Different sensitivities of rabbit isolated blood vessels exhibiting co-transmission to the slow calcium channel blocker, nifedipine

¹J.M. Bulloch, ²A. MacDonald & J.C. McGrath

Autonomic Physiology Unit, Institute of Physiology, University of Glasgow, Glasgow G12 8QQ

1 Antagonist drugs were used to separate the purinergic and adrenergic contributions as well as the adrenoceptor sub-types involved in the sympathetic vasoconstrictor responses produced by electrical field stimulation in rabbit isolated ileocolic and proximal saphenous arteries. Blocking drugs were applied either alone or in various combinations and sequences.

2 Nifedipine attenuated vasoconstrictor responses to sympathetic nerve stimulation both in the presence and in the absence of α -adrenoceptor blocking agents. However in the presence of α, β -methylene ATP, nifedipine produces at best only a small attenuation of the vasoconstrictor response.

3 These results suggest that the purinergic component of the response to sympathetic nerve stimulation, at least in these tissues, can be antagonized by nifedipine, whereas the α_1 -adrenoceptor-mediated response is relatively resistant.

Keywords: rabbit blood vessels; purinergic nerve stimulation; co-transmission; nifedipine

Introduction

There is strong evidence for co-transmission involving a purine most likely adenosine 5'-triphosphate (ATP), as well as noradrenaline at sympathetic nerve terminals in the vas deferens (Sneddon *et al.*, 1983; Sneddon & Burnstock, 1984a; Stjärne & Astrand, 1984; 1985) and in blood vessels *in vitro* (Sneddon & Burnstock, 1984b; Ishikawa, 1985; Kennedy *et al.*, 1986; Muramatsu, 1986; Burnstock & Warland, 1987) as well as *in vivo* (Bulloch & McGrath, 1986; 1988a,b).

An interesting feature of the earlier studies on vas deferens was that the non-adrenergic component, subsequently shown to be purinergic (Sneddon & Burnstock, 1984a), was blocked by the calcium channel blocking agent, nifedipine, an effect shown to be due to blockade of the propagation of the action potential in the smooth muscle (Blakeley *et al.*, 1981). Later studies also showed a preferential blocking effect of nifedipine on the purinergic component of the vasopressor response to stimulation of the sympathetic outflow in the pithed rat (Bulloch & McGrath, 1988b). The aim of the present study was to test whether vascular smooth muscle *in vitro* also shows differential susceptibility of the purinergic component to nifedipine.

For this study we have chosen blood vessels where the purinergic contribution to sympathetic nerve stimulation has been demonstrated to varying degrees, for example; the ileocolic artery (von Kugelgen & Starke, 1985; Bulloch & Starke, 1988; 1990) where the purinergic component of the sympathetic nerve-mediated response is dominant; the saphenous artery (Burnstock & Warland, 1987) which also exhibits a large purinergic component but where a large α_1 -adrenoceptor-mediated component is also evident.

A preliminary communication of these results has been published (Bulloch *et al.*, 1988).

Methods

New Zealand White rabbits, of either sex (2–3 kg) were killed by stunning and exsanguination, and the blood vessels to be studied were dissected out and cleared of fatty tissue.

The proximal segment of the saphenous artery was cut into four 4 mm rings. A thin wire (upper) support was inserted

through the lumen of the ring taking care not to damage the endothelial cell lining. A second wire (lower) support, attached to an electrode assembly, was also threaded through the lumen and the tissue was then suspended between the two supports. The upper support was connected by thread to a Grass FT03c transducer for isometric recording. Individual electrode assemblies were then mounted in 30 ml organ baths containing physiological salt solution (PSS) maintained at 37°C, and gassed with 95% O₂:5% CO₂. Tissues were placed under an initial resting tension of 1.0 g and left for 1 h to reach an eventual resting tone of approximately 0.5 g. Sections from the ileocolic artery were similarly prepared.

For perfusion studies, the proximal section of the largest ramus arteriae ileocolicae was dissected out and cleared of fatty tissue. Side branches were tied off and the arteries were cannulated at both ends before being mounted vertically in a 4 ml organ bath. They were then perfused at a constant rate of flow (2.7 ml min⁻¹) in such a way that the perfusate did not mix with the bathing fluid.

