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The effect of reserpine on sympathetic, purinergic neurotransmission in the isolated mesenteric artery of the dog: a pharmacological study

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1 Electrical transmural stimulation evoked a transient contraction in the isolated mesenteric artery of the dog. This contraction was abolished by guanethidine or tetrodotoxin and was partially inhibited by prazosin. Noradrenaline was competitively antagonized by prazosin.

2 Similarly, in the reserpine-treated artery, electrical transmural stimulation produced a transient contraction which was abolished by guanethidine or tetrodotoxin. However, prazosin failed to inhibit this contraction. The contraction to noradrenaline was not significantly different from the response it produced in control vessels.

3 Tyramine (10^{-5} M), which acts on sympathetic nerves to release noradrenaline, evoked a tonic contraction in the untreated artery. This contraction was abolished or markedly attenuated by prazosin or guanethidine. The response was not observed in the reserpine-treated artery, indicating that reserpine had depleted the nerves of noradrenaline.

4 In the control vessel α,β-methylene-ATP produced a transient contraction which was followed by a complete relaxation to the basal level. This contractile response was not significantly different in the presence of guanethidine or prazosin or in the reserpine-treated artery.

5 After desensitization of the vessel to α,β-methylene ATP (5×10^{-6} M) the prazosin-resistant contractions induced by electrical transmural stimulation were abolished both in reserpine-treated and untreated arteries. Also the contractile responses to ATP and α,β-methylene-ATP were abolished but the responses to tyramine (control vessels), noradrenaline and KCl were not affected.

6 8-Phenyltheophylline (10^{-5} M) showed no inhibitory effect on the contractile responses to electrical transmural stimulation, tyramine, ATP or α,β-methylene-ATP.

7 Neuropeptide Y, peptide YY, vasoactive intestinal polypeptide, bombesin and substance P (10^{-7} and 10^{-6} M for each peptide) caused no contractile response in the dog mesenteric artery.

8 These experiments provide further evidence that the sympathetic contraction of the isolated mesenteric artery of the dog induced by electrical transmural stimulation consists of an adrenergic and a purinergic component and that the latter component is mediated through postsynaptic P_2-purinoceptors.

Introduction

Sympathetic response in blood vessels has been considered to be caused exclusively by noradrenaline released from the sympathetic nerve terminals. However, there is evidence that the sympathetic responses in some arteries are not completely blocked by α-adrenoceptor antagonists (Hirst & Neild, 1980; Holman & Surprenant, 1980; Muramatsu *et al.*, 1981; Kuriyama & Makita, 1983). Recent studies have revealed that the α-adrenoceptor antagonist-resistant responses may be produced by substances other than noradrenaline (ATP or a related substance: Muramatsu *et al.*, 1981; Sneddon & Westfall, 1984; Sneddon & Burnstock, 1984; Kennedy *et al.*, 1986; Burnstock & Warland, 1987; neuropeptide Y: Lundberg & Tatemoto, 1982).

In the dog mesenteric artery, prazosin or other α-adrenoceptor antagonists do not completely inhibit the contractions induced by electrical transmural stimulation or nicotine (Muramatsu *et al.*, 1984). Recently, it was demonstrated that the prazosin-resistant contraction of the dog mesenteric artery was completely inhibited after desensitization of P_2-purin-

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oceptor with α,β-methylene ATP (α,β-Me-ATP), suggesting a possibility of purinergic transmission (Muramatsu, 1986). The present study was performed to obtain further evidence for sympathetic, purinergic transmission in the dog mesenteric artery. The sympathetic responses were evoked by electrical transmural stimulation and tyramine, and the effects of prazosin, 8-phenyltheophylline and desensitization to α,β-Me-ATP in control and reserpine-treated vessels were examined. Contractile responses of neuropeptides such as neuropeptide Y were also studied.

