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Roles of norepinephrine and ATP in sympathetically evoked vasoconstriction in rat tail and hindlimb in vivo

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Johnson, Christopher D., Andrew M. Coney, and Janice M. Marshall. Roles of norepinephrine and ATP in sympathetically evoked vasoconstriction in rat tail and hindlimb in vivo. *Am J Physiol Heart Circ Physiol* 281: H2432–H2440, 2001.—In anesthetized rats, we characterized the contributions of norepinephrine (NE) and ATP to changes in tail and hindlimb (femoral) vascular resistances (TVR and FVR, respectively) evoked by three patterns of sympathetic stimulation: *1*) couplets (2 impulses at 20 Hz), *2*) short trains (20 impulses at 20 Hz), and *3*) a natural irregular pattern previously recorded from a sympathetic fiber innervating the rat tail artery. All stimuli evoked greater changes in TVR than FVR. Judging from the effects of the α-adrenoceptor antagonist phentolamine, the purinergic receptor antagonist suramin, or α,β-methylene ATP (which desensitizes P2X receptors), we propose that NE has a major role in the constriction evoked by the couplet, as well as by the short train and by the low- and high-frequency components of the natural pattern, but that considerable synergy occurred between the actions of ATP and NE. This contrasts with previous in vitro studies that indicated that ATP dominates vascular responses evoked by sympathetic stimulation with a few impulses at low frequency and that NE dominates responses to longer trains or at high frequencies.

cotransmission; synergy; cutaneous circulation; skeletal muscle circulation

THE CONCEPT OF COTRANSMISSION has been accepted for some years (11) and is now thought to occur as a rule, not an exception (28). A number of transmitters and modulatory substances are released from sympathetic nerves with norepinephrine (NE) and appear to be highly specific to particular vessels in individual vascular beds and species (28). In the caudal ventral artery of the rat tail, these are known to include ATP and possibly neuropeptide Y (NPY) (1, 3, 36). In vitro studies (4, 21, 35) on rat mesenteric and tail arteries and on rabbit ear arteries have suggested that ATP makes the major contribution to sympathetically mediated vasoconstriction when sympathetic fibres are stimulated with a few impulses, whereas NE makes the major contribution when longer trains of impulses at a constant frequency are used. However, in vivo recordings in several species, including rats and humans, have shown that sympathetic nerve activity tends to occur intermittently, with single impulses or bursts containing two to seven impulses, at intraburst instantaneous frequencies of up to 20–50 Hz (13, 14, 23). Furthermore, single sympathetic fibres innervating the caudal ventral artery and lateral veins of the rat tail can show a very robust bursting rhythm (16–18). This raises the possibility that the patterning of sympathetic nerve activity that normally occurs in vivo may have a profound influence on the contribution that each transmitter makes to sympathetically evoked responses.

To date, most studies on the contribution made by different transmitters to vascular responses evoked by different patterns of sympathetic stimulation have been conducted in vitro. However, in a recent in vivo study, Morris (26) demonstrated that single impulses or short trains (2–20) of impulses at 2–20 Hz did not evoke constriction in small ear arteries of the guinea pig and that ATP played no role in vasoconstriction evoked by stimulation with longer trains (50–300 impulses) at 10–20 Hz. Therefore, the major aim of the present study was to investigate the contributions that NE and ATP make to responses evoked in the circulations of the rat tail and hindlimb in vivo by three different patterns of stimulation applied to the sympathetic chain. Modeled patterns, which showed clear differences in the parts played by each transmitter in in vitro preparations of rat tail and mesenteric arteries (Refs. 3 and 34, respectively), were chosen. We also used a pattern of nerve activity previously recorded from a single sympathetic fiber innervating the rat caudal ventral artery. These patterns were delivered before and after antagonists of the actions of NE and/or ATP. We addressed the following questions: *1*) Does the number of nerve impulses applied affect the contributions that ATP and NE make to responses evoked in the tail (cutaneous) and hindlimb (muscle) circulations? *2*) Are there differences between the contributions made by NE and ATP in responses evoked in tail and hindlimb circulations? and *3*) Are responses evoked by modelled patterns of stimuli comparable with those evoked by natural patterns of activity?

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Preliminary accounts of this work have been published as abstracts (15, 19, 20).

