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Presynaptic α_2-autoinhibition in a vascular neuroeffector junction where ATP and noradrenaline act as co-transmitters

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1 α_2-Autoinhibition of transmitter release was investigated in the largest rami caecales of the rabbit ileocolic artery. Vasoconstriction, elicited by electrical field stimulation or by exogenous agonists, was measured as an increase in perfusion pressure.

2 Short periods of electrical stimulation elicited monophasic vasoconstriction, whereas longer periods (>10 s) produced biphasic vasoconstriction. Prazosin had no significant effect on the first component of the biphasic vasoconstriction elicited by electrical stimulation, but did reduce the second component at higher frequencies. α,β-Methylene ATP significantly attenuated the first component whilst the second component was relatively resistant.

3 The α_2-adrenoceptor antagonist yohimbine did not change responses evoked by very short pulse trains (<2 s) but enhanced responses to longer pulse trains. When vasoconstriction was biphasic, both phases were potentiated by yohimbine.

4 The results indicate that the vasoconstriction elicited by brief trains of sympathetic nerve impulses is mainly or exclusively mediated by ATP, whereas at longer pulse trains a noradrenergic component comes into play. The potentiation produced by yohimbine is due to interruption of presynaptic α_2-adrenoceptor-mediated autoinhibition of transmitter release. The autoinhibition affects both purinergic and adrenergic components of sympathetic neurotransmission.

Introduction

Investigations have now revealed numerous sympathetic neuroeffector systems where adenosine 5'-triphosphate (ATP) and noradrenaline (NA) act as co-transmitters. These systems include the vas deferens (Sneddon *et al.*, 1983; Sneddon & Burnstock, 1984a; Stjärne & Åstrand, 1984; 1985), as well as a variety of blood vessels, such as rat tail artery (Sneddon & Burnstock, 1984b), rabbit and guinea-pig mesenteric arteries (Ishikawa, 1985), guinea-pig saphenous artery (Cheung & Fujioka, 1986), rabbit central ear artery (Kennedy *et al.*, 1986), dog mesenteric artery (Muramatsu, 1986) and rabbit saphenous artery (Burnstock & Warland, 1987). Apart from these *in vitro* observations NA-ATP co-transmission has also been shown in the vasculature of the pithed rat (Grant *et al.*, 1985; Flavahan *et al.*, 1985; Bulloch & McGrath, 1986; 1988a,b).

The role of presynaptic α_2-adrenoceptor-mediated autoinhibition of transmitter release in a system where ATP and NA act as co-transmitters has been studied previously in the rabbit ileocolic artery (von Kügelgen & Starke, 1985) and in the smaller jejunal branches of the mesenteric artery of the rabbit (Ramme *et al.*, 1987). The α_2-adrenoceptor antagonist yohimbine increased the electrically evoked release of previously stored [^{3}H]-NA in these tissues. In jejunal arteries, yohimbine increased also the excitatory junction potential (e.j.p.) amplitudes from the fourth e.j.p. onwards in a series of e.j.ps evoked by 15 pulses at 2 Hz. In the rabbit ileocolic artery, the vasoconstrictor responses produced by electric field stimulation (5 Hz 100 pulses) were significantly increased by yohimbine.

The aim of the present study was to investigate the potentiation of vasoconstriction by yohimbine under a variety of stimulation parameters, in order to verify whether this action was in fact due to interruption of the α_2-autoinhibition (which does not operate at short pulse trains: Story *et al.*, 1981; Auch-Schwelk *et al.*, 1983) and not to some other effect of yohimbine, such as sensitization of vascular smooth muscle to the contractile effect of NA (Auch-Schwelk *et al.*, 1983). Our second question was whether it was possible to investigate the role of α_2-autoinhibition on the release of NA and ATP separately.

A preliminary communication of these results has been published (Bulloch & Starke, 1988).

Methods

Rabbits of either sex (2–3 kg) were decapitated and the proximal section of the largest ramus caecalis arteriae ileocolicae (Barone *et al.*, 1973) was dissected out and cleared of fatty tissue. Side branches were tied up and ileocolic arteries were cannulated at both ends before being mounted vertically in a 4 ml organ bath. They were 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 (von Kügelgen & Starke, 1985).

Vasoconstriction produced by drugs or 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 via a Statham P23 Db pressure transducer). The perfusate and bathing medium contained (mmol l^{-1}) NaCl 118, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 0.9, $NaHCO_3$ 25, glucose 11, Na_2 EDTA 0.03 and ascorbic acid 0.3. The medium was saturated with 95% O_2–5% CO_2 and maintained at 37°C.

The arteries were allowed to equilibrate for 1 h before electrical stimulation or the addition of exogenous agents.