Methods

Dogs of either sex, weighing 8 to 15 kg, were anaesthetized with thiopentone sodium (20 mg kg^{-1}, i.v.), exsanguinated from the common carotid arteries, and the mesenteric artery was isolated. Helical strips, approximately 2 mm in width and 15 mm in length, were prepared under a dissecting microscope. In order to avoid the possible involvement of endothelium-derived relaxing factor in the mechanical response, the endothelial cells were removed by rubbing them with filter paper (Furchgott, 1981), and the functional loss of endothelial cells was confirmed by the loss of relaxant response to acetylcholine (10^{-6} M) in noradrenaline (3×10^{-7} M) precontracted arteries (Muramatsu *et al.*, 1986). Each strip was mounted vertically in an organ bath containing 20 ml of Krebs-Henseleit solution of the following composition (mM): NaCl 112, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2, $NaHCO_3$ 25, $NaHPO_4$ 1.2, and glucose 11.5. To block the β-adrenoceptors, propranolol (10^{-6} M) was added to the bath solution throughout the experiment. The bath medium was maintained at 37°C, pH 7.4, and was equilibrated with a gas mixture consisting of 95% O_2 and 5% CO_2. Resting tension of 1.0 g was applied to each artery and the tension was recorded isometrically through a force-displacement transducer. The preparations were equilibrated for 90 min before starting the experiments.

Drugs were added directly to the bath. Cumulative concentration-response curves were determined by the step-wise increases in the concentration of an agonist, as soon as a peak response to the previous administration had been achieved. In the case of α,β-Me-ATP or ATP, when carrying out a non-cumulative concentration-response curve, the drugs were added as single additions at intervals of 40 min with repeated washing between each addition. Antagonists were added 20 min before application of the agonist. In order to produce desensitization of the P_2-purinoceptors (Kasakov & Burnstock, 1983), the tissues were treated with α,β-Me-ATP (5×10^{-6} M) for more than 20 min, during which time the transient contraction induced by the drug had returned to the original resting level.

Electrical transmural stimulation was applied through a pair of platinum-wire electrodes (Muramatsu *et al.*, 1983). Stimulus parameters were 0.3 ms duration, a frequency of 10 Hz and supramaximum voltage (10 V) for 5 s.

For the treatment with reserpine, the drug was given i.p. once daily for 4 days (on the 1st day, 0.5 mg kg^{-1} and the following days, 1.0 mg kg^{-1}). Twenty-four hours after the last injection, the dogs were exsanguinated from the common carotid arteries under thiopentone anaesthesia and the mesenteric artery was isolated. Reserpine is widely known to deplete sympathetic nerves of 98–99% of their noradrenaline (Langer & Pinto, 1976; Suzuki *et al.*, 1984; Kügelgen & Starke, 1985). In this experiment the depletion of noradrenaline from sympathetic nerves was confirmed either by the lack of tyramine responses in the mesenteric artery and vein, carotid and femoral arteries, or by the abolition of responses to electrical transmural stimulation in the carotid and femoral arteries (Muramatsu *et al.*, 1984).

The following drugs were used: lithium salt of α,β-methylene ATP (α,β-Me-ATP) (Sigma, MO, U.S.A.), (−)-noradrenaline bitartrate, (±)-propranolol hydrochloride, tyramine hydrochloride (Nakarai, Kyoto, Japan), guanethidine sulphate (Tokyo-Kase, Tokyo, Japan), tetrodotoxin (Sankyo, Tokyo, Japan), reserpine as Apoplon (Daiichi Seiyaku, Tokyo, Japan), prazosin hydrochloride (Taito-Pfizer, Tokyo, Japan), adenosine triphosphate (Kohjin, Tokyo, Japan), 8-phenyltheophylline (Calbiochem, CA, U.S.A.), neuropeptide Y, peptide YY (Peninsula, CA, U.S.A.), bombesin, porcine vasoactive intestinal polypeptide, substance P (Protein Institute Inc., Osaka, Japan).

Experimental values are given as a mean ± standard error of mean (s.e.mean). Results have been analysed using Student's *t* test (paired and unpaired as appropriate) and a probability of less than 0.05 was considered significant.

Results

Responses to electrical transmural stimulation

Electrical transmural stimulation evoked a transient contraction in the dog mesenteric artery. This contraction was abolished by guanethidine (3×10^{-6} M) or tetrodotoxin (10^{-7} M) and partially inhibited by prazosin (10^{-7} M) (Table 1). Figure 1 shows contractile responses to electrical transmural stimulation before and after desensitization of the vessel to α,β-Me-ATP. α,β-Me-ATP (5×10^{-6} M) itself produced a large contraction, but the response was transient, resulting in a complete relaxation to the original level within 20 min. After such treatment with α,β-Me-ATP and in the

Table 1 Effects of various drugs on the contractile responses induced by electrical transmural stimulation (10 Hz, 5 s, maximum voltage) in the mesenteric arteries isolated from untreated or reserpine-treated dogs

