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COMPARISON BETWEEN SYMPATHETIC ADRENERGIC AND PURINERGIC TRANSMISSION IN THE DOG MESENTERIC ARTERY

BY IKUNOBU MURAMATSU, TSUYOSHI OHMURA AND MASAFUMI OSHITA

From the Department of Pharmacology, Fukui Medical School, Matsuoka, Fukui 910–11, Japan

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SUMMARY

1. Electrical transmural stimulation evoked a sympathetic contraction in the isolated dog mesenteric artery. This contraction consisted of adrenergic and purinergic components, which were separately observed under conditions where postjunctional P_2 purinoceptors were desensitized with α,β-methylene ATP or postjunctional α_1 adrenoceptors were blocked by prazosin, respectively.

2. The purinergic component was transient and developed immediately after the start of stimulation and declined rapidly after a peak. In contrast, the adrenergic contraction slowly developed and lasted longer than the purinergic component. Thus, the purinergic and adrenergic components predominantly contributed to the early and later phases of the total sympathetic response, respectively.

3. The peak amplitudes of contraction of both components were similar at 1 Hz, but the adrenergic component occurred more dominantly in the responses to higher frequency stimulation.

4. Cocaine, a neuronal uptake inhibitor of noradrenaline, potentiated the adrenergic component but attenuated the purinergic component. This attenuation was reversed by DG-5128, a prejunctional α_2 adrenoceptor antagonist. Treatment with DG-5128 alone augmented both adrenergic and purinergic components.

5. 8-Phenyltheophylline, a P_1 purinoceptor antagonist, potentiated the purinergic component without affecting the adrenergic component.

6. Exogenous noradrenaline produced a sustained contraction, which was potentiated by cocaine and was competitively inhibited by prazosin. α,β-Methylene ATP and 8-phenyltheophylline had no effect on the response to noradrenaline. Exogenous ATP produced a transient contraction, which was abolished under conditions where postjunctional P_2 purinoceptors were desensitized with α,β-methylene ATP. 8-Phenyltheophylline potentiated but cocaine or prazosin did not affect the ATP response.

7. Electrical stimulation produced an increase in ^3H efflux from the sympathetic nerve terminals in the mesenteric arteries pre-incubated with [^3H]noradrenaline. The evoked efflux was significantly augmented by cocaine or DG-5128 and was inhibited by guanethidine or tetrodotoxin. α,β-Methylene ATP and 8-phenyltheophylline were without effect. Adenosine reduced the ^3H efflux and the inhibition was suppressed by 8-phenyltheophylline.

8. These results suggest that sympathetic contraction of the dog mesenteric artery is caused through not only adrenergic but also purinergic mechanisms, and that both the sympathetic transmissions are predominantly modulated through prejunctional adrenergic mechanisms.

INTRODUCTION

Blood vessels are innervated by sympathetic nerves and the muscle tonus is regulated by noradrenaline released from the nerve terminals. However, there is recent evidence that the sympathetic responses of some blood vessels are caused by not only noradrenaline but also other substances which may be released concomitantly with noradrenaline (Ganten, Lang, Archelos & Unger, 1984; Haynes, 1986; Burnstock, 1988).

In the dog mesenteric artery, α adrenoceptor antagonists fail to inhibit completely the sympathetic contraction induced by perivascular nerve stimulation (Muramatsu, Kigoshi & Oshita, 1984) and the resistant component is abolished after desensitization of postjunctional P_2 purinoceptors using α,β-methylene ATP, thus suggesting that the sympathetic contraction consists of adrenergic and purinergic components (Muramatsu, 1986, 1987; Machaly, Dalziel & Sneddon, 1988). Such purinergic responses have also been demonstrated in other blood vessels (Muramatsu, Fujiwara, Miura & Sakakibara, 1981; Sneddon & Burnstock, 1984*a*; Kügelgen & Starke, 1985; Kennedy, Saville & Burnstock, 1986; Burnstock & Warland, 1987; Muramatsu & Kigoshi, 1987), vas deferens (Fedan, Hogaboom, O'Donnell, Colby & Westfall, 1981; Sneddon & Burnstock, 1984*b*; Sneddon & Westfall, 1984), urinary bladder (Kasakov & Burnstock, 1983; Theobald, 1983; Westfall, Fedan, Colby, Hogaboom & O'Donnell, 1983) and seminal vesicle (Meldrum & Burnstock, 1985).

Sympathetic adrenergic transmission is influenced by complex factors. For example, release of noradrenaline is negatively regulated by feed-back mechanisms mediated through prejunctional α_2 adrenoceptors, and the released noradrenaline is largely reabsorbed into the nerve terminals (Langer, 1974; Starke, 1977; Vanhoutte, Verbeuren & Webb, 1981). However, virtually nothing is known about feed-back mechanisms of the sympathetic co-transmitter ATP and its interactions with noradrenaline. In one report on prejunctional action in the vas deferens, ATP, from stimulated nerve terminals, was shown to act back on prejunctional P_1 purinoceptors and to modify ATP release (Sneddon, Meldrum & Burnstock, 1984). In the present study, we compared the sympathetic adrenergic and purinergic transmission in the dog mesenteric artery and assessed how both transmitters contribute to the total sympathetic response.

METHODS

Dogs of either sex, weighing 8–15 kg, were anaesthetized with thiopental sodium (20 mg/kg, I.V.), exsanguinated from the common carotid arteries, and the mesenteric arteries were isolated. The vessels were helically cut, approximately 2 mm in width and 15 mm in length, under a dissecting microscope and the endothelial cells were removed by rubbing them with filter paper. The strips were 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; NaH_2PO_4, 1·2; glucose, 11·5. To block the β adrenoceptors, 10^{-6} M-propranol 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. A resting tension of 0·5 g was applied and the responses were recorded isometrically through force-displacement transducers. All preparations were equilibrated for 90 min before starting the experiments. Drugs were directly added to the bath. Cumulative concentration–response curves were determined by the stepwise increases in the concentration of an agonist by a factor 3 or 10, as soon as a steady response to the previous administration had been achieved. Lack of possible involvement of endothelium-derived factors in the mechanical response was confirmed by the lack of a relaxant response to acetylcholine (10^{-6} M) in noradrenaline (3×10^{-7} M)-precontracted arteries (Muramatsu, Hollenberg & Lederis, 1986).