Drugs used were adenosine 5'-triphosphate dipotassium salt (ATP) (Sigma), α,β-methylene adenosine 5'-triphosphate lithium salt (mATP) (Sigma), (−)-noradrenaline bitartrate (Sigma), prazosin HCl (Pfizer), SK&F 104078 maleate (6-chloro-9-[(3-methyl-2-butenyl)oxyl]-3-methyl-1H-2,3,4,5-tetrahydro-3-benzazepine) (gift from SK&F, U.S.A.), tetrodotoxin (TTX) (Sigma), yohimbine HCl (Roth). Stock solutions of drugs were prepared with distilled water. NA, ATP and mATP were added only to the organ bath. The antagonists prazosin, SK&F 104078 and yohimbine were added to both the perfusing fluid and the organ bath.

The means and s.e.mean are given throughout. Means were compared by use of paired Student's *t* test. *P* values of 0.05 or

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less were considered to be significant. n is the number of preparations.

Results

The effect of yohimbine on peak responses to electrical field stimulation

Stimulation of perfused ileocolic arteries produced reproducible vasoconstrictions observed as increases in perfusion pressure. These vasoconstrictions were pulse number and frequency-dependent. Yohimbine ($0.3\,\mu mol\,l^{-1}$) (Figure 1) increased by up to 100% vasoconstrictor responses to 100 or 200 pulses at 10 Hz and 100 pulses at 5 Hz. Yohimbine did not change the responses elicited by 5 or 10 pulses at these frequencies or by 6 or 8 pulses at 40 Hz. Hence, the potentiation by yohimbine was only observed when train lengths $\geqslant 10$ s were employed.

Analysis of the components of the nerve-mediated vasoconstrictor response

When the recording apparatus was run at a faster chart speed, the responses to pulse trains of up to 10 s duration appeared to be monophasic, whereas it was possible to distinguish two phases in the response to 20 s pulse trains (Figure 2). In most tissues the two phases were separated by an intervening dip in tension, or at higher frequencies, by a plateau. The first phase of the contraction reached a peak within 5–9 s, with the second phase peaking within 18–23 s after onset of stimulation. The relative proportion of the two phases was frequency-dependent. At lower frequencies (5 Hz) the first phase was nearly always larger than the second phase but at higher frequencies (20 Hz) the latter dominated the response.

We used the P_{2x}-purinoceptor desensitizing agent, mATP and the α_1-adrenoceptor antagonist prazosin, in an attempt to identify the (main) transmitter for each of the two components of the vasoconstriction. Selected responses from individual experiments are shown in Figure 3. mATP itself elicited marked but transient vasoconstrictions when given at five cumulative concentrations of $3\,\mu mol\,l^{-1}$ each (not shown). Subsequently, the first phase of the vasoconstrictor response

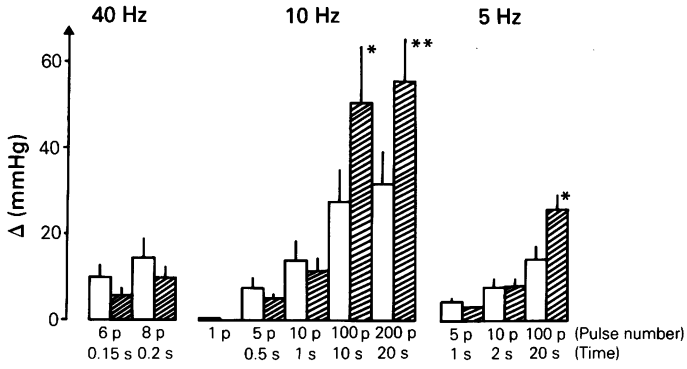

Figure 1 The effect of yohimbine on peak vasoconstrictor responses. Arteries were stimulated with different pulse numbers first at 40 Hz frequency, then at 10 Hz and finally at 5 Hz in the order shown in the graph. Intervals between stimulation periods were 5 min. After an initial sequence in the absence of yohimbine (open columns), the sequence was repeated 10 min after the addition of yohimbine ($0.3\,\mu mol\,l^{-1}$) to the perfusate (hatched columns). Columns represent means of seven experiments; bars show s.e.mean. Significant differences from pre-yohimbine values, for a paired t test: $^*P < 0.05$, $^{**}P < 0.01$. In control tissues (stimulation repeated in the absence of yohimbine) there were no significant differences between the corresponding responses within each tissue. Only two out of seven tissues responded to one pulse. This response was then abolished by yohimbine.

to electrical stimulation was greatly reduced whereas the second phase persisted (Figure 3Bb). Prazosin, in contrast, reduced the second phase with little effect on the first (Figure 3Ab). The combination of prazosin and mATP abolished vasoconstrictor responses almost entirely (Figure 3Ac).