Treatments	*% response*[a] *Untreated*	*Reserpine-treated*
Prazosin 10^{-7} M	42 ± 3[c] (10)	95 ± 3 (5)
α,β-Me-ATP 5×10^{-6} M	80 ± 2[c] (10)	2 ± 1[c] (5)
Prazosin 10^{-7} M + α,β-Me-ATP 5×10^{-6} M	3 ± 1[c] (10)	0[c] (3)
8-Phenyltheophylline 10^{-5} M	89 ± 5 (5)	—[b]
Guanethidine 3×10^{-6} M	0[c] (10)	0[c] (5)
Tetrodotoxin 10^{-7} M	0[c] (7)	0[c] (5)

[a] % response compared with that before treatment with drug (control).
[b] Not examined.
[c] Significantly different from the control (paired t test, $P < 0.01$).
Number in parentheses represents the number of experiments.

absence of prazosin, the responses to electrical transmural stimulation were slightly attenuated (Figure 1a). On the other hand, in the preparations pretreated with prazosin (10^{-7} M), the response to electrical stimulation was completely inhibited after desensitization of the vessel to α,β-Me-ATP (Figure 1b). Washing the tissues free of the drugs reversed the inhibitory action of α,β-Me-ATP more rapidly than that of prazosin. 8-Phenyltheophylline (10^{-5} M) had no significant effect on the response to electrical stimulation (Table 1).

Figure 2 shows the responses to electrical transmural stimulation of a mesenteric artery isolated from a reserpine-treated dog. Prazosin failed to cause an inhibitory effect on the response, whereas guanethidine and tetrodotoxin abolished the contraction (Figure 2a, Table 1). α,β-Me-ATP also produced a transient contraction in the reserpine-treated artery; after total desensitization of the vessel to α,β-Me-ATP the response to electrical transmural stimulation was abolished. This complete inhibition was produced even in the absence of prazosin treatment (Figure 2b, Table 1).

Response to tyramine

Tyramine (10^{-5} M) produced a tonic contraction in the dog mesenteric artery (Figure 1). This response was abolished or markedly attenuated by prazosin (10^{-7} M) and by guanethidine (3×10^{-6} M) (Table 2). After desensitization of the tissue to α,β-Me-ATP, the tyramine response was not significantly altered from

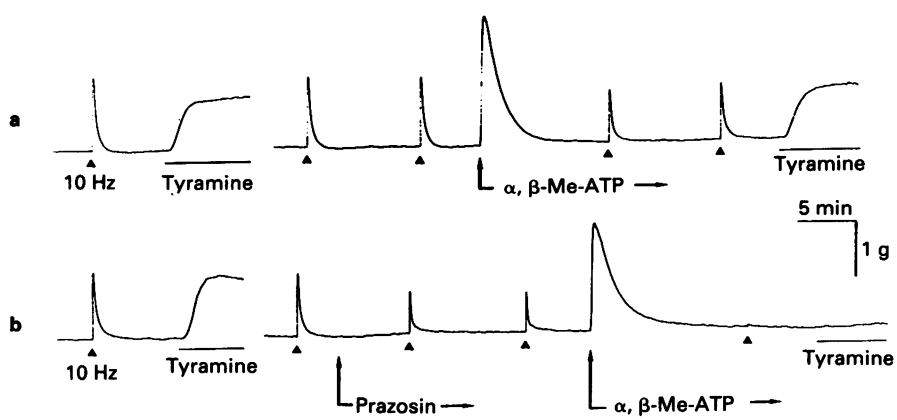

Figure 1 Effects of α,β-methylene ATP (α,β-Me-ATP, 5×10^{-6} M) on the contractile responses of the dog mesenteric arteries to electrical transmural stimulation (10 Hz, at maximum voltage for 5 s) and tyramine (10^{-5} M) in the absence (a) and presence (b) of prazosin (10^{-7} M). Endothelium of the preparations had been removed. Propranolol (10^{-6} M) was present throughout the experiments.