In order to desensitize postjunctional P_2 purinoceptors (Kasakov & Burnstock, 1983), the tissues were treated with 5×10^{-6} M-α,β-methylene ATP 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, 1987). In this case, the preparation was placed in parallel between the electrodes. The distance between the electrodes was approximately 2 mm. Stimulus parameters were 0·3 ms in duration and supramaximal voltage (10 V). A total of fifty pulses were delivered at various frequencies, unless mentioned otherwise.

The release of [^{3}H]noradrenaline was determined according to the method of Muramatsu *et al.* (1981). The mesenteric arterial strips were pre-incubated with 2×10^{-7} M-[^{3}H]noradrenaline in Krebs–Henseleit solution containing $5{\cdot}7 \times 10^{-4}$ M-ascorbic acid for 90 min at 37 °C. The strips were then suspended between a pair of parallel platinum wire electrodes under 0·5 g of tension. The Krebs–Henseleit solution containing $5{\cdot}7 \times 10^{-4}$ M-ascorbic acid was bubbled with 95% O_2 and 5% CO_2 at 37 °C and was superfused using a peristaltic pump at a flow rate of 1 ml/min. Before the start of the experiments, each strip was equilibrated for 90 min, after which electrical stimulation was applied through a pair of electrodes. Stimulus parameters were of a frequency of 10 Hz, duration of 0·1 ms and supramaximal voltage (7·5 V) for 5 s. Superfusate solution was collected every 1 min and the radioactivity was determined by counting in a Packard Tri-Carb liquid scintillation spectrometer. The increase in ^{3}H efflux induced by electrical stimulation was calculated to be the net ^{3}H efflux by stimulation. The drugs to be tested were superfused 10 min before the initiation of electrical stimulation.

The following drugs were used: lithium salt of α,β-methylene ATP (Sigma, MO, USA), L-noradrenaline bitartrate, D,L-propranolol hydrochloride (Nakarai, Kyoto, Japan), guanethidine sulphate (Tokyo-Kasei, Tokyo, Japan), tetrodotoxin (Sankyo, Tokyo, Japan), DG-5128 (Daiichi Seiyaku, Tokyo, Japan), prazosin (Taito-Pfizer, Tokyo, Japan), 8-phenyltheophylline (Calbiochem, CA, USA), cocaine hydrochloride (Takeda, Osaka, Japan), L-[ring-2,5,6-^{3}H]noradrenaline (43·7 Ci/mmol, NEN Research Product, Boston, MA, USA)

Experimental values are given as mean ± S.E.M. The effects of drugs were considered significant if $P < 0{\cdot}05$ by Student's t test for unpaired data.

RESULTS

Separation of sympathetic contraction into adrenergic and purinergic components by prazosin and α,β-methylene ATP

Electrical transmural stimulation (fifty pulses) elicited a transient contraction in the dog mesenteric artery. This contraction was abolished by 3×10^{-6} M-guanethidine or 10^{-7} M-tetrodotoxin, suggesting that the response is sympathetic in origin. Figure 1 shows the effects of 10^{-7} M-prazosin and 5×10^{-6} M-α,β-methylene ATP on the responses evoked at 1 and 10 Hz. The contractile responses before and after treatment with each drug were superimposed. The sympathetic contraction was partially attenuated by each drug and was abolished after the combined treatments. As described in later sections, prazosin at the concentration used selectively inhibited noradrenaline-induced contraction, and continuous treatment with α,β-methylene

ATP abolished ATP-induced contraction. Furthermore, prazosin and α,β-methylene ATP failed to affect [^{3}H]noradrenaline release from the sympathetic nerve terminals. Therefore, we designated the resistant contractions after treatment with prazosin or α,β-methylene ATP as 'purinergic' or 'adrenergic' component, respectively.

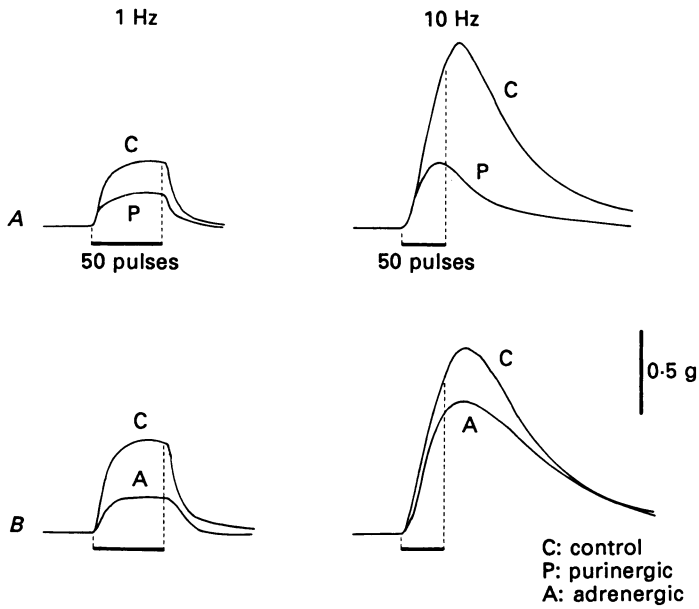

Fig. 1. Time courses of purinergic and adrenergic components in sympathetic contractions of dog mesenteric arteries evoked by electrical transmural stimulation. The arteries were electrically stimulated at 1 Hz for 50 s or at 10 Hz for 5 s. C, control response recorded before treatment with drugs. P, purinergic component recorded after treatment with 5×10^{-7} M-prazosin. A, adrenergic component recorded after treatment with 5×10^{-6} M-α,β-methylene ATP. Propranolol (10^{-6} M) was present throughout the experiments.