A statistical evaluation of these experiments is presented in Figures 4 and 5. mATP consistently attenuated the first component as well as the monophasic responses to trains of up to 10 s duration but had little effect on the second component (Figure 4). Prazosin tended to reduce the second phase, although this effect was significant only for stimulation at 20 Hz. Prazosin did not change the first phase (except for a reduction of monophasic responses to 12 pulses delivered at 40 Hz) (Figure 5). When mATP was given after prazosin, all responses were abolished (not shown, but see Figure 3Ac).

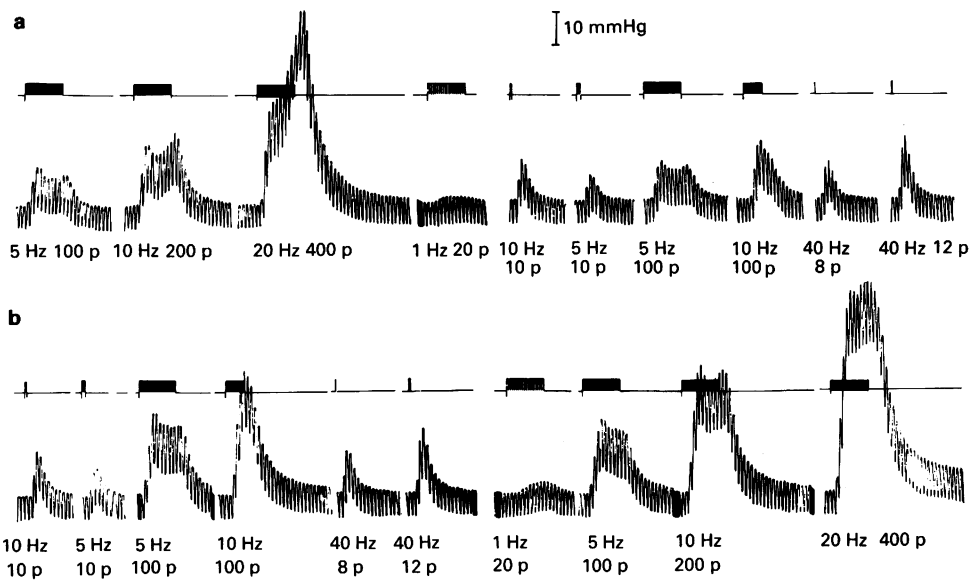

Figure 2 Mono- and biphasic vasoconstrictor responses of the ileocolic artery and the effect of yohimbine. Vessels were stimulated by trains of pulses and in the order defined in the graph. Duration of stimulation is also illustrated above each response. Intervals between stimulation periods were 5 min. The fluctuations in perfusion pressure were due to the pulsatile movements of the pump. (a) Shows the control responses to the first cycle of stimulation, and (b), the responses to the second cycle of stimulation carried out in the presence of yohimbine ($0.3\,\mu mol\,l^{-1}$). Yohimbine was added 10 min before the cycle of stimulations was repeated.