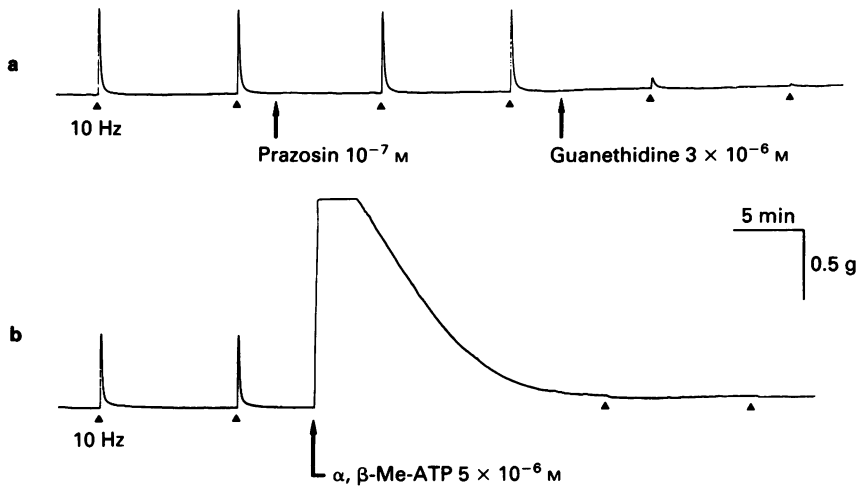

Figure 2 Effects of prazosin, guanethidine and α,β-Me-ATP on the contractile responses to electrical transmural stimulation in reserpine-treated dog mesenteric artery. A transient, contractile response to α,β-Me-ATP exceeded the recording range. Other experimental conditions were the same as those in Figure 1.

its control response (Figure 1a and Table 2). In the reserpine-treated arteries, tyramine (10^{-5} M) did not evoke a significant contraction (Table 2).

Responses to ATP, α,β-Me-ATP, noradrenaline and KCl

ATP at concentrations over 10^{-6} M and α,β-Me-ATP at concentrations over 10^{-8} M produced a contraction in the dog mesenteric artery. These responses were concentration-dependent; the maximum amplitudes induced by 3×10^{-3} M ATP and 10^{-5} M α,β-Me-ATP (non-cumulative application) were $64 \pm 2\%$ ($n = 8$) and $68 \pm 1\%$ ($n = 8$) of that of noradrenaline, respectively. The responses were transient, thus resulting in a gradual decline of tension irrespective of the presence of the drug (Figure 3). However, there were no significant differences ($P > 0.05$) in the potency (the concentration of purine to produce 50% maximal noradrenaline contraction) of the ATP or α,β-Me-ATP contractile response induced by cumulative [ATP: $(2.7 \pm 0.6) \times 10^{-4}$ M, $n = 4$; α,β-Me-ATP: $(3.7 \pm 1.2) \times 10^{-7}$ M, $n = 4$] and non-cumulative [ATP: $(2.1 \pm 0.4) \times 10^{-4}$ M, $n = 4$; α,β-Me-ATP: $(2.0 \pm 0.4) \times 10^{-7}$ M, $n = 4$] protocols. Each concentration of the cumulative concentration-response curves to ATP and α,β-Me-ATP in control preparations was not significantly affected ($P > 0.05$) by guanethidine (3×10^{-6} M, $n = 5$), prazosin (10^{-7} M, $n = 5$) or 8-phenyltheophylline (10^{-5} M, Figure 4), and was not significantly different from those in the reserpine-treated arteries (Figure 4). However, ATP and α,β-Me-ATP caused no contractile response when the preparations had been desensitized with α,β-Me-ATP 5×10^{-6} M beforehand (Figure 4).

Noradrenaline and KCl (in the presence of guanethidine) each produced concentration-dependent contractions. At each concentration these contractions were not significantly affected by pretreatment with α,β-Me-ATP 5×10^{-6} M (Figure 5). The EC_{50} value of noradrenaline in control arteries [$(4.8 \pm 1.3) \times 10^{-7}$ M, $n = 6$] was not significantly dif-

Table 2 Effects of various drugs on the contractile response to tyramine (10^{-5} M) in the dog isolated mesenteric arteries

Treatment	*% response*[a]
Prazosin 10^{-7} M	3 ± 1[c] (7)
α, β-Me-ATP 5×10^{-6} M	95 ± 3 (9)
8-Phenyltheophylline 10^{-5} M	102 ± 2 (4)
Guanethidine 3×10^{-6} M	21 ± 4[c] (5)
Reserpine	5.3[b] (10)

[a] % response compared with that before treatment with drug (control).
[b] The mean value of contractile amplitudes induced by 10^{-5} M tyramine in reserpine-treated arteries was compared with that in untreated arteries. The actual amplitudes of contraction in reserpine-treated dog mesenteric arteries were significantly different from the untreated vessels (unpaired t test, $P < 0.01$).
[c] Significantly different from the control (paired t test, $P < 0.01$).
Number in parentheses represents the number of experiments.

