitatory junction potentials which are largely resistant to α-adrenoceptor antagonists (Holman & Surprenant, 1980). Guanethidine blocks these responses (Burnstock & Warland, 1987), which suggests that they are generated by substances released from sympathetic nerves. Recently it has been demonstrated, using α,β-methylene ATP which desensitizes P_2-purinoceptors (Kasakov & Burnstock, 1983), that the large non-adrenergic component of the response is likely to be due to ATP coreleased with noradrenaline from sympathetic nerves (Burnstock & Warland, 1987). Cotransmission involving ATP and noradrenaline from sympathetic nerves has also been demonstrated in a number of other vessels (Sneddon & Burnstock, 1984a; Ishikawa, 1985; Kügelgen & Starke, 1985; Allcorn *et al.*, 1985; Cheung & Fujioka, 1986; Muramatsu, 1986; Kennedy *et al.*, 1986, Vidal *et al.*, 1986), as well as in the vas deferens (Meldrum & Burnstock, 1983; Sneddon & Westfall, 1984; Sneddon & Burnstock, 1984b; Stjärne & Åstrand, 1985) and the nictitating membrane (Duval *et al.*, 1985).

The aim of the present study was to see if the evidence obtained following treatment of the isolated saphenous artery of the rabbit with reserpine and with 6-hydroxydopamine, supports the proposal that ATP is a cotransmitter with noradrenaline in perivascular sympathetic nerves supplying this vessel.

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Methods

Pharmacology

Male New Zealand white rabbits (2.8–3.5 kg) were killed by a blow to the back of the neck 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 a tension of 1 g, and left to equilibrate for 1–2 h in a bath of oxygenated Krebs solution at 37°C as detailed previously (Burnstock & Warland, 1987). Isometric tension changes of the circular muscle were recorded by use of a Grass FT03 transducer and displayed on a Grass ink-writing oscillograph (Model 79).

Electrical stimulation was delivered via platinum electrodes connected to a Grass S11 stimulator. Submaximal voltage (50–70V) and a pulse duration of 0.1 ms were used throughout the experiments. Such parameters did not directly stimulate the muscle, but instigated nerve-mediated responses that were totally abolished by tetrodotoxin (3.1 μM). The artery was stimulated electrically over a range of frequencies (4–64 Hz) for a period of 1 s at 4 min intervals.

In order to produce agonist concentration-response curves, noradrenaline and histamine were added cumulatively to the bath, but ATP, which rapidly desensitized its own receptors, was added non-cumulatively at 20 min intervals (see Burnstock & Warland, 1987). Desensitization of the P_2-purinoceptor was achieved by several exposures of the vessel to α,β-methylene ATP for approximately 5 min at 10 min intervals until no further contractile response was elicited and tone had returned to baseline. α,β-Methylene ATP was then readded to the bath and was present during subsequent measurements of α,β-methylene ATP-resistant responses. Prazosin was added to the bath 20 min before the measurement of prazosin-resistant responses. α,β-Methylene ATP and prazosin were each used at 10 μM concentration; this concentration was selective in blocking purinergic and adrenergic responses, respectively (Burnstock & Warland, 1987).

These procedures were carried out in reserpine-treated and control (vehicle-injected) vessels and in 6-hydroxydopamine-treated and control (preincubated in Krebs solution containing ascorbic acid for 4 h) vessels.

Reserpine treatment

Animals were injected intraperitoneally with reserpine 3 mg kg^{-1} 48 h, and 5 mg kg^{-1} 24 h before being killed. Reserpine was dissolved in a minimum volume of Tween 80 and then made up with saline to the volume required so as to inject the animal with 1 ml of solution. For the control experiments, rabbits were injected over the same time scale and under the same conditions with Tween 80 dissolved in 1 ml saline.

In vitro *6-hydroxydopamine treatment*

The vessel was removed from untreated animals, cleared of excess connective tissue, and incubated in oxygenated Krebs solution containing 6-hydroxydopamine (0.4 mM) and ascorbic acid (0.1%) at 37°C for 4 h. For the control experiments, isolated vessels were incubated for 4 h at 37°C in oxygenated Krebs solution containing ascorbic acid (0.1%). Preparations were then transferred to normal Krebs solution, mounted horizontally and subjected to a tension of 1 g and left to equilibrate for 1 h before the start of the experiment.