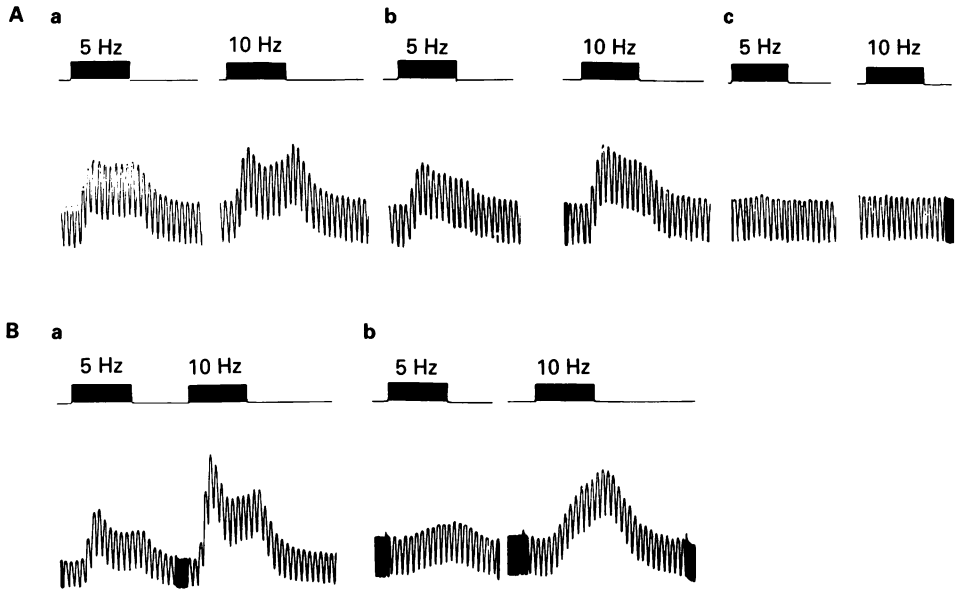

Figure 3 Biphasic vasoconstrictor responses of the ileocolic artery and the effect of prazosin and α,β-methylene ATP (mATP). Vessels were electrically stimulated according to the protocol illustrated in Figure 2, but only the responses to 5 Hz and 10 Hz, 20 s each, are shown here. (A) and (B) represent experiments on two different tissues. (Aa) and (Ba) show control responses to the first cycle of stimulations. (Ab) Shows responses to the second cycle of stimulations, carried out in the presence of prazosin ($0.3\,\mu mol\,l^{-1}$), and (Ac) responses to a third cycle of stimulations, after exposure to mATP ($15\,\mu mol\,l^{-1}$), given as five additions of $3\,\mu mol\,l^{-1}$ over 30 min (see von Kügelgen & Starke, 1985) and then washed out 5 min before electrical stimulation (prazosin still present). (Bb) Shows responses after exposure to mATP ($15\,\mu mol\,l^{-1}$) alone.

The effect of yohimbine on biphasic responses

It was with this biphasic profile in mind that the effect of yohimbine was re-analysed to take into consideration the components of co-transmission. Figures 2 and 6 show that at stimulations of 10 or 20 s duration, yohimbine potentiated both phases of the responses at each frequency, except for the second component at 400 pulses 20 Hz (and for the very small responses to 1 Hz). In contrast, none of the responses to shorter stimulation periods were enhanced. In particular, yohimbine did not change the vasoconstriction elicited by 8 or 12 pulses at 40 Hz even though these responses were of a comparable size to those obtained at 5 Hz 100 pulses and 10 Hz 100 pulses.

The effect of prazosin ($0.3\,\mu mol\,l^{-1}$) in the presence of yohimbine ($0.3\,\mu mol\,l^{-1}$) (Figure 7) on the biphasic response elicited by electrical field stimulation at 5 Hz 100 pulses, was compared to its effect in the absence of yohimbine (Figure 5). In tissues exposed to yohimbine ($0.3\,\mu mol\,l^{-1}$), prazosin ($0.3\,\mu mol\,l^{-1}$) significantly attenuated both components of the response.

The effect of yohimbine on responses to exogenously administered agonists

The introduction of NA ($3\,\mu mol\,l^{-1}$–$30\,\mu mol\,l^{-1}$) to the bathing fluid, produced concentration-dependent vasoconstrictions. These responses were reproducible within any one

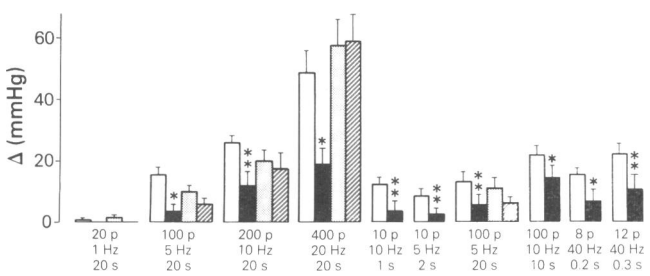

Figure 4 The effect of α,β-methylene ATP (mATP) on the two phases of the vasoconstrictor responses in the ileocolic artery. Arteries were stimulated according to the protocol illustrated in Figure 2, first before the addition of mATP (open columns – first component; stippled columns - second component where present) and then after desensitisation with mATP ($15\,\mu mol\,l^{-1}$) (solid columns – first component; hatched columns – second component where present). The first phase was taken as the peak constrictor height which occurred 5–9 s after the onset of stimulation, and the second phase, as the peak height between 18–23 s. Columns represent mean values of six experiments; bars show s.e.mean. Significant differences from pretreatment values, for paired t test: $^*P < 0.05$, $^{**}P < 0.01$. In control tissues (stimulation repeated in the absence of mATP) there were no significant differences between the corresponding responses within each tissue.