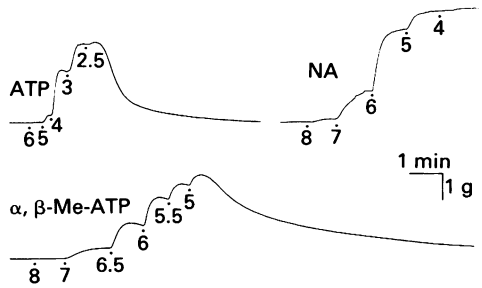

Figure 3 Representative pattern of contractile responses to ATP, α,β-Me-ATP and noradrenaline (NA) in a dog mesenteric artery. Each drug was cumulatively applied. The numbers below each trace represent the negative logarithm concentrations of each drug. Endothelium layer of the preparation had been removed. Propranolol (10^{-6} M) was present throughout the experiments.

ferent from that of the reserpine-treated arteries [$(2.6 \pm 0.5) \times 10^{-7}$ M, $n = 5$)]. The response to noradrenaline was competitively antagonized by prazosin; the pA_2 value being 8.3 ± 0.1 ($n = 5$) for control vessels.

Responses to various neuropeptides

Neuropeptide Y, peptide YY, vasoactive intestinal polypeptide, bombesin and substance P at concentrations of 10^{-7} and 10^{-6} M evoked no contractile response in the dog mesenteric artery ($n = 4$ for each peptide). Rather, vasoactive intestinal polypeptide slightly reduced the resting tone ($n = 4$).

Discussion

Electrical transmural stimulation produced a contraction in the dog isolated mesenteric artery. This contraction was abolished by guanethidine and by tetrodotoxin, suggesting that the response is sympathetic in origin. However, the response was not completely inhibited by prazosin. Prazosin acted as a competitive α_1-adrenoceptor antagonist in the dog mesenteric artery; the pA_2 value being much the same as those in other vessels (Agrawal *et al.*, 1984; Muramatsu *et al.*, 1984). Furthermore, the sensitivity of postjunctional α-adrenoceptors to noradrenaline was not different from those reported in other blood vessels (Sakakibara *et al.*, 1982). Thus, it is unlikely that the inability of prazosin to abolish the sympathetic response is due to atypical features of postsynaptic α-adrenoceptors of the mesenteric artery.

A contraction to electrical transmural stimulation was also observed in reserpine-treated dog isolated mesenteric arteries: prazosin failed to affect this res-

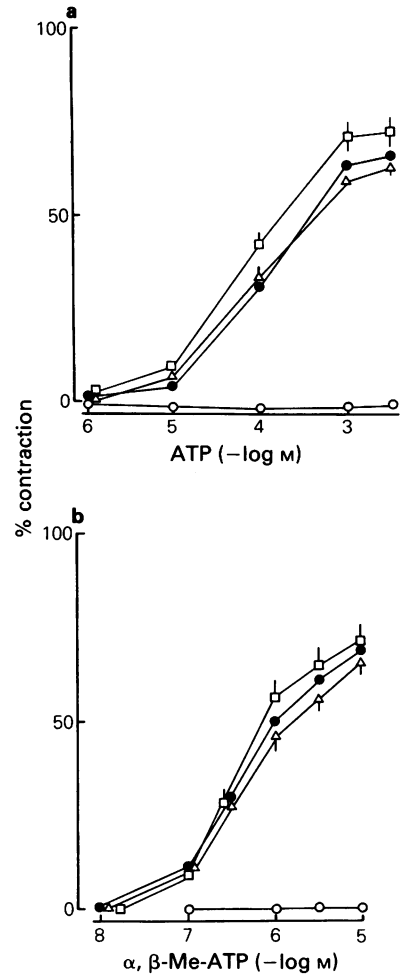

Figure 4 Concentration-response curves of ATP (a) and α,β-Me-ATP (b) in reserpine-treated and untreated dog mesenteric arteries, and the effects of 8-phenyltheophylline (10^{-5} M) and α,β-Me-ATP (5×10^{-6} M). Effects of 8-phenyltheophylline and α,β-Me-ATP were examined in reserpine-untreated arteries. The maximum contraction induced by noradrenaline (10^{-4} M) in each preparation was taken as 100%. (●) Control response in reserpine-untreated dog mesenteric arteries ($n = 12$); (△) response 20 min after treatment with 8-phenyltheophylline ($n = 6$); (○) response 20 min after treatment with α,β-Me-ATP ($n = 6$); (□) response in reserpine-treated dog mesenteric arteries ($n = 5$). Each value is the mean with s.e.mean shown by vertical lines. Other experimental conditions were the same as those in Figure 3.