Fluorescence histochemistry to detect noradrenaline-containing nerves

The presence or absence of adrenergic nerves in whole-mount stretch preparations of the proximal rabbit saphenous artery (control and treated tissues) was investigated by the fluorescence histochemical method using glyoxylic acid (Lindvall & Björklund, 1974; Furness & Costa, 1975). Vessel segments, cleaned of excess fat, were immersed in a freshly prepared solution of 2% w/v glyoxylic acid in 0.1 M phosphate buffer (pH 7.2) at room temperature for 1.5 h. At the termination of the incubation the portions of artery were opened longitudinally. They were stretched to the approximate *in situ* length on a glass microscope slide, adventitial surface uppermost, and air-dried until they assumed a transparent appearance. The stretched preparations were incubated at 100°C for 4 min and then mounted in liquid paraffin and were viewed with a Zeiss photomicroscope, fitted with an epi-fluorescence condenser 111 RS. A high pressure mercury light source was used (Osram HB50) with excitation filters (BP 436/8), barrier filter (LP 470) and dichroic mirror (FT 460). Selected areas were photographed on Kodak Tri X Pan film (Din 27).

Noradrenaline assay

Noradrenaline was estimated by electrochemical detection following high performance liquid chromatography (h.p.l.c.). After samples of rabbit saphenous artery (control or reserpine-treated) were cleaned of fat and connective tissue and cleared of blood, they were frozen immediately and stored in liquid nitrogen until assayed. The extraction procedure, slightly modified by the addition of 0.1 mM EDTA in the solution used for washing the alumina, was essentially that of Keller *et al.* (1976). Chromatography was carried out at a flow rate of

1.0 ml min^{-1} using a mobile phase consisting of 0.1 M sodium dihydrogen phosphate, 0.1 mM EDTA, 5 mM heptane sulphonate (pH 5.0) containing 10% (v/v) methanol on μ-Bondapak C-18 reverse phase column. Detection and quantification were accomplished with a waxy carbon paste electrode set at a potential of + 0.72 V. Noradrenaline levels were corrected for recovery using dihydroxybenzylamine as an internal standard.

Sources of chemicals and drugs

Drugs: reserpine, 6-hydroxydopamine hydrobromide, α,β-methylene adenosine 5′-triphosphate (α,β-methylene ATP, sodium salt), adenosine 5′-triphosphate (ATP, sodium salt), (−)-noradrenaline bitartrate, histamine dihydrochloride and tetrodotoxin were all obtained from Sigma Chemical Company. Prazosin hydrochloride was a gift from Pfizer Ltd. Guanethidine sulphate (Ismelin) was obtained from Ciba Laboratories. All drugs, except for reserpine which was initially dissolved in Tween 80 and then made up to the required volume in saline, were dissolved in distilled water. Ascorbic acid (100 μM) was added to the noradrenaline solution. All drugs except tetrodotoxin and guanethidine were prepared fresh each day.

Chemicals: alumina, polyoxyethylenesorbitan monooleate (Tween 80), glyoxylic acid monohydrate, Tris buffer, EDTA and sodium bisulphite were obtained from Sigma Chemical Co. Perchloric acid and 0.1 M sodium dihydrogen phosphate were obtained from BDH. Paraformaldehyde and paraffin liquid were obtained from Fisons, phosphate-buffered saline from Oxoid, dihydroxybenzylamine from Aldrich, heptane sulphonate from Fluka, h.p.l.c. methanol from Rathburn Chemical Co. and μ-Bondapak from Waters.