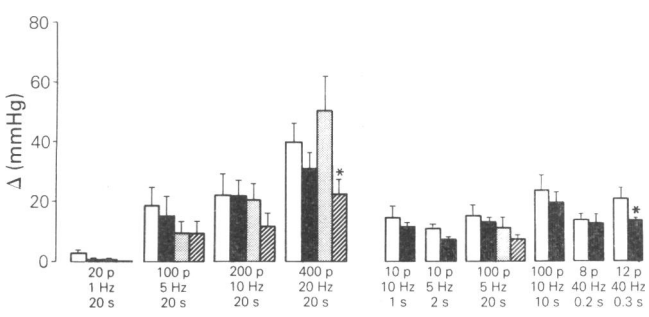

Figure 5 The effect of prazosin on the two phases of the vasoconstrictor response. Arteries were stimulated according to the protocol illustrated in Figure 2, first in the absence of prazosin (open columns – first component; stippled columns – second component where present) and then in the presence of prazosin ($0.3\,\mu mol\,l^{-1}$) (solid columns – first component; hatched columns – second component where present). The subsequent administration of α,β-methylene ATP (mATP, $15\,\mu mol\,l^{-1}$) completely abolished the responses (not shown). The first phase was taken as the peak constrictor height which occurred 5–9 s after the onset of stimulation, and the second phase, as the peak height between 18–23 s. Columns represent mean values of six experiments; bars show s.e.mean. Significant differences from pretreatment values, for paired t test: $^*P < 0.05$, $^{**}P < 0.01$. In control tissues (stimulation repeated in the absence of prazosin) there were no significant differences between the corresponding responses within each tissue.

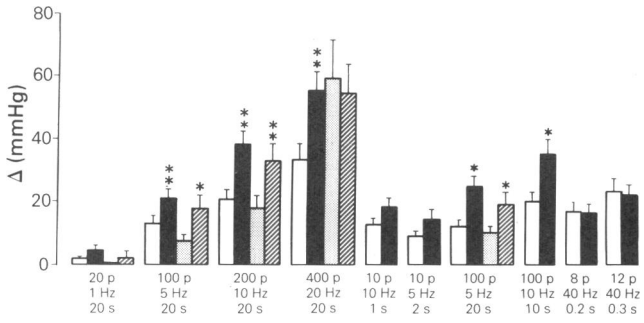

Figure 6 The effect of yohimbine on the two phases of the vasoconstrictor response. Arteries were stimulated according to the protocol illustrated in Figure 2, first in the absence (open columns – first component; stippled columns – second component where present) and then in the presence of yohimbine (0.3 μmol l^{-1}) (solid columns – first component; hatched columns – second component where present). The first phase was taken as the peak constrictor height which occurred 5–9 s after the onset of stimulation, and the second phase as the peak height between 18–23 s. Columns represent values of nine experiments; vertical bars show s.e.mean. Significant differences from pretreatment values, for paired t test: * $P < 0.05$, ** $P < 0.01$. In control tissues (stimulation repeated in the absence of yohimbine) there was no significant difference between the corresponding responses within each tissue.

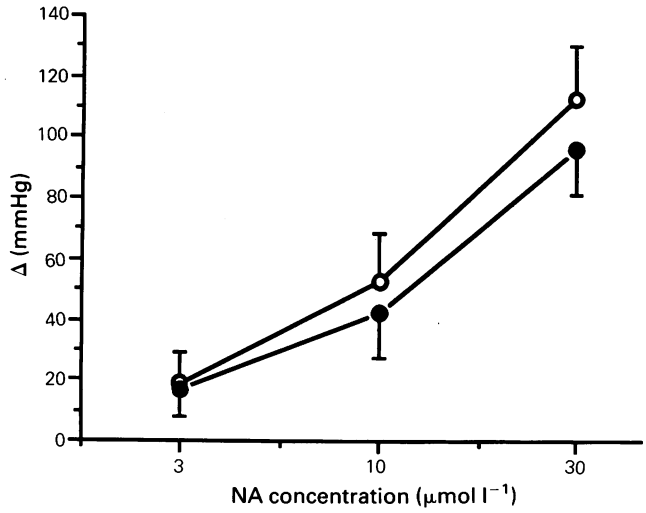

Figure 8 The effect of α,β-methylene ATP (mATP) on vasoconstrictor responses to exogenous noradrenaline (NA). A concentration-response curve was constructed before (○) and after treatment (●) with mATP (15 μmol l^{-1}; given as five additions of 3 μmol l^{-1} over 30 min and then washed out 5 min before the addition of NA). Values are means of five experiments; vertical lines show s.e.mean. In mATP-treated tissues and in control tissues (no treatment with mATP) there was no significant difference (paired t test) between the corresponding responses within each tissue.

preparation and were not affected by yohimbine (0.3 μmol l^{-1}) (Table 1).

ATP (300 μmol l^{-1}) administered to the bathing fluid at intervals of 30 min, to avoid problems of tachyphylaxis, produced transient vasoconstrictions which were unaffected by yohimbine (0.3 μmol l^{-1}) (Table 1).