Electrical stimulation (parameters and protocol)

Ring segments taken from the saphenous artery and ileocolic artery were electrically stimulated at various frequencies for 1 s duration (35–40 V, at frequencies of 4, 8, 16, 32 and 64 Hz, pulse width 0.1–0.3 ms) to produce isometric contractions. Additional stimuli of 4 Hz for 10 s, or 5 Hz and 10 Hz for 20 s, were used in some preparations as examples of long duration stimuli. The interval between trains of pulses, during frequency response curves (FRC), was 5 min.

In the perfused ileocolic artery, vasoconstriction produced by electrical field stimulation (platinum electrodes: 0.3 ms pulse width; supramaximal current strength of 200 mA; variable frequencies and train lengths) was observed as an increase in perfusion pressure (monitored by a Satham P23 Db pressure transducer).

Drugs and solutions

The composition of the PSS used for the saphenous artery was (mM): NaCl 118.4, KCl 4.7, CaCl₂ 2.5, MgSO₄ · 7H₂O 1.2, NaHCO₃ 24.9, KH₂PO₄ 1.2 and glucose 11.1. Na₂ EDTA, 23 μ M, was included in all the above experiments to prevent oxidative degradation of NA. The composition of the PSS used for the ileocolic artery was (mM): NaCl 118, KCl 4.8, CaCl₂ 2.5, NaHCO₃ 25, KH₂PO₄ 0.9, glucose 11, Na₂ EDTA

¹ Author for correspondence.

² Present address: Department of Biological Sciences, Glasgow College, Glasgow G4 0BA.

0.03 and ascorbic acid 0.3. The medium was saturated with 95% O₂: 5% CO₂ and maintained at 37°C.

The following drugs were used: prazosin HCl (Pfizer); rau-wolscine HCl (Roth); $\alpha\beta$ -methylene ATP (Sigma); nifedipine (Bayer); yohimbine HCl (Roth).

The blocking drugs were applied at least 20 min before starting each series of stimulations, either alone or in various combinations and sequences. The choice of these agents and concentrations used were based on their selectivity of blockade in previous analyses on vas deferens and isolated blood vessels (see Bulloch & Starke, 1990; McGrath *et al.*, 1990); prazosin, post-junctional α_1 -adrenoceptors (Cambridge *et al.*, 1977); rauwolscine, pre- and post-junctional α_2 -adrenoceptors (Weitzell *et al.*, 1979); $\alpha\beta$ -methylene ATP ($\alpha\beta$ -MeATP), post-junctional P_{2x}-purinoceptors (Sneddon & Burnstock, 1984a,b); nifedipine, postjunctional blockade of transmission propagated by action potentials sustained by slow Ca²⁺ channels (Blakeley *et al.*, 1981).

All drugs were dissolved in distilled water and added to the organ baths in a volume of 30 μ l or less, except nifedipine which was dissolved in ethanol and water (1:4) before being added to the organ bath.

The means and standard error of the means were calculated and compared by a paired or unpaired *t* test. *P* values of 0.05 or less were considered to be significant; *n* is the number of preparations.

Results

The perfused ileocolic artery

Stimulation of the isolated perfused ileocolic arteries, produced reproducible vasoconstrictions, observed as increases in perfusion pressure, which were pulse number- and frequency-dependent.

Short periods of electrical stimulation (1 s) elicited monophasic vasoconstriction, whereas longer periods (20 s) produced biphasic vasoconstriction of which the first component was mainly mediated by ATP (selectively inhibited by $\alpha\beta$ -MeATP) whereas the second component although still mainly purinergic, had a large noradrenergic component (antagonized by prazosin) (see Bulloch & Starke, 1990).

Electrically evoked vasopressor responses of the isolated perfused ileocolic artery (5 Hz, 50 V, 10 and 100 pulses), were not significantly affected by the addition of nifedipine (10 μ mol l⁻¹) on its own to the bathing medium (Figure 1a). In the presence of prazosin (0.3 μ mol l⁻¹) (Figure 1b) the effect of nifedipine (10 μ mol l⁻¹) was more variable between tissues but did show a significant attenuation at 10 Hz 100 pulses.