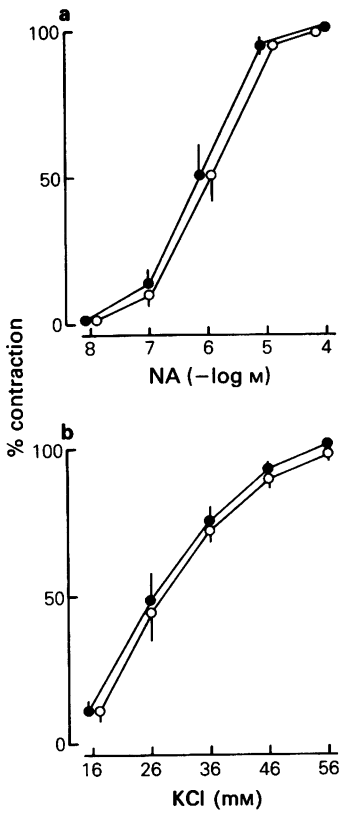

Figure 5 Effects of α,β-Me-ATP on the contractile responses to noradrenaline (NA) and KCl in the dog mesenteric arteries. The concentration-response curves of noradrenaline and KCl were recorded in the presence of propranolol (10^{-6} M) and guanethidine (3×10^{-6} M), respectively. (●) Control; (○) 20 min after treatment with α,β-Me-ATP (5×10^{-6} M). The maximum contraction induced by noradrenaline or KCl before treatment with α,β-Me-ATP was taken as 100%. Each value is the mean of 6 experiments with s.e.mean shown by vertical lines.

ponse significantly, thus showing an apparent lack of an adrenergic component in these reserpine-treated arteries. It has been demonstrated in other tissues that reserpine depletes 98% of noradrenaline from sympathetic nerves (Langer & Pinto, 1976; Suzuki *et al.*, 1984; Kügelgen & Starke, 1985). This lack of an adrenergic component is substantiated in this experiment by the fact that tyramine, which produced a contraction in control arteries, did not cause any response in reserpine-treated arteries. Tyramine often causes contraction of vessels by acting indirectly on sympathetic nerves to release noradrenaline which in turn acts postjunctionally to produce a vasoconstriction (Vanhoutte *et al.*, 1981), such a response is abolished or is far less potent in the reserpine-pretreated vessel (Lee *et al.*, 1981; Muramatsu *et al.*, 1984). This evidence suggests that in the dog mesenteric artery the prazosin-resistant contraction is caused by substance(s) other than noradrenaline.

Recently, it has been demonstrated that many neuropeptides coexist in sympathetic or other nerves together with classical transmitters (Hökfelt *et al.*, 1980; O'Donohue *et al.*, 1985). Neuropeptide Y is present in and released from the sympathetic nerve terminals, causing an α-adrenoceptor antagonist resistant vasoconstriction in the cat submandibular gland (Lundberg & Tatemoto, 1982) and cat spleen (Lundberg *et al.*, 1986). In order to examine a possible involvement of neuropeptides in the sympathetic response of the dog mesenteric artery, effects of several neuropeptides at relatively high concentrations were examined. However, all peptides tested (neuropeptide Y, peptide YY, vasoactive intestinal polypeptide, bombesin and substance P) did not cause any contractile response. Thus, it is likely that these peptides are not involved in the prazosin-resistant contraction in this artery, although it may well be that neuropeptide Y coexists in the sympathetic nerves along with noradrenaline and ATP and may have a modulatory role on neurotransmission (Haynes, 1986).

On the other hand, ATP and its stable analogue, α,β-Me-ATP, produced a potent contraction in the dog mesenteric artery. In a number of tissues the P_2-purinoceptor has been shown to be desensitized by persistent treatment with α,β-Me-ATP (Kasakov & Burnstock, 1983; Burnstock & Kennedy, 1985; Katsuragi & Furukawa, 1985). Likewise in the dog mesenteric artery the purine nucleotide-mediated contractile responses are abolished after desensitization of the P_2-purinoceptor with α,β-Me-ATP. On the other hand, the transient contractions of ATP and α,β-Me-ATP were not inhibited by guanethidine, prazosin or 8-phenyltheophylline (P_1-antagonist; Griffith *et al.*, 1981) nor were they reduced in the reserpine-treated artery. These results suggest that ATP and α,β-Me-ATP produced a contraction through the activation of postsynaptic P_2-purinoceptors in the dog mesenteric artery.