Statistical analysis

Data are expressed as mean ± standard error of the mean (s.e.mean); for each experiment n refers to the number of animals from which vessels were used, for each result, unless specified, only one preparation was taken from each animal. Noradrenaline content was expressed as μg g^{-1} wet weight of tissue. Neurogenic responses were expressed as a percentage of the maximal contraction to histamine. The pD_2 value for a drug was calculated from the mean − log (concentration of the drug) ± s.e.mean which produced 50% of its maximal response. In the case of ATP, since a pD_2 value could not be estimated, the '$-\log[ATP]_{30}$' was defined as the concentration of ATP required to produce 30% of the maximal histamine response. The maximal contraction to different agonists was measured in g tension. Comparison of neurogenic responses, agonist potencies and their maximal contractions between reserpine-treated and 6-hydroxydopamine-treated vessels and their control vessels were analysed by Student's unpaired t tests. Comparison of agonist potencies and neurogenic contractions before and after α,β-methylene ATP or prazosin treatment were analysed by paired t tests. The level of probability <0.05 was taken to be statistically significant.

Results

Neurogenic responses in reserpine-treated and control vessels

Transmural electrical stimulation of control vessels (vehicle injected) for a period of 1 s induced rapid frequency-dependent contractions which quickly fell back to baseline at the end of the stimulation period. Responses could be repeated every 3–4 min without fatigue. In the presence of prazosin (10 μM) there was a partial (significant, $P<0.05$) reduction in the neurogenic contraction at the higher frequencies of stimulation (32 and 64 Hz) (Figure 1a). After desensitization of the P_2-purinoceptor with α,β-methylene ATP (10 μM), this residual prazosin-resistant response was abolished (Figure 1a). Alternatively, in vessels treated with α,β-methylene ATP (10 μM) but not prazosin, the neurogenic response was markedly reduced from control responses particularly at lower frequencies of stimulation (Figure 1b). On treatment with prazosin these α,β-methylene ATP-resistant responses were abolished (Figure 1b) (see Burnstock & Warland, 1987).

Vessels pretreated with reserpine still produced sizable contractions to transmural electrical stimulation, which were frequency-dependent and were totally abolished by tetrodotoxin (3.1 μM, $n = 4$) and by guanethidine (3.4 μM, $n = 5$). On treatment with prazosin (10 μM) these neurogenic contractions were not significantly reduced at any frequency of stimulation tested ($P<0.05$, paired t test, $n = 8$) (Figure 1c). On the other hand, in the absence of prazosin, and after desensitization of the P_2-purinoceptor with α,β-methylene ATP, the reserpine-resistant neurogenic contraction at each frequency of stimulation was totally abolished (Figure 1d).

At stimulation frequencies of 16 and 32 Hz, the response in reserpine-treated vessels was not significantly different from the prazosin-resistant neurogenic response of control (vehicle-injected) vessels. However, at 4 and 8 Hz the response in reserpine-treated vessels was smaller, and at 64 Hz it was slightly greater than the equivalent prazosin-resistant response of control vessels (significant, $P<0.05$, unpaired t test, $n = 10–20$ from 5–9 animals).