The ileocolic artery ring preparation: the effect of increasing concentrations of nifedipine

The effect of nifedipine (0.3–30 μ mol l⁻¹) on vasoconstrictions produced by electrical field stimulation (10 Hz, 40 V, 0.3 ms for 20 s) under conditions of α_1 -adrenoceptor blockade (prazosin, 0.3 μ mol l⁻¹) were studied. Prazosin (0.3 μ mol l⁻¹) on its own attenuated the second component of the response to a greater degree (Figure 2). The subsequent administration of nifedipine (0.3 μ mol l⁻¹) significantly attenuated only the first component of the response. Subsequent increasing concentrations of nifedipine (1–10 μ mol l⁻¹) had no further effect. The subsequent administration of nifedipine (30 μ mol l⁻¹) significantly attenuated the first, but not the second, component of the vasoconstrictor response further (Figure 2).

The ileocolic artery ring preparation: the effect of nifedipine on its own and after α_1 -adrenoceptor blockade or P_{2x}-purinoceptor desensitization

For this series of experiments, the highest concentration of nifedipine was used (30 μ mol l⁻¹) and consequently a higher

Figure 1 The effect of nifedipine on responses at different frequencies and pulse trains in the isolated perfused ileocolic artery of the rabbit is illustrated (a) in the absence of prazosin and (b) in the presence of prazosin (0.3 μ mol l⁻¹). Arteries were stimulated electrically at various frequencies and pulse durations in the order shown in the figure to produce vasopressor responses. Additional stimuli of 1 Hz, 5 Hz and 10 Hz for 20 s, were also performed 30 min before the start of each experiment. The interval between trains of pulses was 5 min. Arteries were stimulated, first before the addition of drugs (open columns), then after (a) nifedipine (10 μ mol l⁻¹) (solid columns); or (b) after prazosin (0.3 μ mol l⁻¹) (hatched columns) and subsequently after nifedipine (3 μ mol l⁻¹) (solid columns). Data are presented as the change in perfusion pressure (mmHg) produced as a result of electrical stimulation within any one artery. Columns represent mean values of ten experiments; bars show s.e.mean. Significant difference from pretreatment values, for paired *t* test: **P* < 0.05. In control tissues (stimulation repeated in the absence of drugs) there were no significant differences between the corresponding responses within each tissue.

concentration of ethanol (from the nifedipine solution) was present in the bathing medium. The effect of ethanol was therefore taken into consideration.

The effect of nifedipine on vasoconstrictions produced by electrical field stimulation (4–32 Hz, 40 V, 0.3 ms for 1 s) were studied. On its own, nifedipine (30 μ mol l⁻¹) significantly attenuated the nerve-mediated responses at 8, 16 and 32 Hz frequencies (Figure 3a). With longer trains of pulses (5 Hz and 10 Hz for 20 s), biphasic responses were produced. With these longer durations of stimulation only the first phase of the response was reduced by nifedipine (30 μ mol l⁻¹), whilst the second phase was not significantly affected (Figure 3b). The subsequent addition of prazosin (0.3 μ mol l⁻¹) and $\alpha\beta$ -MeATP (3 μ mol l⁻¹) completely abolished the responses (Figure 3a and b).

In the presence of prazosin (0.3 μ mol l⁻¹) the attenuation of the electrically evoked vasoconstrictor responses by nifedipine (30 μ mol l⁻¹) was more marked (Figure 4a and b). The attenuation was significant at all parameters of stimulation studied. The subsequent administration $\alpha\beta$ -MeATP (3 μ mol l⁻¹) completely abolished the responses (Figure 4a and b).

Figure 2 The effect of increasing concentrations of nifedipine in conditions of α -adrenoceptor blockade on biphasic vasoconstrictor responses of the ileocolic artery. Arteries were stimulated electrically at 10 Hz for 20 s repeatedly to produce biphasic vasoconstrictions. The interval between trains of pulses was 5 min. Ring preparations of artery were electrically stimulated, first before the addition of antagonist, then after prazosin (Praz, $0.3 \mu\text{mol l}^{-1}$), then after subsequent increasing concentrations of nifedipine (Nif, 0.3 – $30 \mu\text{mol l}^{-1}$). Data are presented as a percentage of the maximum control response at each phase produced as a result of electrical stimulation within any one artery. The first phase (open columns) at 10 Hz 20 s was taken as the peak constrictor height which occurred 5–9 s after the onset of stimulation, and the second phase (solid columns) as the peak height between 18–23 s. Columns represent mean values of five experiments; bars show s.e.mean. Significant difference from pretreatment values, for paired t test: * $P < 0.05$. In control tissues (stimulation repeated in the absence of drugs) there were no significant differences between the corresponding responses within each tissue.