The selectivity of mATP was also tested against exogenously administered NA and ATP. The vasoconstrictor responses produced by NA (3 μmol l^{-1}–30 μmol l^{-1}) were unaffected by mATP (15 μmol l^{-1}) (Figure 8), but those produced by ATP (300 μmol l^{-1}) were abolished (not illustrated).

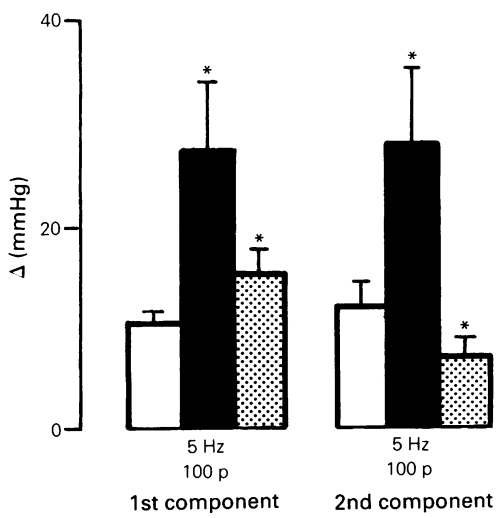

Figure 7 The effect of prazosin administered in the presence of yohimbine on the two phases of the vasoconstrictor response elicited by 5 Hz 100 pulses. The protocol followed here was different from that adopted in previous figures. A frequency-response curve was obtained first (5, 10, and 20 Hz, all for 20 s) and then stimulation by 100 pulses at 5 Hz (20 s) was repeated every 5 min for the remainder of the experiment. Shown are the last responses to 100 pulses, 5 Hz, before drug addition (open columns), responses 10 min after addition of yohimbine (0.3 μmol l^{-1}) (solid columns), and responses 10 min after addition of prazosin (0.3 μmol l^{-1}), still in the presence of yohimbine (stippled columns). Columns represent mean values of five experiments. Significant difference from preceding values, for paired t test: * $P < 0.05$, ** $P < 0.01$. In control tissues (stimulations repeated in the absence of yohimbine and prazosin) there was no significant difference between the corresponding responses within each tissue.

The effect of other agents on vasoconstrictor responses to nerve stimulation

The addition of tetrodotoxin (TTX) (0.5 μmol l^{-1}) abolished nerve-mediated vasoconstrictions at all frequencies studied (results not shown).

The effect of the 'selective' postjunctional α_2-adrenoceptor antagonist SK&F 104078 (Ruffolo *et al.*, 1987) was also tested against nerve-mediated vasoconstrictions, as well as those elicited by exogenous NA. SK&F 104078 (3 μmol l^{-1}) potentiated the first component of the electrically-induced vasoconstrictor response, but an attenuation was observed for the second component at 20 Hz (Figure 9). SK&F 104078 (3 μmol l^{-1}) attenuated the response to exogenous NA (10–30 μmol l^{-1}) (Table 1).

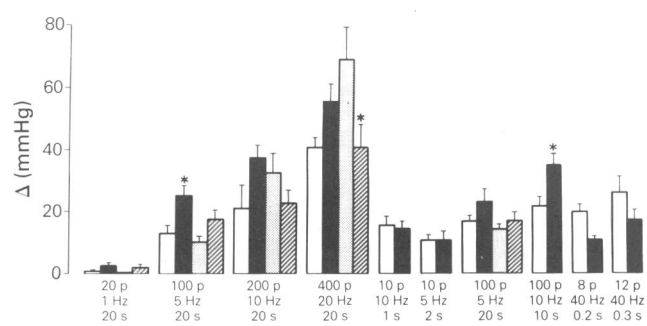

Figure 9 The effect of SK&F 104078 on the two phases of the vasoconstrictor response. Arteries were stimulated according to the protocol illustrated in Figure 2, first in the absence (open columns – first component; stippled columns – second component where present) and then in the presence of SK&F 104078 (3 μmol l^{-1}) (solid columns – first component; hatched columns – second component where present). The first phase was taken as the peak constrictor height which occurred 5–9 s after the onset of stimulation, and the second phase as the peak height between 18–23 s. Columns represent mean values of six experiments; vertical bars show s.e.mean. Significant difference from pretreatment values, for paired t test: * $P < 0.05$, ** $P < 0.01$. In control tissues (stimulation repeated in the absence of SK&F 104078) there was no significant difference between the corresponding responses within each tissue.