METHODS

Experiments were conducted on 34 male Wistar rats (200–270 g) anesthetized initially with a oxygen-halothane mixture (3.5% halothane) and then maintained on a constant intravenous infusion of Saffan (Schering-Plough Animal Health; Welwyn Garden City, UK) delivered at 7–12 $mg \cdot kg^{-1} \cdot h^{-1}$. The depth of anesthesia was monitored continuously, and boluses of Saffan were given if required, as judged from *1*) the stability of blood pressure and respiratory movements, *2*) the size of pupils, and *3*) palpebral and paw-pinch reflexes. The animals were killed by an overdose of pentobarbitone sodium (given intravenously) at the end of each experiment.

A brachial artery and vein were cannulated to monitor arterial blood pressure (ABP) and administer drugs, respectively. The other brachial artery was cannulated to supply samples of arterial blood for blood gas analysis. The trachea was cannulated low in the neck, and the spontaneously breathing animals were supplied with O_2-enriched room air. Arterial blood samples (150 μl) were taken regularly for analysis of blood gasses and pH and were kept within the following ranges: pH, 7.35–7.45; P_{CO_2}, 38–45 mmHg; and P_{O_2}, 90–120 mmHg. Esophageal temperature was monitored and maintained at 37.0 ± 0.5°C. A stimulating electrode was attached to the lumbar sympathetic chains (LSCs) to deliver the required patterns of impulses to the sympathetic innervation of blood vessels to the tail and hindlimbs. To this end, the LSCs were exposed by a laparotomy, and a silver wire bipolar electrode was wrapped around them between the third and fourth ganglia. The LSCs were then cut centrally between the second and third ganglia. Both sympathetic chains and electrode tips were enclosed in insulating material (President, Light Body; Coltène, Switzerland). Stimulus patterns were generated (Master-8 pulse generator, Intracel) and delivered via an isolated stimulator (DS2A, Digitimer). Tail blood flow (TBF; via the ventral artery) and femoral blood flow (FBF) were recorded via perivascular flow probes (0.7 V, Transonic Systems; Ithaca, NY) connected to a flowmeter (model T206, Transonic Systems). Vascular resistances in the tail and femoral circulations (TVR and FVR, respectively) were computed on-line as TBF or FBF divided by ABP with a calculation frequency of 5 Hz (SPIKE2, Cambridge Electronic Design).

Protocols

Responses of tail and hindlimb vascular beds to couplets and short trains before and after α-adrenergic and P2 purinergic receptor antagonists. Two types of stimulation (1-ms pulse duration and at least three times suprathreshold, 6–30 V) were used: couplets at 20 Hz and short trains of 20 impulses at 20 Hz. The changes in TVR and FVR evoked by these stimuli were assessed under control conditions and then after either the α-adrenoceptor antagonist phentolamine (10 mg/kg iv, *group 1*, $n = 10$) or the purinergic P2 receptor antagonist suramin (15 mg/kg iv, *group 2*, $n = 7$; FVR was not recorded in one of these experiments). The nonselective α-adrenoceptor antagonist was used because in vitro studies (3) have shown that both postjunctional α_1- and α_2-receptor subtypes contribute to constrictor responses evoked by nerve stimulation in the rat tail artery. In each group, the other antagonist was then given to establish the combined effects of a blockade of α-adrenergic and P2 purinergic receptors upon the evoked changes in TVR and FVR. Some additional experiments were carried out in the presence of α,β-methylene ATP (α,β-meATP), which desensitizes P2X receptors. This was administered according to the protocol used by Bulloch and McGrath (10), in which increasing doses were given every minute, the first two doses at 0.05 mg/kg iv followed by five doses at 0.5 mg/kg (see Fig. 6*B*). In one experiment, the P2X preceptor antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) was administered as a bolus (30 mg/kg iv).