In the presence of a P_{2x} -purinoceptor desensitizing concentration of α, β -MeATP ($3 \mu\text{mol l}^{-1}$) (see McGrath *et al.*, 1990) the addition of nifedipine ($30 \mu\text{mol l}^{-1}$) in ethanol produced no further attenuation of the responses and indeed enhanced them significantly at all parameters studied (Figure 5a and b). After the addition of α, β -MeATP ($3 \mu\text{mol l}^{-1}$), the same concentration of ethanol (0.66%) as used in the nifedipine solution, produced potentiations of the nerve-mediated responses especially at higher frequencies and longer pulse durations of stimulation (Figure 6a and b). On its own, ethanol potentiated the second component of the vasoconstrictor response at 10 Hz 20 s and responses at 16 Hz and 32 Hz for 1 s. In the presence of prazosin ($0.3 \mu\text{mol l}^{-1}$), ethanol had no effect (Bullock & McGrath, unpublished observations). The further addition of prazosin ($0.3 \mu\text{mol l}^{-1}$) after nifedipine abolished the residual responses (Figure 5a and b).

Proximal saphenous artery

Contractile responses of the rabbit isolated saphenous artery to electrical stimulation (4–64 Hz, 25–30 V, 0.1 ms pulse width for 1 s) were reduced but not abolished by $1 \mu\text{mol l}^{-1}$ prazosin (Figure 7a). The remaining prazosin-resistant component was significantly attenuated and almost completely abolished at lower frequencies by desensitization with $3 \mu\text{mol l}^{-1}$ α, β -MeATP (results not shown).

The relatively low dose of nifedipine ($1 \mu\text{mol l}^{-1}$) produced a significant reduction of the responses that remained after the addition of prazosin ($1 \mu\text{mol l}^{-1}$) by around 50% (Figure 7a). The remaining nifedipine resistant component was almost completely abolished by α, β -MeATP (not shown) confirming that the nifedipine-resistant component that remained after prazosin treatment, was purinergic.

Nifedipine ($1 \mu\text{mol l}^{-1}$) had no effect on responses after α, β -MeATP addition (Figure 7b). The further addition of prazosin after α, β -MeATP and nifedipine, abolished the responses (not shown), confirming that the nifedipine resistant component after α, β -MeATP treatment was adrenergic.

Nifedipine ($1 \mu\text{mol l}^{-1}$) was dissolved in ethanol to give a final bath concentration of 0.02%. This concentration of ethanol on its own has no significant effect on nerve-mediated responses (Bullock & McGrath, unpublished observations).

Figure 3 The effect of nifedipine on responses at different frequencies in the rabbit ileocolic artery is illustrated in (a). Arteries were stimulated electrically at various frequencies for 1 s duration (35–40 V, at frequencies of 4, 8, 16 and 32 Hz, pulse width 0.1–0.3 ms) to produce isometric contractions. Additional stimuli of 5 Hz and 10 Hz for 20 s, were performed before each frequency response curve as examples of a low frequency/long duration stimuli. The interval between trains of pulses, during frequency response curves (FRC), was 5 min. Arterial rings were electrically stimulated, (a) first before the addition of nifedipine (○), then after nifedipine ($30 \mu\text{mol l}^{-1}$) (△), then after prazosin ($0.3 \mu\text{mol l}^{-1}$) (■) and finally after desensitization with α, β -methylene ATP (α, β -MeATP) ($3 \mu\text{mol l}^{-1}$) (▲). Data are presented as a percentage of the maximum response produced as a result of electrical stimulation within any one artery. The effect of nifedipine on the biphasic responses in the ileocolic artery is illustrated in (b). The first phase at 5 Hz (open columns) and 10 Hz (stippled columns) was taken as the peak constrictor height which occurred 5–9 s after the onset of stimulation, and the second phase at 5 Hz (solid columns) and 10 Hz (hatched columns) as the peak height between 18–23 s. Columns represent mean values of five experiments; bars show s.e.mean. Significant difference from pretreatment values, for paired t test: * $P < 0.05$. In control tissues (stimulation repeated in the absence of drugs) there were no significant differences between the corresponding responses within each tissue.