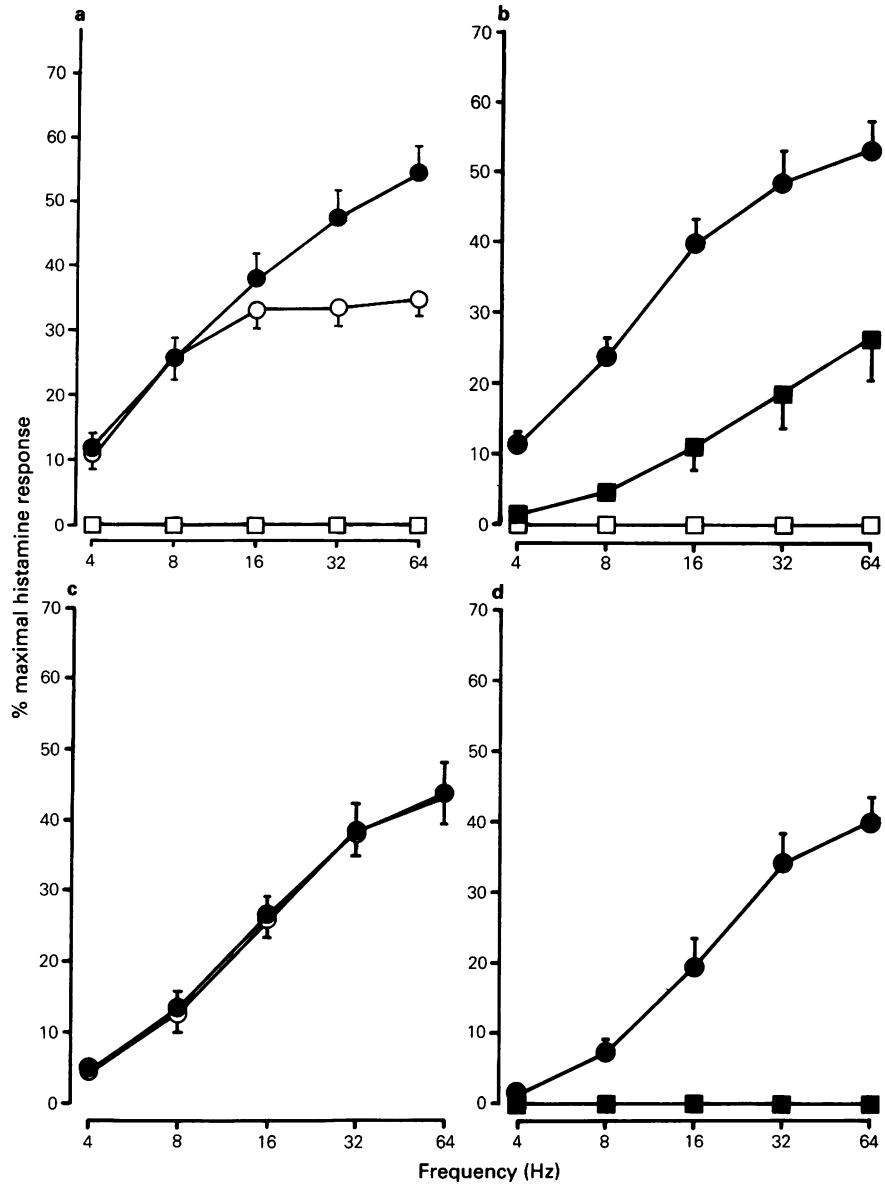

Figure 1 Contractions of isolated saphenous artery from control rabbits (a, $n = 10$, 5 rabbits, vehicle injected; b, $n = 15$) and from rabbits treated with reserpine (c, $n = 8$; d, $n = 8$). Responses to 1 s periods of stimulation (0.1 ms submaximal voltage, 4–64 Hz) are expressed as a percentage of the maximal histamine contraction. The response to each frequency of stimulation was measured in the absence of α,β-methylene ATP and prazosin (●) (all graphs), in the presence of 10 μM prazosin (○) (a, c), in the presence of 10 μM α,β-methylene ATP (■) (b, d), and in the presence of both prazosin and α,β-methylene ATP (□) (a, b). Symbols represent mean response and vertical lines denote s.e. Note that the presence of prazosin or α,β-methylene ATP only partially reduced the contractile response in untreated vessels, whereas in reserpine-treated vessels α,β-methylene ATP completely inhibited the response while prazosin had no significant effect on the response.