The purinergic contraction immediately developed after the start of stimulation; thus, the initial phase overlapped the early phase of the total (control) response recorded before treatment with prazosin (Fig. 1*A*). With the stimulation at 10 Hz for 5 s, the purinergic component reached a peak rapidly and then declined even though the stimulation still continued (Fig. 1*A* right and Table 1). In contrast, the adrenergic contraction developed slowly, with a tendency to become more pronounced in the responses to high-frequency stimulation. Thus, at 10 Hz the time to peak contraction was significantly longer than the purinergic component (Fig. 1*B* and Table 1). Frequency– or pulse number–response relationships (Figs 2 and 3) revealed the following points: (1) peak amplitudes of purinergic and adrenergic components were similar at low frequencies, but (2) the adrenergic component became greater than the purinergic component at higher frequencies, and (3) the second point resulted from less dependence of the purinergic component on frequency or pulse number; that is, the purinergic component reached a peak and then declined rapidly. Therefore, it was likely that the later phase of total (control)

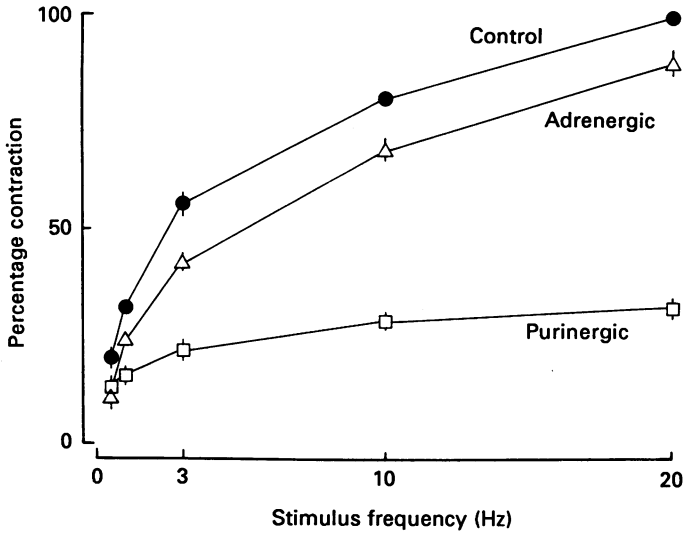

Fig. 2. Relation between stimulus frequency and sympathetic contractions in the dog mesenteric arteries. Electrical transmural stimulation (fifty pulses) was applied at the frequencies stated in the abscissa and each response was normalized against the maximum contraction evoked at 20 Hz before treatment with drugs in each preparation. Each value is the mean ± S.E.M. of six to ten experiments. The other experimental conditions are the same as in Fig. 1.

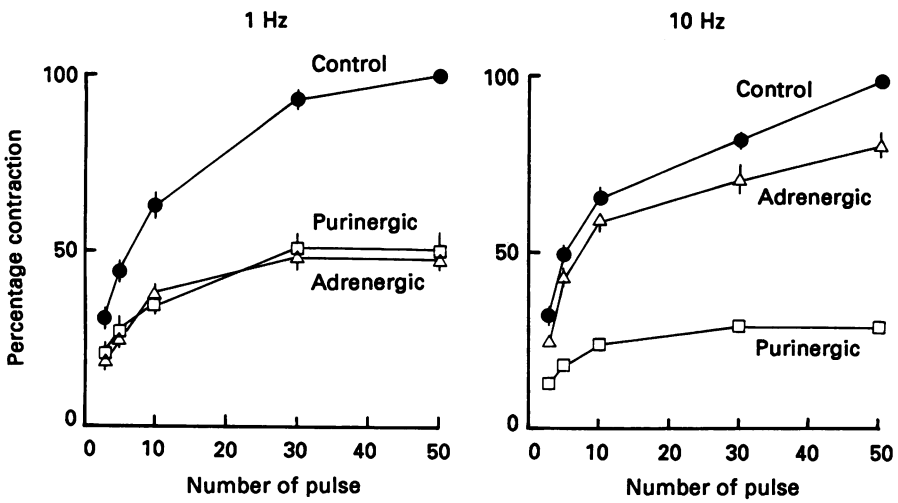

Fig. 3. Relation between stimulus pulse number and sympathetic contraction in the dog mesenteric arteries. The mesenteric arteries were electrically stimulated at 1 or 10 Hz and the responses were normalized against the response evoked with fifty pulses before treatment with drugs in each preparation. Mean ± S.E.M. of six to twelve experiments. The other experimental conditions are the same as those in Fig. 1.

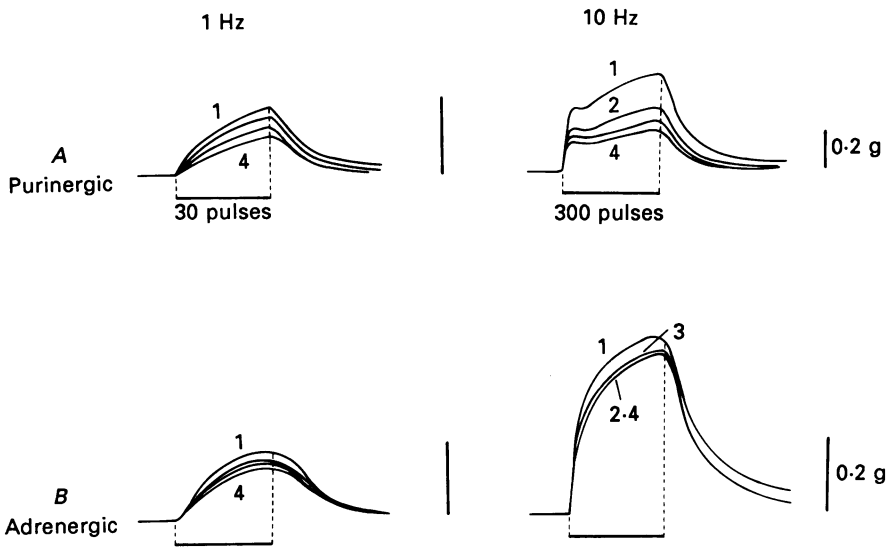

Fig. 4. Purinergic and adrenergic responses induced by repeated application of electrical stimulation. Four trains of stimuli (1 or 10 Hz for 30 s) were applied at intervals of 1·5 min and the responses were superimposed. Purinergic (*A*) and adrenergic (*B*) responses were recorded after treatment with 10^{-7} M-prazosin or 5×10^{-6} M-α,β-methylene ATP, respectively. Numbers on the trace represent the order of trials.