Responses of tail and hindlimb vascular beds to a natural pattern of activity before and after α-adrenergic and P2 purinergic receptor antagonists. In these experiments (*group 3*, $n = 9$), a natural pattern of activity that had been recorded over 7 min from a single sympathetic fiber innervating the tail artery in a previous experiment (for methods, see Ref. 17) was used to stimulate the LSCs. The TVR and FVR recordings were analyzed over the whole period of stimulation under control conditions, after phentolamine and then after suramin, or with suramin given as the first antagonist; the antagonists were given at the doses indicated above. The neural recording had been made in a preparation in which the core temperature was slowly increasing (from 37 to 38°C) and activity in the sympathetic unit was of relatively low mean frequency; the section of recording contained a range of frequencies from 0.038 to 40 Hz (mean, 0.38 Hz). The mean changes in TVR and FVR were computed over the full 7 min of the recording and compared with the baseline values before stimulation (see *Data Analysis*). In addition, responses evoked by two individual components of the nerve activity were analyzed in more detail: those evoked by two single impulses separated by 2 s (low-frequency stimulation, a couplet at 0.5 Hz) and by bursts of higher frequency (5 impulses, instantaneous frequencies of 5–40 Hz: high-frequency stimulation, lasting 1.5 s). These were assessed before and after phentolamine. This is similar to the analysis carried out by Sjöblom-Widfeldt and Nilsson (35), who applied single fiber activity that had been recorded from the peroneal nerve of human subjects to paravascular nerves that supplied in vitro preparations of a small mesenteric artery of the rat.

Data Analysis

All results are expressed as means ± SE. TVR and FVR were computed on-line in millimeters of mercury per milliliter per minute, as shown in Figs. 1, 3, and 6–8 and in Tables 1 and 2. Effects of the antagonist on baseline were tested by Student's paired *t*-tests. To quantify the evoked changes in TVR and FVR, the integrated area under the appropriate recordings of TVR and FVR and above the baseline recorded for an equivalent period before the stimulus was computed and expressed in resistance units (RU; see Figs. 2, 4, and 9). The evoked changes in integrated TVR and FVR after an antagonist were also calculated as a percentage of the control response recorded before the antagonist. Comparisons were made between absolute values by one-way ANOVA and, when $P < 0.05$ and $n \geq 5$, Fisher's post hoc test was used as appropriate. In addition, mean and peak changes in TVR and FVR and their durations were calculated to provide further description of the responses; these were not subjected to statistical analysis because we considered the integral of the change in vascular resistance to be the most meaningful expression of the constrictor response. In some groups of experiments, FBF was not recorded; the *n* values for TVR were, therefore, greater than those for FVR.

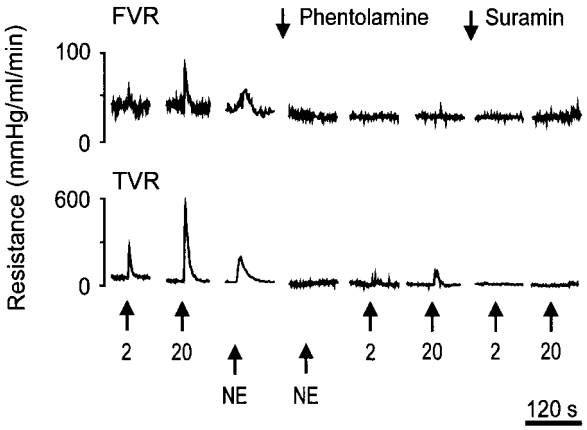

Fig. 1. Mean responses evoked in femoral vascular resistance (FVR) and tail vascular resistance (TVR) by couplet and short train stimulation in the presence and absence of the actions of NE (*group 1*). Example of responses of FVR (*top trace*) and TVR (*bottom trace*) evoked by stimulation with couplets (*arrow 2*), short trains (*arrow 20*), and intravenous boluses of norepinephrine (NE; 10 μg/kg iv) are shown during control, in the presence of phentolamine (10 mg/kg iv), and in the additional presence of suramin (15 mg/kg iv). Data were recorded simultaneously. Note the different resistance axis scales between TVR and FVR.

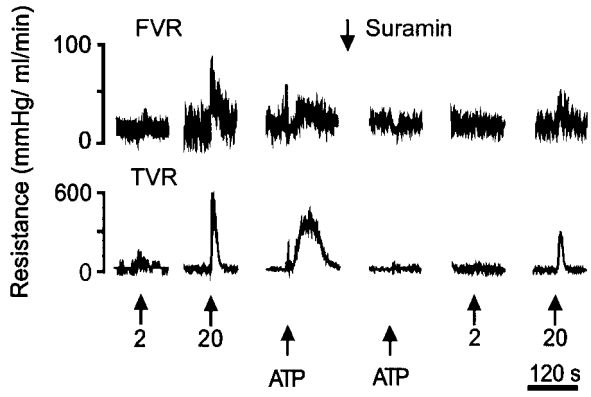

Fig. 3. Responses evoked in FVR and TVR by couplet and short train stimulation in the presence and absence of the actions of ATP (*group 2*). Example of responses of FVR (*top trace*) and TVR (*bottom trace*) evoked by stimulation with couplets (*arrow 2*), short trains (*arrow 20*) at 20 Hz, and intravenous boluses of ATP (2.2 mg/kg iv) are shown during control and in the presence of suramin (15 mg/kg iv). Data were recorded simultaneously. Note the different resistance axis scales between TVR and FVR.