Discussion

The ileocolic artery and the saphenous artery of the rabbit are blood vessels in which noradrenaline (NA)-ATP co-transmission have been demonstrated (von Kgelgen & Starke, 1985; Burnstock & Warland, 1987; Bullock & Starke, 1988; 1990). The degree of involvement of either the adrenergic or purinergic components of the response to sympathetic nerve stimulation in these blood vessels depends on the

Figure 4 The effect of nifedipine in the presence of prazosin on responses of the ileocolic artery at different frequencies, as detailed in Figure 3, is illustrated. (a) Arterial rings were electrically stimulated, first before the addition of antagonists (○), then after prazosin ($0.3 \mu\text{mol l}^{-1}$) (■), then after nifedipine ($30 \mu\text{mol l}^{-1}$) (△) and finally after desensitization with α, β -methylene ATP ($3 \mu\text{mol l}^{-1}$) (▲). Data are presented as a percentage of the maximum response produced as a result of electrical stimulation within any one artery. The effect of nifedipine on the biphasic responses in the ileocolic artery is illustrated in (b). The first phase at 5 Hz (open columns) and 10 Hz (stippled columns) was taken as the peak constrictor height which occurred 5–9 s after the onset of stimulation, and the second phase at 5 Hz (solid columns) and 10 Hz (hatched columns) as the peak height between 18–23 s. Columns represent mean values of five experiments; bars show s.e.mean. Praz, prazosin; Nif, nifedipine; α, β -MeATP, α, β -methylene ATP. Significant difference from pretreatment values, for paired *t* test: * $P < 0.05$. In control tissues (stimulation repeated in the absence of drugs) there were no significant differences between the corresponding responses within each tissue.

parameters of stimulation employed (Bullock & Starke, 1988; 1990; McGrath *et al.*, 1990). At lower frequencies (and a stimulation period of 1 s) the purinergic component dominates the response, with the noradrenergic component playing an increasing role at higher frequencies (32 Hz) (Figures 3a, 4a and 5a and see Bullock & Starke, 1990). For longer trains of pulses (train lengths of 20 s) two distinct phases of the contraction can be differentiated, the first one was greatly reduced by α, β -MeATP and hence was mediated by ATP, whilst the second one was partly sensitive to prazosin and hence was, to a considerable degree, noradrenergic (Figures 3b, 4b and 5b).

Where P_{2x} -purinoceptors had already been desensitized with α, β -MeATP (Figure 3), the subsequent addition of nifedipine (dissolved in ethanol and distilled water giving a

Figure 5 The effect of nifedipine in the presence of α, β -methylene ATP (α, β -MeATP) on responses of the ileocolic artery at different frequencies, as detailed in Figure 3, is illustrated in (a). Arterial rings were electrically stimulated, first before the addition of drugs (○), then after desensitization with α, β -MeATP ($3 \mu\text{mol l}^{-1}$) (▲), then after nifedipine ($30 \mu\text{mol l}^{-1}$) (△) and finally after prazosin ($0.3 \mu\text{mol l}^{-1}$) (■). Data are presented as a percentage of the maximum response produced as a result of electrical stimulation within any one artery. The effect of nifedipine on the biphasic responses in the ileocolic artery is illustrated in (b). The first phase at 5 Hz (open columns) and 10 Hz (stippled columns) was taken as the peak constrictor height which occurred 5–9 s after the onset of stimulation, and the second phase at 5 Hz (solid columns) and 10 Hz (hatched columns) as the peak height between 18–23 s. Columns represent mean values of five experiments; bars show s.e.mean. Nif, nifedipine; Praz, prazosin. Significant difference from pretreatment values, for paired *t* test: * $P < 0.05$. In control tissues (stimulation repeated in the absence of drugs) there were no significant differences between the corresponding responses within each tissue.

final bath concentration of 0.66%) appeared to potentiate the responses. However, this effect can be attributed solely to ethanol, which selectively potentiates the adrenergic component of the sympathetic nerve-mediated responses in this tissue (Bullock & McGrath, unpublished observations; and see Figure 6). The inhibitory action of nifedipine is best seen where the adrenergic component has been antagonized by prazosin (Figure 4) but it is also evident in the absence of prazosin (Figure 3), since in this tissue the purinergic component dominates the response.