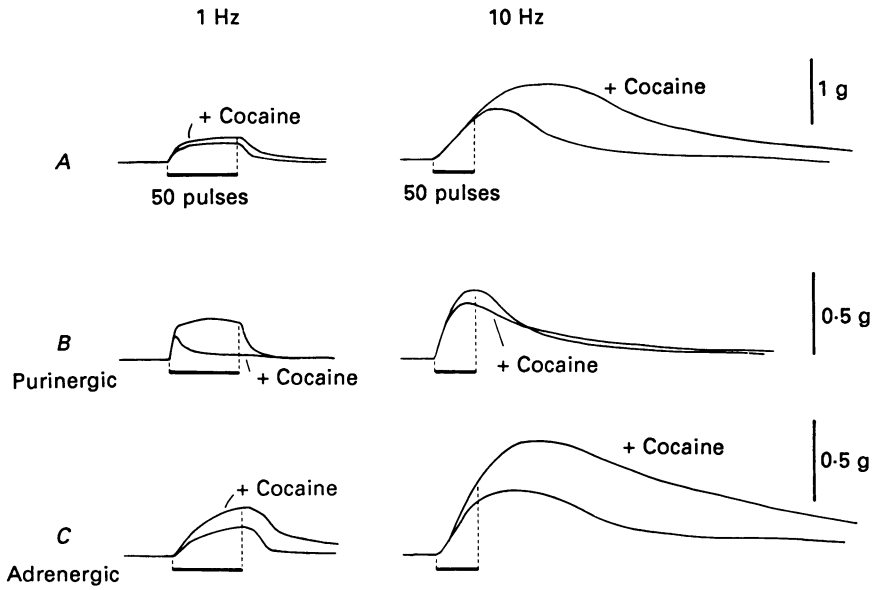

Fig. 5. Effects of cocaine (3×10^{-6} M) on the sympathetic contractions of the dog mesenteric arteries evoked by electrical transmural stimulation. The arteries were electrically stimulated at 1 Hz for 50 s or at 10 Hz for 5 s. *A*, control response in the absence of prazosin and α,β-methylene ATP. *B*, purinergic response recorded under treatment with 10^{-7} M-prazosin. *C*, adrenergic response recorded under treatment with 5×10^{-6} M-α,β-methylene ATP.

TABLE 1. Effects of drugs on the sympathetic contractions induced by electrical transmural stimulation (0·3 ms pulse width, fifty pulses at 1 or 10 Hz) in dog mesenteric arteries

	Time to peak* (s)		50% duration† (s)		Amplitude‡ (%)	
Treatments	1 Hz	10 Hz	1 Hz	10 Hz	1 Hz	10 Hz
None	44·9 ± 1·3 (41)	7·9 ± 0·2 (40)	51·2 ± 0·5 (41)	12·6 ± 0·5 (40)	100	100
Prazosin (10^{-7} M)	39·5 ± 2·7 (13)	5·3 ± 0·2§ (13)	59·2 ± 1·9 (13)	14·2 ± 1·4 (13)	42 ± 6§ (13)	31 ± 2§ (13)
α,β-Methylene ATP (5×10^{-6} M)	39·5 ± 2·4 (12)	8·1 ± 0·2 (12)	54·8 ± 0·9 (12)	14·9 ± 0·6 (12)	53 ± 4§ (12)	80 ± 2§ (12)
Cocaine (3×10^{-6} M)	46·4 ± 3·4 (11)	15·6 ± 1·4§ (10)	55·2 ± 1·1 (11)	27·3 ± 2·2§ (10)	131 ± 11 (11)	156 ± 6§ (10)
DG-5128 (10^{-5} M)	42·6 ± 2·0 (8)	10·5 ± 0·6§ (7)	49·4 ± 0·2 (8)	15·1 ± 0·7§ (7)	331 ± 29§ (8)	147 ± 5§ (7)
8-Phenyltheophylline (10^{-5} M)	48·6 ± 1·2 (8)	7·6 ± 0·2 (8)	53·1 ± 1·4 (8)	14·8 ± 0·8 (8)	108 ± 12 (8)	99 ± 3 (8)

* Time to peak contraction after the start of stimulation.
† Duration at half-maximum contraction.
‡ The peak amplitude of contraction compared with the control response.
§ Significantly different from the control ($P < 0·05$).
Number in parentheses represents the number of experiments.

response at high frequencies mainly reflected the adrenergic component (Fig. 1 and Table 1).

The sympathetic contractions were reproducible when electrical stimulation was applied at intervals of 10 min or more. At shorter intervals, however, the purinergic and adrenergic components showed different reproducibility. In Fig. 4, 30 s trains

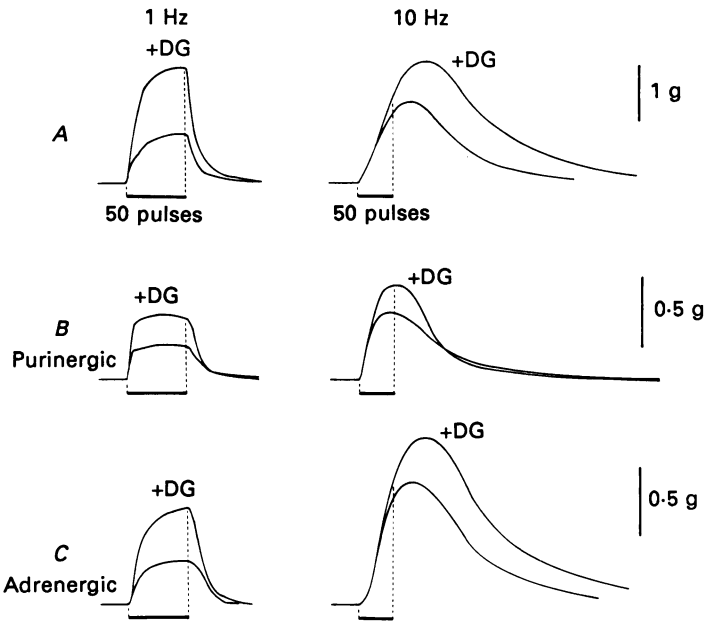

Fig. 6. Effects of DG-5128 (10^{-5} M) on the sympathetic contractions of the dog mesenteric arteries evoked by electrical transmural stimulation. The experimental conditions are the same as those in Fig. 5.