RESULTS

Responses to Couplets and Trains in Control Conditions

In *groups 1* and *2* under control conditions (see Figs. 1-4), stimulation with a couplet evoked a large increase in TVR. The change evoked in FVR was much less marked than in TVR: a couplet evoked an increase in FVR in 4 of 10 *group 1* animals and in 6 of 7 *group 2* animals. This difference reflected that both the duration and magnitude of the evoked response were much greater in TVR than in FVR (Table 1). Although the TVR response to the couplet was somewhat smaller in *group 2*, differences between groups were not significant, probably reflecting the degree of variability between animals. In each vascular bed, the response evoked by train stimulation was much greater than that evoked by the couplet, but again, the change evoked in TVR was greater than that evoked in FVR (see Figs. 1–4), reflecting a greater magnitude and duration of the TVR response (Table 1).

Responses Evoked After α-Adrenoceptor Blockade: Group 1

Phentolamine decreased mean baseline levels of ABP, TVR, and FVR, which partially recovered after 5–10 min to the values shown in Table 2. Thus, at this time, TVR was not significantly changed by phentolamine, but TBF was reduced (from 2.07 ± 0.24 to 1.72 ± 0.21 ml/min, $P < 0.05$). In contrast, FVR was

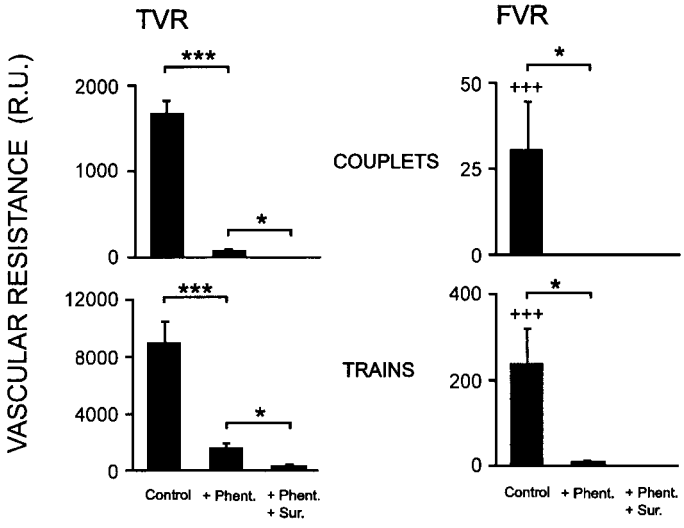

Fig. 2. Responses evoked in FVR and TVR by couplet and short train stimulation in the presence and absence of the actions of NE (*group 1*). RU, resistance units. ***$P < 0.001$, control vs. phentolamine (Phent); *$P < 0.05$, control vs. phentolamine or phentolamine vs. phentolamine + suramin (Sur); +++$P < 0.001$, control TVR vs. control FVR.

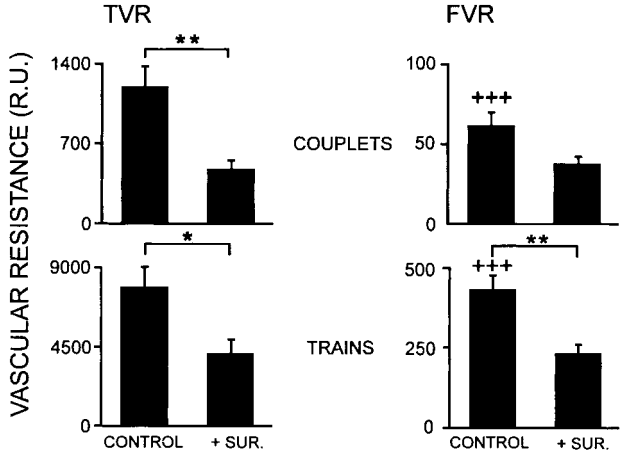

Fig. 4. Mean responses evoked in FVR and TVR by couplet and short train stimulation in the presence and absence of the actions of ATP (*group 2*). *$P < 0.05$ and **$P < 0.01$, control vs. suramin; +++$P < 0.001$, control TVR vs. control FVR.