The purinergic component of the response to stimulation of the sympathetic nerves was reduced by about 50% by nifedipine, whereas the adrenergic component was relatively resistant. This is similar to the situation found in the rat iso-

Figure 6 The effect of ethanol in the presence of α, β -methylene ATP (α, β -MeATP) on responses of the ileocolic artery at different frequencies, as detailed in Figure 3, is illustrated in (a). Arterial rings were electrically stimulated, first before the addition of drugs ($\circ$), then after desensitization with α, β -MeATP ($3 \mu\text{mol l}^{-1}$) ($\blacktriangle$), then after ethanol (0.66%) ($\triangle$) and finally after prazosin ($0.3 \mu\text{mol l}^{-1}$) ($\blacksquare$). Data are presented as a percentage of the maximum response produced as a result of electrical stimulation within any one artery. The effect of ethanol on the biphasic responses in the ileocolic artery is illustrated in (b). The first phase at 5 Hz (open columns) and 10 Hz (stippled columns) was taken as the peak constrictor height which occurred 5–9 s after the onset of stimulation, and the second phase at 5 Hz (solid columns) and 10 Hz (hatched columns) as the peak height between 18–23 s. Columns represent mean values of five experiments; bars show s.e.mean. Praz, prazosin. Significant difference from pretreatment values, for paired *t* test: **P* < 0.05. In control tissues (stimulation repeated in the absence of drugs) there were no significant differences between the corresponding responses within each tissue.

lated vas deferens, where nifedipine showed selectivity against the purinergic component of transmission (Blakeley *et al.*, 1981; Brown *et al.*, 1983).

It should be noted however that both in the pithed rat (Bullock & McGrath, 1988b, Figures 1, 3 and 4) and the isolated vas deferens of the rat (Brown *et al.*, 1983, Figure 6) there was a component of the non-adrenergic response which was resistant to nifedipine, although in both cases it was a relatively smaller component of the overall response than in the present study. This may indicate that in the isolated blood vessels used in this present study a relatively smaller part of the purinergic response is propagated via the action potential than in small resistance vessels (as found in the pithed rat) or the tightly coupled smooth muscle cells of the vas deferens.

Studies on small mesenteric arteries from the rat show a marked sensitivity to lowered calcium, of responses at low fre-

Figure 7 The effect of nifedipine in the presence of (a) prazosin and (b) α, β -methylene ATP (α, β -MeATP) is illustrated on responses of the proximal saphenous artery. Arteries were stimulated electrically at varying frequencies for one second duration (35–40 V, at frequencies of 4, 8, 16, 32 and 64 Hz, pulse width 0.1–0.3 ms) to produce isometric contractions. The interval between trains of pulses, during frequency-response curves (FRC), was 5 min. Arterial rings were electrically stimulated (a) before the addition of drugs (open columns), then after prazosin ($0.3 \mu\text{mol l}^{-1}$) (hatched columns), then after nifedipine ($1 \mu\text{mol l}^{-1}$) (solid columns) and (b) before the addition of drugs (open columns), then after desensitization with α, β -MeATP ($3 \mu\text{mol l}^{-1}$) (hatched columns), then after nifedipine ($1 \mu\text{mol l}^{-1}$) (solid columns). Data are presented as a percentage of the maximum response produced as a result of electrical stimulation within any one artery. Columns represent mean values of five experiments; bars show s.e.mean. Significant difference from pretreatment values, for paired *t* test: **P* < 0.05. In control tissues (stimulation repeated in the absence of drugs) there were no significant differences between the corresponding responses within each tissue.

quency nerve stimulation and much less sensitivity at higher frequencies (Sjöblom-Widfeldt & Nilsson, 1989). The latter authors concluded that this effect could not simply be attributed to a reduction in transmitter output as a result of a decreased availability of calcium since this effect was best seen at low frequencies and suggested that the transmitter responsible for the low-frequency response (possibly ATP) may differ with respect to calcium sensitivity. The results from this present study note a greater effect of nifedipine on the purinergic component of the electrically mediated vasoconstrictor responses.