of stimulation at 1 or 10 Hz were applied four times at intervals of 1·5 min. The purinergic component gradually reduced upon repeated stimulation, the amplitudes at the fourth trial being 57 ± 4% at 1 Hz and 48 ± 1% at 10 Hz of the corresponding first responses (five experiments). On the other hand, reduction of the adrenergic component was small or negligible (82 ± 2% or 94 ± 4% of the first response at 1 or 10 Hz, respectively, five experiments).

Effects of various drugs on sympathetic contractions

Cocaine

Cocaine (3×10^{-6} M) significantly potentiated the adrenergic component and prolonged the time to peak contraction and duration (Fig. 5*C* and Table 2). On the other hand, the purinergic component was inhibited by cocaine. The extent of inhibition was greater at lower frequencies of stimulation; at 1 Hz the purinergic contraction was not maintained during the stimulation period, resulting in a monophasic contraction (Fig. 5*B*). Total sympathetic response showed complex behaviour in response to cocaine. At 1 Hz, cocaine potentiated the total response in

TABLE 2. Effects of drugs on the adrenergic contractions induced by electrical transmural stimulation (0·3 ms pulse width, fifty pulses at 1 or 10 Hz) in dog mesenteric arteries treated with 5×10^{-6} M-α,β-methylene ATP

	Time to peak* (s)		50% duration† (s)		Amplitude‡ (%)	
Treatments	1 Hz	10 Hz	1 Hz	10 Hz	1 Hz	10 Hz
None	40·3 ± 1·8 (19)	8·6 ± 0·3 (19)	53·8 ± 0·8 (19)	17·6 ± 1·1 (19)	100	100
Cocaine (3×10^{-6} M)	44·2 ± 4·2 (7)	13·3 ± 0·6§ (7)	59·8 ± 1·3§ (7)	27·5 ± 2·0§ (7)	184 ± 10§ (7)	188 ± 7§ (7)
DG-5128 (10^{-5} M)	41·3 ± 3·9 (5)	10·8 ± 0·6 (5)	51·5 ± 1·0 (5)	17·9 ± 1·6 (5)	321 ± 23§ (5)	153 ± 7§ (7)
Cocaine (3×10^{-6} M) + DG-5128 (10^{-5} M)	49·3 ± 0·8§ (9)	14·2 ± 0·6§ (9)	58·7 ± 3·3 (9)	31·2 ± 2·2§ (9)	532 ± 46§ (9)	209 ± 8§ (9)
8-Phenyltheophylline (10^{-5} M)	43·7 ± 2·3 (7)	9·7 ± 0·5 (7)	52·6 ± 1·4 (7)	23·1 ± 2·1 (7)	97 ± 5 (7)	90 ± 4 (7)

* Time to peak contraction after the start of stimulation.
† Duration at half-maximum contraction.
‡ The peak amplitude of contraction compared with the control response.
§ Significantly different from the control ($P < 0·05$).
Number in parentheses represents the number of experiments.

TABLE 3. Effects of drugs on the purinergic contractions induced by electrical transmural stimulation (0·3 ms pulse width, fifty pulses at 1 or 10 Hz) in dog mesenteric arteries treated with 10^{-7} M-prazosin

	Time to peak* (s)		50% duration† (s)		Amplitude‡ (%)	
Treatments	1 Hz	10 Hz	1 Hz	10 Hz	1 Hz	10 Hz
None	42·5 ± 1·9 (18)	5·5 ± 0·2 (18)	56·7 ± 1·8 (18)	14·1 ± 1·1 (18)	100	100
Cocaine (3×10^{-6} M)	4·6 ± 0·2§ (5)	4·1 ± 0·3§ (6)	24·3 ± 8·0§ (5)	18·5 ± 3·6 (6)	45 ± 7§ (5)	82 ± 3§ (6)
DG-5128 (10^{-5} M)	39·3 ± 5·7 (5)	5·5 ± 1·9 (5)	53·8 ± 1·9 (5)	10·4 ± 2·2 (5)	181 ± 9§ (5)	149 ± 7§ (5)
Cocaine (3×10^{-6} M) + DG-5128 (10^{-5} M)	15·2 ± 6·6§ (6)	4·9 ± 0·1 (6)	55·9 ± 3·6 (6)	11·2 ± 1·3 (6)	306 ± 28§ (6)	215 ± 49§ (6)
8-Phenyltheophylline (10^{-5} M)	49·4 ± 1·7 (5)	6·8 ± 0·2 (5)	60·6 ± 3·3 (5)	18·4 ± 0·7§ (5)	185 ± 22§ (5)	130 ± 12§ (5)

* Time to peak contraction after the start of stimulation.
† Duration at half-maximum contraction.
‡ The peak amplitude of contraction compared with the control response.
§ Significantly different from the control ($P < 0·05$).
Number in parentheses represents the number of experiments.

eight out of eleven preparations while it attenuated the amplitude in the remaining three preparations. At 10 Hz, an increase in the contractile amplitude and a prolongation of time to peak and duration were produced in all preparations tested (Table 1).