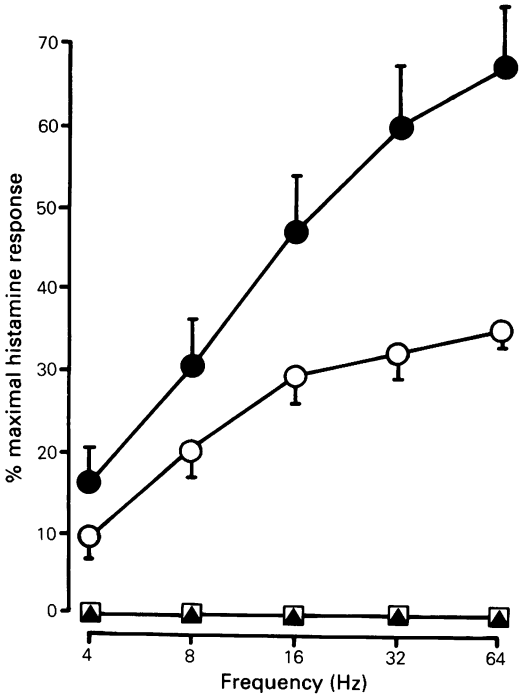

Figure 2 Neurogenic responses of rabbit isolated saphenous artery treated *in vitro* in Krebs solution containing 6-hydroxydopamine and 0.1% ascorbic acid for 4 h, and untreated control arteries incubated for 4 h in Krebs containing 0.1% ascorbic acid. Responses to 1 s periods of stimulation (0.1 ms, submaximal voltage, 4–64 Hz) are expressed as a % of the maximal histamine contraction. In the control preparations the responses to each frequency of stimulation were measured in the absence of prazosin and α,β-methylene ATP (●), in the presence of 10 μM prazosin (○), and in the presence of prazosin and 10 μM α,β-methylene ATP (□). In the 6-hydroxydopamine-treated vessels in the absence of prazosin and α,β-methylene ATP no neurogenic response was observed ($n = 7$) (▲). Symbols represent mean response and vertical lines denote s.e.

Neurogenic responses in control and 6-hydroxydopamine-treated vessels

Untreated saphenous arteries that had been incubated for 4 h in oxygenated Krebs at 37°C containing ascorbic acid (0.1%) produced frequency-dependent contractions to neurogenic stimulation (Figure 2). These contractions at each frequency of stimulation, although they appeared enhanced, were not significantly different from those responses produced in preparations used soon after excision from the animal ($P > 0.05$, unpaired t test, $n = 7$). This enhanced neurogenic response is probably due to enhanced sensitivity to noradrenaline in these tissues (see drug results below). After prazosin treatment there was a significant reduction in contractions produced at 32 and 64 Hz but not at lower frequencies of stimulation (Figure 2); this corresponds with the results obtained from freshly prepared vessels. These results confirm that the tissue's neurogenic responses are not significantly affected by the prolonged incubation in oxygenated Krebs containing ascorbic acid. On the other hand, in vessels which were preincubated for 4 h in oxygenated Krebs containing ascorbic acid and 6-hydroxydopamine (0.4 mM), no response to neurogenic stimulation was observed at any of the frequencies tested (Figure 2).

Responses to drugs in reserpine-treated, 6-hydroxydopamine-treated and control vessels

Histamine (0.1 μM–0.3 mM), noradrenaline (0.1 μM–0.1 mM) and ATP (0.1–30 mM) produced reproducible concentration-dependent contractions of the isolated saphenous artery of the rabbit. Responses to histamine and noradrenaline were well sustained and therefore these agents were added cumulatively to the bath, whereas those to ATP were rapidly desensitized and so ATP was added as single additions at 20 min intervals (see Burnstock & Warland, 1987).

The potencies of histamine (pD_2 value) and ATP (the concentration of ATP required to produce 30% of the maximal histamine contraction) were not significantly different between reserpine-treated and 6-hydroxydopamine-treated vessels and their control vessels (Table 1). However, there was a significant enhancement in potency to noradrenaline in reserpine-treated and 6-hydroxydopamine-treated vessels compared with their respective control vessels (Table 1). The maximal contractions to histamine and to noradrenaline were not significantly enhanced in the reserpine-treated and 6-hydroxydopamine-treated vessels compared with their control vessels (Table 1). ATP at the concentrations tested did not evoke maximal contraction.

The potencies of histamine and ATP, and the maximal contraction to histamine and noradrenaline were not significantly different between freshly prepared vessels and vessels incubated for 4 h in Krebs solution containing ascorbic acid ($P < 0.05$, unpaired t test, $n = 6–16$). However, the potency of noradrenaline was slightly greater (significant, $P < 0.05$) in the control vessel that had been preincubated for 4 h than in the freshly prepared control vessels (Table 1).