Wide occurrence of sympathetic purinergic response in blood vessels has been demonstrated with the use of α,β-Me-ATP (Sneddon & Burnstock, 1984; Kügelgen & Starke, 1985; Kennedy *et al.*, 1986; Muramatsu, 1986; Vidal *et al.*, 1986; Burnstock & Warland, 1987). The present results clearly show that the prazosin-resistant contraction of the dog mesenteric artery is abolished after treatment with α,β-Me-ATP both in reserpinized vessels and in prazosin-treated control vessels. This inhibition is probably due to the selective desensitization of P_2-purinoceptors, since the responses to noradrenaline, tyramine and KCl were not significantly affected after treatment with

α,β-Me-ATP. It has been shown that α,β-Me-ATP has little or no prejunctional action in the rabbit mesenteric artery (Kügelgen & Starke, 1985), rat tail artery (Vidal *et al.*, 1986) and guinea-pig and mouse vas deferens (Stjärne & Astrand, 1985; Westfall *et al.*, 1986).

In conclusion, the present study provides further evidence that the sympathetic contraction in the dog isolated mesenteric artery consists of adrenergic and purinergic components and that the purinergic response is mediated through postjunctional P_2-purinoceptors. Recent work has proved α,β-Me-ATP to be a potentially useful probe for analysing such purinergic transmission.

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References

AGRAWAL, D.K., TRIGGLE, C.R. & DANIEL, E.E. (1984). Pharmacological characterization of the postsynaptic alpha adrenoceptors in vascular smooth muscle from canine and rat mesenteric vascular beds. *J. Pharmac. exp. Ther.*, **229**, 831–838.

BURNSTOCK, G. & KENNEDY, C. (1985). Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen. Pharmac.*, **16**, 433–440.

BURNSTOCK, G. & WARLAND, J.J.I. (1987). A pharmacological study of the rabbit saphenous artery in vitro: a vessel with large purinergic contractile response to sympathetic nerve stimulation. *Br. J. Pharmac.*, **90**, 111–120.

FURCHGOTT, R.F. (1981). The requirement for endothelial cells in the relaxation of arteries by acetylcholine and some vasodilators. *Trends Pharmac Sci.*, **2**, 173–176.

GRIFFITH, S.G., MEGHJI, P., MOODY, C.J. & BURNSTOCK, G. (1981). 8-Phenyltheophylline: A potent P_1-purinoceptor antagonist. *Eur. J. Pharmac.*, **75**, 61–64.

HAYNES, L.W. (1986). Peptide neuroregulation of vascular tone by the sympathetic nervous system. *Trends Pharmac. Sci.*, **7**, 383–384.

HIRST, G.D.S. & NEILD, T.O. (1980). Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle. *Nature, Lond.*, **283**, 767–768.

HÖKFELT, T., JOHANSSON, O., LJUNGDAHL, Å., LUNDBERG, J.M. & SCHULTZBERG, M. (1980). Peptidergic neurones. *Nature, Lond.*, **284**, 515–521.

HOLMAN, M.E. & SURPRENANT, A. (1980). An electrophysiological analysis of the effects of noradrenaline and α-receptor antagonists on neuromuscular transmission in mammalian muscular arteries. *Br. J. Pharmac.*, **71**, 651–661.

KASAKOV, L. & BURNSTOCK, G. (1983). The use of the slowly degradable analog, α,β-methylene ATP, to produce desensitization of the P_2-purinoceptor: effect on non-adrenergic, non-cholinergic responses of the guinea-pig urinary bladder. *Eur. J. Pharmac.*, **86**, 291–294.

KATSURAGI, T. & FURUKAWA, T. (1985). Novel, selective purinoceptor antagonists: investigation of ATP as a neurotransmitter. *Trends Pharmac. Sci.*, **6**, 337–339.

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. Pharmac.*, **122**, 291–300.

KÜGELGEN, I.V. & STARKE, K. (1985). Noradrenaline and adenosine triphosphate as co-transmitters of neurogenic vasoconstriction in rabbit mesenteric artery. *J. Physiol.*, **367**, 435–455.

KURIYAMA, H. & MAKITA, Y. (1983). Modulation of noradrenergic transmission in the guinea-pig mesenteric artery: An electrophysiological study. *J. Physiol.*, **335**, 609–627.