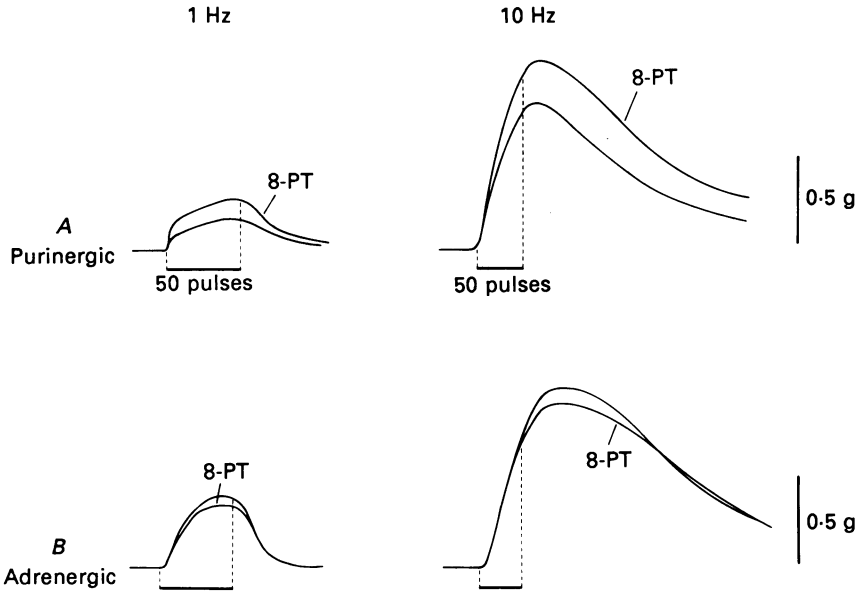

Fig. 7. Effects of 8-phenyltheophylline (8-PT, 10^{-5} M) on the purinergic and adrenergic contractions of the dog mesenteric arteries evoked by electrical stimulation. The experimental conditions are the same as those in Fig. 5.

DG-5128

We previously demonstrated in the dog mesenteric artery that DG-5128 selectively inhibits prejunctional α_2 adrenoceptors without affecting the α_1 adrenoceptors, resulting in an augmentation of sympathetic contraction (Muramatsu, Oshita & Yamanaka 1983). In the present experiments, such a potentiating effect was produced in each component of sympathetic contraction (Fig. 6). The extent of potentiation was greater in the responses at 1 Hz than at 10 Hz (Tables 1, 2 and 3). Combined treatments with DG-5128 and cocaine also potentiated adrenergic and purinergic contractions; thus the inhibitory effect of cocaine on the purinergic component was not observed.

8-*Phenyltheophylline*

8-Phenyltheophylline (10^{-5} M) had no large effect on the time course and amplitude of total or adrenergic contractions (Tables 1 and 2, Fig. 7*B*). However, the drug enhanced the purinergic component; the potentiation was somewhat larger at lower frequencies of stimulation (Table 3, Fig. 7).

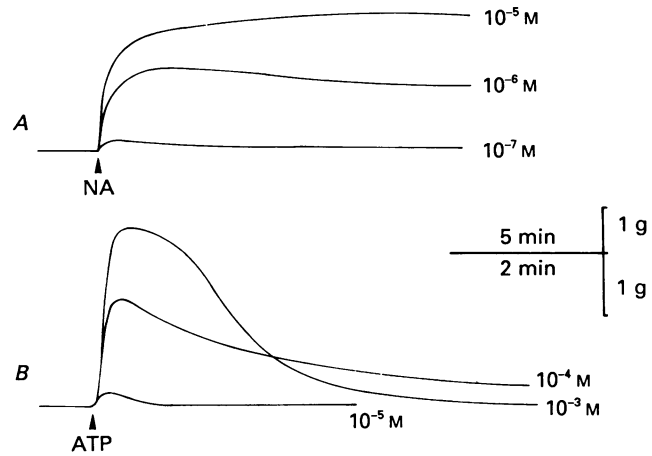

Fig. 8. Contractile responses induced by noradrenaline (NA) and ATP in the dog mesenteric arteries. Each drug was non-cumulatively applied and the responses were superimposed.

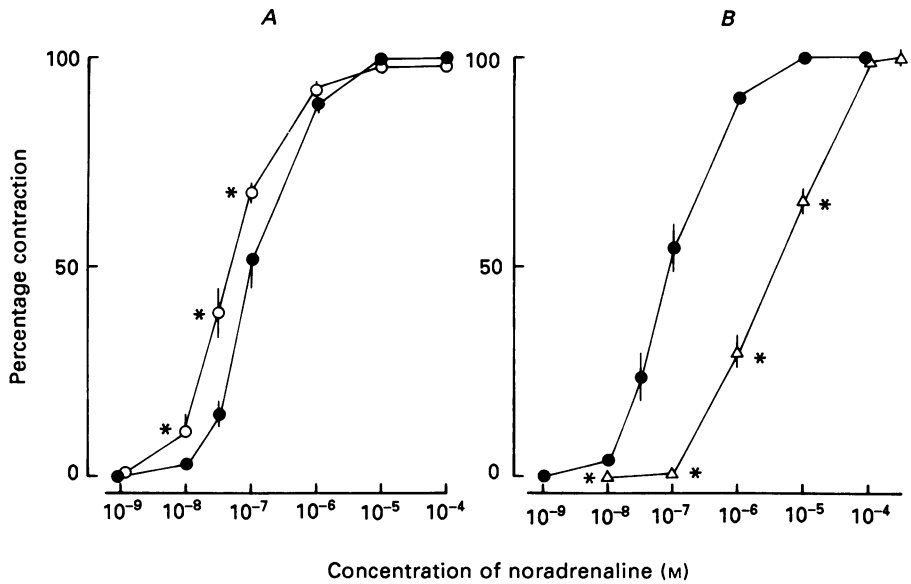

Fig. 9. Effects of cocaine (3×10^{-6} M, *A*) and prazosin (10^{-7} M, *B*) on the contractile responses to noradrenaline. Noradrenaline was applied cumulatively and the maximum contraction before treatment with drug was taken as 100%. Mean ± S.E.M. of six experiments. * Significantly different from the control, $P < 0.05$. ●, control, ○ 15 min after cocaine; △, 30 min after prazosin.