Contractions to ATP (3 mM) were totally abolished after desensitization of the P_2-purinoceptor with α,β-methylene ATP (10 μM) in reserpine-treated vessels and their control vessels (Table 2); in some cases the contraction was replaced by a small relaxation to

Table 1 Potency and maximal contraction of various agonists on isolated saphenous artery of the rabbit: comparison between 6-hydroxydopamine (6-OHDA)-treated and reserpine-treated tissue and their control tissues

Histamine			*Maximal*	
Treatment	pD_2	P‡	*contraction* (g)	P‡
Control (reserpine)†	5.28 ± 0.05 (8)	NS	4.42 ± 0.37 (8)	NS
Reserpine	5.43 ± 0.08 (13)		4.30 ± 0.24 (13)	
Control (6-OHDA)	5.14 ± 0.13 (6)	NS	4.28 ± 0.40 (6)	NS
6-OHDA	5.08 ± 0.09 (8)		4.27 ± 0.35 (8)	
Noradrenaline			*Maximal*	
Treatment	pD_2	P‡	*contraction* (g)	P‡
Control (reserpine)†	4.96 ± 0.08 (10)	*	4.11 ± 0.25 (16)	NS
Reserpine	5.47 ± 0.04 (13)		4.38 ± 0.22 (13)	
Control (6-OHDA)	5.37 ± 0.07 (10)	*	4.00 ± 0.30 (10)	NS
6-OHDA	5.82 ± 0.10 (13)		4.24 ± 0.35 (13)	
ATP				
Treatment	$-log\ [ATP]_{30}$	P‡		
Control (reserpine)†	2.71 ± 0.10 (13)	NS		
Reserpine	2.74 ± 0.09 (18)			
Control (6-OHDA)	2.70 ± 0.16 (6)	NS		
6-OHDA	2.66 ± 0.07 (7)			

All values are given as mean ± s.e.mean with the number of observations n in parentheses, one preparation taken from each animal unless indicated.
†Results from 5 animals.
‡Student's unpaired t test: NS = no significant difference between control and experimental mean values ($P > 0.05$).
*Significant difference between values ($P < 0.05$).
§Since ATP did not reach maximal response at the concentrations tested its potency is measured as the concentration of ATP required to produce 30% maximal histamine contraction $-\log [ATP]_{30}$.

ATP. In these vessels there was only a small (but significant) reduction in the potency of noradrenaline and in the maximal contraction to noradrenaline after α,β-methylene ATP treatment (Table 2). This is consistent with the claim that α,β-methylene ATP is selective for P_2-purinoceptors. Prazosin (10 μM) antagonized the noradrenaline response both in control and reserpine-treated vessels (Table 2). The extent of the shift was not significantly different between control and reserpine-treated vessels (Table 2). Prazosin (10 μM) did not significantly affect the contractile potency of ATP in control or reserpine-treated tissues (Table 2). It is therefore a selective antagonist towards the α-adrenoceptors in this vessel.

Fluorescence histochemistry

All control tissues for the reserpine-treated and 6-hydroxydopamine-treated vessels showed positive noradrenergic nerves using the glyoxylic acid fluorescence treatment ($n = 7$). There was no positive fluorescence in reserpine-treated vessels ($n = 11$) or in 6-hydroxydopamine-treated vessels (n = 7).

Noradrenaline content in control and reserpine-treated tissue

The mean content of noradrenaline in control saphenous artery segments was 0.75 ± 0.15 μg g^{-1} wet weight of tissue ($n = 7$), whereas in segments from the reserpine-treated rabbit, the noradrenaline content was 0.032 ± 0.02 μg g^{-1} ($n = 5$), 4.3% of the control tissue value.

Discussion

The results of this study substantiate the results of our previous work (Burnstock & Warland, 1987). Electrical field stimulation of the perivascular sympathetic nerves of the isolated saphenous artery of the rabbit (vehicle injected) produced contractions of the vessel that were likely to be mediated by both ATP (or a related purine) via P_2-purinoceptors and noradrenaline via α_1-adrenoceptors. Prazosin (10 μM), which selectively antagonized the responses to exogenous noradrenaline but not ATP, only partially reduced neurogenic contractions. Likewise α,β-methylene ATP

Table 2 Effect of prazosin (10 μM) and α,β-methylene ATP (10 μM) on the potency and maximal contraction of noradrenaline (NA) and on the potency of ATP in control (vehicle-injected) (C) and reserpine-treated (R) rabbit isolated saphenous artery