LANGER, S.Z. & PINTO, J.E.B. (1976). Possible involvement of a transmitter different from norepinephrine in the residual responses to nerve stimulation of the cat nictitating membrane after pretreatment with reserpine. *J. Pharmac. exp. Ther.*, **196**, 697–713.

LEE, T.J.-F., ARAKI, H. & SU, C. (1981). Tyramine-induced constriction in rabbit basilar and ear arteries. *J. cardiovasc. Pharmac.*, **3**, 965–976.

LUNDBERG, J.M., FRIED, G., PERNOW, J., THEODORSSON-NORHEIM, E. & ÄNGGÅRD, A. (1986). NPY–a mediator of reserpine-resistant, non-adrenergic vasoconstriction in cat spleen after preganglionic denervation? *Acta physiol. scand.*, **126**, 151–152.

LUNDBERG, J.M. & TATEMOTO, K. (1982). Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to α-adrenoceptor blockade. *Acta physiol. scand.*, **116**, 393–402.

MURAMATSU, I. (1986). Evidence for sympathetic, purinergic transmission in the mesenteric artery of the dog. *Br. J. Pharmac.*, **87**, 478–480.

MURAMATSU, I., HOLLENBERG, M.D. & LEDERIS, K. (1986). Modulation by epidermal growth factor-urogastrone of contraction in isolated canine helical mesenteric arterial strips. *Can. J. Physiol. Pharmac.*, **64**, 1561–1565.

MURAMATSU, I., FUJIWARA, M., MIURA, A. & SAKAKIBARA, Y. (1981). Possible involvement of adenine nucleotides in sympathetic neuroeffector mechanisms of dog basilar artery. *J. Pharmac. exp. Ther.*, **216**, 401–409.

MURAMATSU, I., KIGOSHI, S. & OSHITA, M. (1984). Non-adrenergic nature of prazosin-resistant, sympathetic contraction in dog mesenteric artery. *J. Pharmac. exp. Ther.*, **229**, 532–538.

MURAMATSU, I., OSHITA, M. & YAMANAKA, K. (1983). Selective alpha-2 blocking action of DG-5128 in the dog mesenteric artery and rat vas deferens. *J. Pharmac. exp. Ther.*, **227**, 194–198.

O'DONOHUE, T.L., MILLINGTON, W.R., HANDELMANN, G.E., CONTRERAS, P.C. & CHRONWALL, B.M. (1985). On the 50th anniversary of Dale's law: multiple neurotransmitter neurons. *Trends Pharmac. Sci.*, **6**, 305–308.

SAKAKIBARA, Y., FUJIWARA, M. & MURAMATSU, I.

(1982). Pharmacological characterization of the alpha adrenoceptors of the dog basilar artery. *Naunyn-Schmiedebergs Arch. Pharmac.*, **319,** 1–7.

SNEDDON, P. & BURNSTOCK, G. (1984). ATP as a neurotransmitter in rat tail artery. *Eur. J. Pharmac.*, **106,** 149–152.

SNEDDON, P. & WESTFALL, D.P. (1984). Pharmacological evidence that adenosine triphosphate and noradrenaline are co-transmitters in the guinea-pig vas deferens. *J. Physiol.*, **347,** 561–580.

STJÄRNE, L. & ASTRAND, P. (1985). Relative pre- and postjunctional roles of noradrenaline and adenosine 5′-triphosphate as neurotransmitters of the sympathetic nerves of guinea-pig and mouse vas deferens. *Neuroscience*, **14,** 929–946.

SUZUKI, H., MISHIMA, S. & MIYAHARA, H. (1984). Effects of reserpine treatment on electrical responses induced by perivascular nerve stimulation in the rabbit ear artery. *Biomed. Res.*, **5,** 259–265.

VANHOUTTE, P.M., VERBEUREN, T.J. & WEBB, R.C. (1981). Local modulation of adrenergic neuroeffector interaction in the blood vessel wall. *Physiol. Rev.*, **61,** 151–247.

VIDAL, M., HICKS, P.E. & LANGER, S.Z. (1986). Differential effects of α,β-methylene ATP on responses to nerve stimulation in SHR and WKY tail arteries. *Naunyn-Schmiedebergs Arch. Pharmac.*, **332,** 384–390.

WESTFALL, D.P., BJUR, R.A. & SEDAA, K. (1986). Effects of α,β-methylene ATP on release of norepinephrine from guinea-pig vas deferens. *Fedn Proc.*, **45,** 801.

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