Responses to noradrenaline and ATP

Noradrenaline at concentrations over 10^{-8} M evoked a concentration-dependent contraction. This contraction lasted for more than 20 min if the bath medium was not exchanged with the drug-free medium (Fig. 8). Prazosin competitively

antagonized the noradrenaline response; the pA_2 value being 8·3 ± 0·1 (six experiments, Fig. 9*B*). Cocaine at 3×10^{-6} M augmented the contractile responses to low concentrations of noradrenaline (Fig. 9*A*). α,β-Methylene ATP (5×10^{-6} M), DG-5128 (10^{-5} M) and 8-phenyltheophylline (10^{-5} M) were without effect on the noradrenaline response (five experiments for each drug).

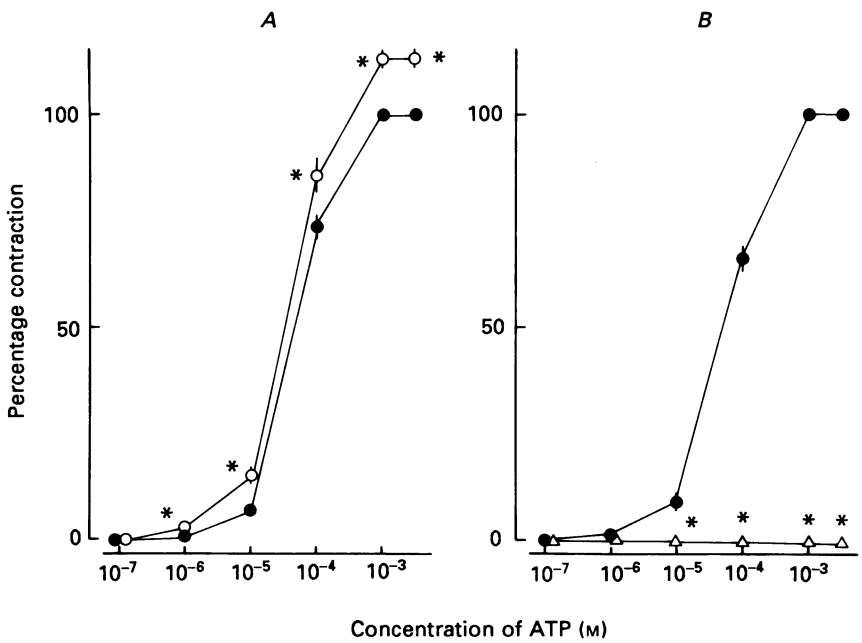

Fig. 10. Effects of 8-phenyltheophylline (10^{-5} M, *A*) and α,β-methylene ATP (5×10^{-6} M, *B*) on the contractile response to ATP. ATP was cumulatively applied and the maximum contraction before treatment with drug was taken as 100%. Mean ± S.E.M. of five experiments. ●, control; ○, 15 min after 8-phenyltheophylline; △, 15 min after α,β-methylene ATP.

ATP likewise produced a concentration-dependent contraction, but the time course of response was much faster and more transient (Fig. 8*B*). The contractile action of ATP did not occur in the preparations treated with 5×10^{-6} M-α,β-methylene ATP (Fig. 10*B*). 8-Phenyltheophylline (10^{-5} M) potentiated the ATP response (Fig. 10*A*), but prazosin (10^{-5} M), cocaine (3×10^{-6} M) and DG-5128 (10^{-5} M) were without effect (five experiments for each drug).

Effects of various drugs on [^{3}H]noradrenaline release

Electrical stimulation (10 Hz for 5 s) elicited an increase in ^{3}H efflux from the arteries pre-incubated with [^{3}H]noradrenaline. This efflux was inhibited or abolished by 3×10^{-6} M-guanethidine or 10^{-7} M-tetrodotoxin. Cocaine (3×10^{-6} M) and DG-5128 (10^{-5} M) increased the ^{3}H efflux to more than two times the control. Adenosine

(10^{-6} M) inhibited the ^3H efflux, which was completely antagonized by 10^{-5} M-8-phenyltheophylline. However, 8-phenyltheophylline itself was without effect. Also, α,β-methylene ATP (5×10^{-6} M, 3 or 15 min treatment) did not affect the ^3H efflux. These results are summarized in Table 4.

TABLE 4. Effects of various drugs on ^3H efflux induced by electrical transmural stimulation in dog mesenteric arteries

Treatments	^3H efflux (% of the first response)*
None	97 ± 4 (8)
Prazosin (10^{-7} M)	121 ± 12 (5)
Cocaine (3×10^{-6} M)	230 ± 29† (4)
DG-5128 (10^{-5} M)	214 ± 18† (4)
8-Phenyltheophylline (10^{-5} M)	100 ± 10 (4)
Adenosine (10^{-6} M)	64 ± 7† (4)
8-Phenyltheophylline (10^{-5} M) + adenosine (10^{-6} M)	111 ± 8 (4)
α,β-Methylene ATP (5×10^{-6} M) 3 min	90 ± 10 (4)
15 min	91 ± 6 (8)

* The preparations were stimulated twice electrically (10 Hz, 5 s), and the ^3H efflux induced by the second stimulation was compared with the efflux induced by the first stimulation. Each drug was treated for 10 min before and during the second stimulation, but α,β-methylene ATP was treated for 3 or 15 min.

† Significantly different from the value without treatment (none) (unpaired t test, $P < 0.05$). Numbers in parentheses represent the number of experiments.

DISCUSSION

As shown previously (Muramatsu, 1986, 1987; Machaly *et al.* 1988), sympathetic contraction of the dog mesenteric artery was separated into adrenergic and purinergic components, the former by desensitizing the postjunctional P_2 purinoceptors with α,β-methylene ATP (Kasakov & Burnstock, 1983) and the latter by blocking the postjunctional α_1 adrenoceptors with prazosin (Muramatsu, 1987). Since neither α,β-methylene ATP nor prazosin had any significant prejunctional effect, we considered that the residual response after treatment with each drug reflected the response pattern of the individual components.