		Control	*+ Prazosin*	*Relative shift*¶	P‡
$-\log [ATP]_{30}$§	C†	2.75 ± 0.16 (7)	2.80 ± 0.11 (7)	+0.06 ± 0.05 (7)	NS
	R	2.65 ± 0.15 (8)	2.57 ± 0.13 (8)	−0.08 ± 0.05 (8)	NS
pD_2 NA	C†	4.95 ± 0.09 (9)	3.10 ± 0.09 (9)	−1.85 ± 0.13 (9)	*
	R	5.47 ± 0.04 (13)	3.69 ± 0.01 (8)	−1.72 ± 0.07 (7)	*††
NA maximal response (g)	C	4.15 ± 0.34 (11)	not reached (11)		
	R	4.23 ± 0.31 (9)	not reached (9)		
		Control	*+α,β-me ATP*	*Relative shift*¶	P‡
$-\log [ATP]_{30}$§	C†	2.67 ± 0.09 (6)	not reached (6)	—	*
	R	2.81 ± 0.10 (10)	not reached (10)		*
pD_2 NA	C†	4.94 ± 0.09 (7)	4.79 ± 0.11 (7)	−0.15 ± 0.05 (7)	*
	R	5.28 ± 0.08 (15)	5.09 ± 0.06 (15)	−0.19 ± 0.06 (15)	*
NA maximal response (g)	C	4.20 ± 0.17 (8)	4.02 ± 0.20 (8)	−4.30 ± 2.3% (8)	NS
	R	4.04 ± 0.18 (15)	3.72 ± 0.20 (15)	−7.90 ± 2.2% (15)	*

All values are given as mean ± s.e.mean with the number of observations *n* in parentheses, one observation made from each animal unless indicated.
†Results from 5 animals.
‡Student's paired *t* tests: NS = no significant difference between agonist potency ($P > 0.05$) before and after prazosin or α,β-methylene ATP treatment. *Significant difference between values before and after treatment ($P < 0.05$).
§Since ATP did not produce a maximal response at the concentrations tested its potency is measured as the concentration of ATP required to produce 30% maximal histamine contraction $-\log [ATP]_{30}$.
¶ Relative shift in the concentration-response curve was calculated as the difference between that of the experimental and control preparations (paired analysis). Relative shift in the maximal response was expressed as % reduction of maximal response (control) by prazosin/α,β-methylene ATP treatment.
††No significant difference in the relative shift to prazosin between control and reserpine-treated vessels.

(10 μM), which desensitizes P_2-purinoceptors (Kasakov & Burnstock, 1983; Meldrum & Burnstock, 1983), and selectively inhibited contractions to exogenous ATP but not noradrenaline, only partially inhibited neurogenic contractions. However, treatment with a combination of prazosin and α,β-methylene ATP abolished the neurogenic contraction. By studying the vessel after reserpine treatment (so as to deplete noradrenaline from the nerves) it was possible to focus on the purinergic component of the neurogenic response.

Reserpine treatment has been shown to deplete sympathetic nerves of 95% or more of their noradrenaline content (Langer & Pinto, 1976; Suzuki *et al.*, 1984; Duval *et al.*, 1985). ATP is stored in the same terminal vesicles as noradrenaline (Smith 1972; 1979; Lagercrantz, 1976; Haynes, 1986). In chromaffin granules reserpine has been shown to interfere with the noradrenaline, but not the ATP, uptake mechanisms (Winkler *et al.*, 1981). In the vas deferens, the release of ATP from the sympathetic nerves is not reduced after reserpine treatment (Kirkpatrick & Burnstock, 1987). The prazosin-resistant (purinergic) electrical junction potentials of the rat tail artery (Cheung, 1982; Sneddon & Burnstock, 1984a) and rabbit ear artery (Suzuki *et al.*, 1984; Kennedy *et al.*, 1986) are still present after reserpine treatment, although they are reduced in amplitude: reserpine treatment abolished the neurogenic slow depolarizations (due to noradrenaline) in these arteries. In the present study, the reserpine treatment that was employed depleted the sympathetic nerves of the saphenous artery of 95.7% of their noradrenaline. However, a substantial response to transmural stimulation remained which was blocked by tetrodotoxin.