Table 1 The effects of antagonists on agonist-induced vasoconstrictor responses of the ileocolic artery

	Increase in perfusion pressure (Δ mmHg) *elicited by*			
	NA (μmol l^{-1})			ATP (μmol l^{-1})
	3	*10*	*30*	*300*
Before yohimbine	8.4 ± 3.5	23.8 ± 10.4	56.7 ± 15.3	18.3 ± 6.3
Yohimbine (0.3 μmol l^{-1}) present	8.9 ± 3.6	20.3 ± 7.1	54.6 ± 16.5	19.6 ± 7.6
Before SKF	16.2 ± 6.3	48.1 ± 14.1	85.9 ± 13.6	—
SKF (3 μmol l^{-1}) present	7.4 ± 4.0	20.1 ± 12.1*	60.9 ± 12.7*	—

Noradrenaline (NA) and ATP were added at 30 min intervals. In any one tissue the same concentration of agonist was used throughout. Agonist additions were made until consistent responses were obtained and the responses to the final 3 control additions of agonist were averaged. The antagonists yohimbine or SK&F 104078 were introduced both to the perfusing fluid and to the organ bath, after these control additions and 10 min before the final addition of agonist. Values are means ± s.e.mean of five experiments. Significant differences from pretreatment values (paired t test): * $P < 0.05$, ** $P < 0.01$. In control tissues (repeated agonist additions in the absence of antagonists) there was no significant difference between the corresponding responses within each tissue.

Discussion

The rabbit ileocolic artery is one blood vessel in which NA-ATP co-transmission has been demonstrated (von Kügelgen & Starke, 1985). Our experiments show that, for longer pulse trains (train lengths of 20 s), two phases of the contraction can be differentiated; the first one (and the only one detected at short pulse trains) was greatly reduced by mATP, and hence mainly mediated by ATP, the second one was mATP-resistant, but at least partly sensitive to prazosin, and hence had a considerable noradrenergic component (Figures 3, 4 and 5). When interpreting the data, it should be noted that the measured height of at least the second of the two phases comprises both purinergic and adrenergic components, with the second component being superimposed upon the first one. This overlap (with the purinergic component dominating the first phase and the adrenergic component dominating the second phase) is probably responsible for the fact that prazosin reduced significantly only the second phase of the response to 20 Hz, at least in the absence of other drugs; in the presence of yohimbine, it significantly reduced both phases of the response to 5 Hz stimulation as well (Figure 7).

Biphasic vasoconstrictor responses have also been obtained in other vascular neuroeffector sites where NA and ATP act as co-transmitters, with each of the two phases being attributed to a different co-transmitter (Kennedy *et al.*, 1986; Burnstock & Warland, 1987; Machaly *et al.*, 1988).

Another example of a neuroeffector system where ATP and NA act as co-transmitters is the vas deferens (Sneddon *et al.*, 1983; Sneddon & Burnstock, 1984a; Stjärne & Astrand, 1984; 1985). The mechanical response of the vas deferens to sympathetic nerve stimulation is also biphasic; a rapid twitch contraction is followed by a slow tonic contraction. Each of the two components seems to be due to both NA and ATP with only quantitative differences in the contribution of either (Stjärne & Åstrand, 1985). This biphasic profile of the mechanical response is therefore comparable to that identified in the vasculature.

Yohimbine has been shown to potentiate electrically-elicited vasoconstrictor responses (von Kügelgen & Starke, 1985). Although the increase in neurogenic vasoconstriction may be due to the interruption of presynaptic α_2-adrenoceptor-mediated autoinhibition, a postsynaptic potentiation by yohimbine has also been observed (Auch-Schwelk *et al.*, 1983). If a postsynaptic mechanism such as the sensitization of the vascular smooth muscle were responsible for the enhancement of neurogenic vasoconstriction, then the effect of yohimbine should be observed at all parameters of stimulation studied. However, the present results indicate an exclusively presynaptic action of yohimbine, since potentiation was only observed when long trains of stimulation were employed but not when trains were too short for autoinhibition (for example 5 Hz 10 pulses, 10 Hz 10 pulses, 40 Hz 8 pulses and 40 Hz 12 pulses; see Story *et al.*, 1981; Auch-Schwelk *et al.*, 1983). In addition, yohimbine had no effect on contractile response to exogenous NA or ATP (Table 1). An ongoing autoinhibition is, in fact, a prerequisite for the effect of yohimbine, and this effect reveals marked postsynaptic sequelae of a primary presynaptic control mechanism.

The blockade of α_2-autoinhibition by yohimbine potentiated both phases of the responses in the ileocolic artery (except for the second component at 20 Hz (Figure 6)). This could indicate that the release of both ATP and noradrenaline is subject to α_2-autoinhibition, in agreement with recent findings in the dog mesenteric artery (Muramatsu *et al.*, 1989).