Analyses of the time course and frequency–response curves of each component revealed the following points: (1) the purinergic component develops more rapidly than the adrenergic component, (2) the purinergic contraction is transient while the adrenergic contraction lasts longer, and (3) in contrast to the purinergic component, the adrenergic component becomes greater at higher stimulus frequencies. From these results, we can speculate that the relationship of each of the components to the total sympathetic contraction is as follows: the purinergic component is involved more dominantly in the early phase of the total response, while the adrenergic component mainly contributes to the latter phase. Figure 11 shows the time course of each of the components at low and high frequencies.

Rapid and transient responsiveness of the purinergic contraction has been

reported in many tissues where purinergic transmission has been demonstrated (blood vessels: Kügelgen & Starke, 1985; Kennedy *et al.* 1986; Burnstock & Warland, 1987; Muramatsu & Kigoshi, 1987; vas deferens: Fedan *et al.* 1981; Sneddon & Burnstock, 1984*b*; Allcorn, Cunnane & Kirkpatrick, 1986), suggesting that such responsiveness is a common feature of purinergic contraction. Since a

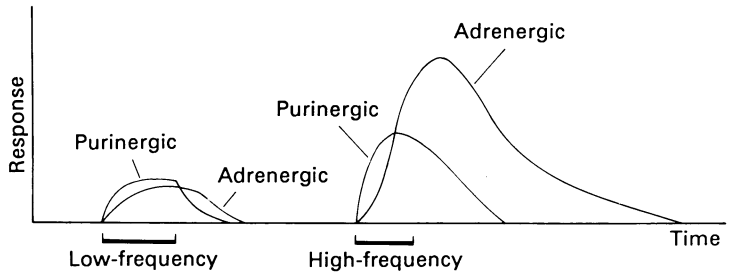

Fig. 11. Schematic comparison of the sympathetic purinergic and adrenergic contractions evoked by electrical stimulation at low and high frequencies.

purinergic transmitter, ATP, produced a rapid and transient contraction and mimicked the purinergic response, it is likely that this feature may reflect the postjunctional rather than prejunctional mechanisms.

Lundberg & Tatemoto (1982) have demonstrated that the sympathetic regulation of blood flow in the cat submandibular gland is mediated by noradrenaline and neuropeptide Y (NPY) concomitantly released from the sympathetic nerve. Furthermore, they have suggested that the peptidergic response develops more slowly than the adrenergic response and that the peptidergic regulation becomes more significant at higher frequencies of stimulation (Lundberg & Hökfelt, 1983). These features are apparently in contrast to the fast, purinergic response mentioned above (Fig. 11). Unfortunately, no peptidergic contraction could be produced by electrical stimulation or by exogenously applied NPY in the dog mesenteric artery (Muramatsu, 1987). However, if sympathetic contraction was caused by three substances (noradrenaline, ATP and NPY) which would co-exist in and be released from the nerve terminals, one would expect each component to be involved in total sympathetic contraction in the following sequence: purinergic, adrenergic and then peptidergic.

Adrenergic transmission has been studied extensively. Released noradrenaline acts on not only postjunctional α adrenoceptors but also prejunctional α_2 adrenoceptors, the latter reducing the release of noradrenaline itself (Langer, 1974; Starke, 1977). A large proportion of noradrenaline released is reabsorbed into the sympathetic nerve terminals (Vanhoutte *et al.* 1981). Such mechanisms did function in the dog mesenteric artery, as cocaine (an inhibitor of noradrenaline reuptake) and DG-5128 (an antagonist for prejunctional α_2 adrenoceptors; Muramatsu *et al.* 1983) potentiated the adrenergic contraction with an increase in [^{3}H]noradrenaline release.

If ATP or a related purine was concomitantly released with noradrenaline from the sympathetic nerve terminals, one might expect purinergic transmission to also be

influenced by the drugs mentioned above in the same way as the adrenergic transmission. In fact, DG-5128 potentiated the purinergic contraction as well as the adrenergic response. However, cocaine attenuated the purinergic contraction in contrast to the potentiation of the adrenergic response. This discrepancy is not unfavourable to the co-transmitter hypothesis; the attenuation of the purinergic response is probably related to a reduction of transmitter release which would be the resultant effect of a more extensive activation of prejunctional α_2 adrenoceptors under treatment with cocaine. In fact, the inhibitory effect of cocaine on purinergic contraction was reversed by DG-5128. Thus, it is likely that the release of purinergic transmitter is also regulated adrenergically in the same manner as is the release of noradrenaline. This result further supports the adrenergic and purinergic co-transmitter hypothesis (Muramatsu, 1986; Machaly *et al.* 1988).

The inhibition of noradrenaline release by adenosine, and its reversal by 8-phenyltheophylline, show the presence of prejunctional P_1 purinoceptor-mediated feed-back mechanisms in the dog mesenteric artery. However 8-phenyltheophylline alone had no prejunctional effect. This result suggests that, even though P_1 purinoceptors exist on the nerve terminals, they may not be stimulated by endogenous purines; this is in contrast to prejunctional α_2 adrenoceptors. Rather, postjunctional P_1 purinoceptors appear to be more functional since purinergic and ATP-induced contractions were both potentiated by 8-phenyltheophylline. Recently, we observed that ATP acts on not only postjunctional P_2 purinoceptors but also on postjunctional P_1 purinoceptors and that 8-phenyltheophylline augments ATP-induced contraction by inhibiting the postjunctional P_1 purinoceptors (I. Muramatsu & T. Ohmura, unpublished observations).

α,β-Methylene ATP is a selective P_2 purinoceptor agonist (Burnstock & Kennedy, 1985). However, this compound rapidly desensitizes the receptors (Kasakov & Burnstock, 1983). Therefore, we examined the prejunctional effect of α,β-methylene ATP with two treatment periods (3 and 15 min). The results obtained show that α,β-methylene ATP had no effect on [^{3}H]noradrenaline release, suggesting that the P_2 purinoceptor is absent or not functioning in the sympathetic nerve terminals.

In conclusion, the present study clearly shows that sympathetic contraction of the dog mesenteric artery is composed of adrenergic and purinergic components and that the release of both transmitters is prejunctionally regulated by adrenergic mechanisms.

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