Vascular smooth muscle from reserpine-treated animals has been shown to be postjunctionally supersensitive to various agonists (Hudgins & Fleming, 1966; Taylor & Green, 1971; Krishnamurty & Gulati, 1980). In this study the reserpine-treated vessel showed a slight supersensitivity to exogenous noradrenaline (leftward shift in pD_2 value), but not to ATP or

histamine. The 6-hydroxydopamine-treated vessel also showed supersensitivity to noradrenaline. Both treatments deplete tissue of noradrenaline; this in turn would result in supersensitivity to noradrenaline (Westfall, 1981). In contrast, the lack of supersensitivity to ATP after reserpine treatment is consistent with the finding that ATP is not depleted from the nerves with this treatment.

At stimulation frequencies of 16 and 32 Hz, neurogenic contractions produced in the reserpine-treated vessels were not significantly different from the prazosin-resistant responses produced in control vessels. This is consistent with the fact that both prazosin and reserpine treatment inhibit the noradrenergic component of the neurogenic response, leaving just the purinergic component. As to the reason for the small differences at 4, 8 and 16 Hz in the neurogenic response in reserpine-treated vessels compared with the prazosin-resistant neurogenic response of control vessels, further investigation is required. It could be due to the absence of a modulatory effect of noradrenaline (Enero & Langer, 1973) or to some direct effect of the reserpine treatment on the purinergic component of the neurogenic response.

All reserpine-resistant neurogenic contractions (4–64 Hz) were totally insensitive to treatment with prazosin, while prazosin selectively reduced the potency of the exogenous noradrenaline response. This suggests that a second neurotransmitter in addition to noradrenaline may be active in the rabbit saphenous artery. After desensitization of the P_2-purinoceptor with α,β-methylene ATP, contractions to exogenous ATP (up to 3 mM) were abolished whereas the potency and maximal contraction to noradrenaline were only slightly reduced. Even in the absence of prazosin, after treatment with α,β-methylene ATP, the neurogenic contractions of the reserpine-treated vessel were totally abolished at all frequencies of stimulation. This effect is unlikely to be due to a prejunctional action of α,β-methylene ATP on noradrenaline release, since noradrenaline has been depleted by 95%. Work by Byrne & Large (1986) has shown that in the rat basilar artery, α,β-methylene ATP desensitization is not selective towards the ATP-mediated depolarization but also blocks the noradrenaline-mediated depolarization. However, α,β-methylene ATP treatment has been shown to block selectively the ATP response and not the noradrenergic response in the current experiments and also in other smooth muscle preparations including the guinea-pig vas deferens (Meldrum & Burnstock, 1983), the cat nictitating membrane (Duval *et al.*, 1985), the mesenteric artery (Kügelgen & Starke, 1985; Ishikawa, 1985; Muramatsu, 1986) and the rabbit ear artery (Kennedy *et al.*, 1986). Hence these results substantiate the evidence of previous work on the rabbit isolated saphenous artery (Burnstock & Warland, 1987) that α,β-methylene ATP is most likely acting to desensitize postjunctional P_2-purinoceptors, that ATP is a cotransmitter with noradrenaline in sympathetic nerves and that ATP, released from these nerves, does not require noradrenaline for its postjunctional action.

6-Hydroxydopamine treatment destroys sympathetic nerves (Theonen & Tranzer, 1968; Bennett *et al.*, 1970). In this study saphenous arteries treated with 6-hydroxydopamine did not respond to transmural electrical stimulation at any frequency tested (4–64 Hz). This result substantiates previous evidence using guanethidine treatment (Burnstock & Warland, 1987), that noradrenaline and ATP are coreleased from sympathetic nerves supplying the isolated saphenous artery of the rabbit. In vessels incubated for 4 h *in vitro* before the start of the experiment (in the absence of 6-hydroxydopamine), the neurogenic response was, although slightly enhanced, not significantly different from that of freshly prepared saphenous arteries. The pD_2 value for noradrenaline was also slightly enhanced in this control tissue, whereas the pD_2 value for histamine and noradrenaline and the response to ATP were not significantly different from results from freshly prepared control tissues. Therefore the protocol used for 6-hydroxydopamine treatment did not hinder vessel responsiveness.

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