It is also unlikely that attenuation of the nerve-mediated response by nifedipine is a result of its interference with presynaptic feedback mechanisms as found in the rat brain (Ilu *et al.*, 1987) since the attenuation of the nerve-mediated response is also evident at very short pulse trains (train lengths of 1 s) where conditions for autoinhibition may not be present (Bullock & Starke, 1990). It is also unlikely that nifedipine is acting presynaptically by directly inhibiting transmitter release, since this inhibition would have to be selective against the release of ATP so leaving the release of its co-transmitter

unaffected as the postjunctional adrenergic response is unaffected (Figures 4 and 5). Studies measuring [^3H]-NA efflux during nerve stimulation in dog mesenteric arteries found no interaction of nifedipine ($0.01\text{--}1\ \mu\text{mol l}^{-1}$) with [^3H]-NA efflux (Omote *et al.*, 1989).

In conclusion, nifedipine shows selectivity for the purinergic component of the electrically evoked nerve mediated response in the rabbit ileocolic artery. However, its inability to block

the purinergic component of the response completely suggests that more than Ca influx is involved in mediating the purinergic component of the sympathetic nerve-mediated response in this tissue.

J.M.B. holds an Intermediate Fellowship from the British Heart Foundation. This work was generously supported by NATO, SERC and the Medical Research Funds of the University of Glasgow.