However, yohimbine changed the vasoconstrictor response not only in size but, apparently, qualitatively as well. In the presence of yohimbine, prazosin attenuated both phases of the response to 5 Hz 100 pulses (Figure 7) and this contrasts to its lack of any significant effect on the same response in the absence of yohimbine (Figure 5). In other words, yohimbine changed a response which was nearly all purinergic (Figure 5) to one which was predominantly adrenergic (Figure 7). This observation opens up the interesting possibility that yohimbine preferentially increased the release of NA. Hence, the storage vesicles in the sympathetic nerve terminals may differ in their ATP/NA ratio, and presynaptic α_2-autoinhibition may decrease (and its interruption enhance) mainly transmitter release from NA-rich vesicles. Another recent observation in our laboratory supports this possibility. (Bulloch & Starke, unpublished observations), where vasoconstriction in the rabbit ileocolic artery was elicited by nicotine or 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP) which act by releasing transmitter from sympathetic nerve terminals through presynaptic nicotinic receptors (see Starke, 1977). The responses to these drugs, although comparable in size to those produced by electrical stimulation at 5 Hz 100 pulses (in the absence of yohimbine) were almost entirely blocked by prazosin (0.3 μmol l^{-1}), again indicating differential storage and release of NA and ATP. Recent work (Trachte, 1985; 1988; Ellis & Burnstock, 1989) has also raised questions on methods of storage and release of NA and ATP.

This study also highlighted the difficulties encountered when analysing prejunctional effects of an antagonist like yohimbine, which is also active at postjunctional α_2-adrenoceptors. It has been proposed that postjunctional α_2-adrenoceptors occur in the vicinity of the postganglionic sympathetic varicosities in the vasculature in the pithed rabbit (McGrath *et al.*, 1982; Bulloch *et al.*, 1987) and the pithed rat (Flavahan *et al.*, 1985). Vasoconstrictor responses of the rabbit ileocolic artery to exogenous NA are mediated exclusively by α_1-adrenoceptors, since they are abolished by relatively low concentrations of prazosin but not attenuated by yohimbine (von Kügelgen & Starke, 1985; and present study). However, released endogenous NA might still exert some of its vasoconstrictor effect by acting on smooth muscle α_2-adrenoceptors located close to the sites of release. If so, the adrenergic component of co-transmission would be greater than revealed by prazosin, and the degree of α_2-autoinhibition also would be greater than revealed by yohimbine, because the latter would block the α_2-adrenoceptor-mediated part of the vasoconstrictor response.

It was for this reason that the 'selective' postjunctional α_2-adrenoceptor antagonist SK&F 104078 (Ruffolo *et al.*, 1987) was employed to study any postjunctional α_2-adrenoceptor component in neurogenic vasoconstriction. The concentration of SK&F 104078 used was $3\,\mu mol\,l^{-1}$ and was therefore under the $10\,\mu mol\,l^{-1}$ concentration at which the antagonist has been postulated to cease to be selective for postjunctional α_2-adrenoceptors (Ruffolo *et al.*, 1987). However, at $3\,\mu mol\,l^{-1}$, SK&F 104078 potentiated the first component of the electrically-mediated response indicating possibly blockade of prejunctional α_2-adrenoceptors (Figure 9). The second component of the response to 20 Hz 400 pulses was, in contrast, attenuated. Interestingly, this was the one set of stimulation parameters, in stimulation periods of sufficient length, where the second component was not potentiated by yohimbine. Hence, there is indeed some evidence that postjunctional α_2-adrenoceptors may come into play when longer pulse trains are delivered at higher frequencies. However, non-selective (α_1-adrenoceptor) antagonism by SK&F 104078 cannot be ruled out. Although yohimbine had no effect on responses to exogenous NA (Table 1), responses to exogenous NA ($10–30\,\mu mol\,l^{-1}$) were significantly reduced by SK&F 104078 (Table 1). There is no evidence from the present study to support the proposal that SK&F 104078 is selective for postjunctional α_2-adrenoceptors. This lack of selectivity is in agreement with recent findings (Connaughton & Docherty, 1988).

In conclusion, NA and ATP are co-transmitters of neurogenic vasoconstriction in the rabbit ileocolic artery. When the sympathetic nerves of the artery are stimulated by relatively long pulse trains (more than 10 s), the vasoconstrictor response becomes biphasic. The initial phase is predominantly or exclusively purinergic whereas the second phase contains a considerable adrenergic component. Both the release of NA and the release of ATP are subject to presynaptic α_2-adrenoceptor-mediated autoinhibition. However, the composition of the co-transmitter mixture may change when autoinhibition is interrupted.

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