References

- BLAKELEY, A.G.H., BROWN, D.A., CUNNANE, T.C., FRENCH, A.M., McGRATH, J.C. & SCOTT, N.C. (1981). Effects of nifedipine on electrical and mechanical responses of rat and guinea pig vas deferens. *Nature*, **294**, 759–761.
- BROWN, D.A., DOCHERTY, J.R., FRENCH, A.M., MacDONALD, A., McGRATH, J.C. & SCOTT, N.C. (1983). Separation of adrenergic and nonadrenergic contractions to field stimulation in the rat vas deferens. *Br. J. Pharmacol.*, **79**, 379–393.
- BULLOCH, J.M., DALY, C.J., MacDONALD, A., McGRATH, J.C. & WILSON, V.G. (1988). The effect of nifedipine on nerve-mediated responses of blood vessels *in vivo* and *in vitro*. *Br. J. Pharmacol.*, **95**, 563P.
- BULLOCH, J.M. & McGRATH, J.C. (1986). Blockade of vasopressor and vas deferens responses by alpha, beta-methylene ATP in the pithed rat. *Br. J. Pharmacol.*, **89**, 577P.
- BULLOCH, J.M. & McGRATH, J.C. (1988a). An investigation of purinergic co-transmission in sympathetic vascular control and in the vas deferens motor response. *Br. J. Pharmacol.*, **94**, 103–108.
- BULLOCH, J.M. & McGRATH, J.C. (1988b). The effect of nifedipine on nerve-mediated pressor responses of blood vessels *in vivo* and *in vitro*. *Br. J. Pharmacol.*, **95**, 695–700.
- BULLOCH, J.M. & STARKE, K. (1988). Presynaptic alpha₂-autoinhibition in the rabbit ileocolic artery: a blood vessel where ATP and noradrenaline act as co-transmitters. In *Proceedings of the International Symposium on Biosignalling in Cardiac and Vascular Systems* ed. Fujiwara, M., Narumiya, S. & Miwa, S. pp. 122–128. Oxford: Pergamon Press.
- BULLOCH, J.M. & STARKE, K. (1990). Presynaptic alpha₂-autoinhibition in the rabbit ileocolic artery: a blood vessel where ATP and noradrenaline act as co-transmitters. *Br. J. Pharmacol.*, **99**, 279–284.
- BURNSTOCK, G. & WARLAND, J.J.I. (1987). A pharmacological study of the rabbit saphenous artery *in vitro*: a vessel with a large purinergic contractile response to sympathetic nerve stimulation. *Br. J. Pharmacol.*, **90**, 111–190.
- CAMBRIDGE, D., DAVEY, M.J. & MASSINGHAM, R. (1977). Prazosin, a selective antagonist of post-synaptic α -adrenoceptors. *Br. J. Pharmacol.*, **54**, 514–515.
- ILU, P.S., LINDGREN, E., JACOBSON, K. & FREDHOLM, B.B. (1987). Interaction of dihydropyridine calcium channel agonists and antagonists with adenosine receptors. *Pharmacol. Toxicol.*, **61**, 121–125.
- ISHIKAWA, S. (1985). Actions of ATP and α,β -methylene ATP on neuromuscular transmission and smooth muscle membrane of the rabbit and guinea pig mesenteric arteries. *Br. J. Pharmacol.*, **86**, 777–787.
- KENNEDY, C., SAVILLE, V.L. & BURNSTOCK, G. (1986). The contributions of noradrenaline and ATP to the responses of the rabbit central ear artery to sympathetic nerve stimulation depend on the parameters of stimulation. *Eur. J. Pharmacol.*, **122**, 291–300.
- VON KÜGELGEN, I. & STARKE, K. (1985). Noradrenaline and adenosine triphosphate as co-transmitters of neurogenic vasoconstriction in rabbit mesenteric artery. *J. Physiol.*, **367**, 435–455.
- McGRATH, J.C., BULLOCH, J.M., DALY, C.J. & MacDONALD, A. (1990). Contributions of different receptors to vasoconstrictor co-transmission in rabbit isolated arteries and veins. *Blood Vessels*, **27**, 44.
- DE MORAES, S. & CAPAZ, F.R. (1984). The effects of ethanol dependence on drug responsiveness of mouse isolated vas deferens. *J. Pharm. Pharmacol.*, **36**, 70–72.
- MURAMATSU, I. (1986). Evidence for sympathetic purinergic transmission in the mesenteric artery of the dog. *Br. J. Pharmacol.*, **87**, 478–480.
- OMOTE, S., KIGOSHI, S. & MURAMATSU, I. (1989). Selective inhibition by nifedipine of the purinergic component of neurogenic vasoconstriction in the dog mesenteric artery. *Eur. J. Pharmacol.*, **160**, 239–245.
- STJÄRNE, L. & ÅSTRAND, P. (1984). Discrete events measure single quanta of adenosine 5'-triphosphate secreted from sympathetic nerves of the guinea pig and mouse vas deferens. *Neuroscience*, **13**, 21–28.
- STJÄRNE, L. & ÅSTRAND, P. (1985). Relative pre- and post-junctional roles of noradrenaline and adenosine 5'-triphosphate as neurotransmitters of sympathetic nerves of the guinea pig and mouse vas deferens. *Neuroscience*, **14**, 929–946.
- SJÖBLOM-WIDFELDT, N. & NILSSON, H. (1989). Sympathetic transmission in small mesenteric arteries from the rat: highly calcium dependent at low stimulation rates. *Acta Physiol. Scand.*, **135**, 505–511.
- SNEDDON, P. & BURNSTOCK, G. (1984a). Inhibition of excitatory junction potentials in guinea pig vas deferens by α,β -methylene ATP: further evidence for ATP and noradrenaline as cotransmitters. *Eur. J. Pharmacol.*, **100**, 85–90.
- SNEDDON, P. & BURNSTOCK, G. (1984b). ATP as co-transmitter in rat tail artery. *Eur. J. Pharmacol.*, **106**, 149–152.
- SNEDDON, P., WESTFALL, D.P. & FEDAN, J.S. (1983). Cotransmitters in the motor nerves of the guinea pig vas deferens: electrophysiological evidence. *Science*, **218**, 693–695.
- WEITZELL, R., TANAKA, T. & STARKE, K. (1979). Pre- and post-synaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. *Naunyn Schmiedeberg Arch. Pharmacol.*, **308**, 127–136.

(Received November 21, 1990

Revised March 7, 1991

Accepted March 11, 1991)