Table 1. *Values for mean resistance and duration of response and peak response evoked by couplets and trains of impulses in TVR and FVR*

	TVR Responses						FVR Responses					
	Couplets			Trains			Couplets			Trains		
	Mean response	Duration	Peak response	Mean response	Duration	Peak response	Mean response	Duration	Peak response	Mean response	Duration	Peak response
Group 1 control ($n = 10$)	45.2 ± 5.7	38.5 ± 2.4	265 ± 32.1	159.3 ± 41.5	66.5 ± 6.9	1,347.6 ± 508.7	2.8 ± 0.7	11.3 ± 4.9	33.0 ± 14.5	6.1 ± 1.7	32.9 ± 5.3	88.8 ± 21.3
Group 2 control ($n = 6$–7)	26.5 ± 4.3	39.2 ± 3.3	106 ± 14.6	162 ± 50.5	44.2 ± 3.5	607.9 ± 168.3	5.3 ± 2.4	22.0 ± 3.0	65.5 ± 16.5	7.1 ± 1.0	44.0 ± 3.3	80.5 ± 9.5

Values are means ± SE; n = no. of experiments. Mean and peak tail (TVR) and femoral vascular resistances (FVR) responses (in mmHg·ml^{-1}·min^{-1}) were measured as responses above the preceding baseline and so are not absolute resistance values; duration was measured in seconds. These values are stated to provide further characterization of the responses evoked by couplets and trains and were not subjected to statistical analyses. This is in addition to the responses measured as resistance units, which were subjected to full statistical analyses (see text).

reduced by phentolamine ($P < 0.05$), but FBF was not changed (2.10 ± 0.24 vs. 2.5 ± 0.33 ml/min). The increases in ABP, TVR, and FVR produced by NE (10 μg/kg iv) were abolished by phentolamine (see Fig. 1). This blockade lasted at least 40 min and so outlasted the remainder of the experiment.

After phentolamine, the TVR response evoked by the couplet was greatly reduced (to 3.1 ± 1.1% of control), whereas the FVR response was abolished (Figs. 1 and 2). The TVR response to the train was also markedly reduced and that evoked in FVR was abolished in 7 of 10 experiments (reduced to 15.6 ± 3.6% and 1.2 ± 0.8%, respectively; Figs. 1, 2, and 5). Considering the percent change in the response, phentolamine had a greater effect on the response evoked in TVR by the couplet than by the train, but this was not the case for FVR (Fig. 5). Subsequent administration of suramin had little effect on baseline TVR or FVR (see Table 2) but abolished the TVR response to the couplet and reduced the response to the train; a small TVR response persisted in 4 of 10 animals (2.2 ± 1.2% of the control response; Figs. 1 and 2). The small FVR response to the train that remained after phentolamine in 3 of 10 animals was abolished (Figs. 1 and 2).

Because phentolamine caused a large fall in ABP, and hence in perfusion pressure for the tail and hindlimb vascular beds, it is possible that this reduced the evoked responses (see DISCUSSION). However, in the three animals that showed the lowest ABP after phentolamine (54 ± 1 mmHg), the increases in TVR evoked by the couplet and train were 1,682 ± 284 and 8,638 ± 202 RU, respectively, and, in the three animals that showed the highest ABP after phentolamine (73 ± 11 mmHg), the TVR increases evoked by the couplet and train were only 1,264 ± 349 and 5,391 ± 958 RU, respectively. In these same two subgroups, the increase in FVR evoked by the train was 130 ± 30 and 334 ± 207 RU, respectively.

Table 2. *Baseline (preantagonist) values for ABP, TVR, and FVR in groups 1–3*

	ABP, mmHg	TVR Baseline, mmHg·ml^{-1}·min^{-1}	FVR Baseline, mmHg·ml^{-1}·min^{-1}
Group 1			
Control	92.4 ± 16.6 ($n = 10$)	44.7 ± 15.5 ($n = 10$)	41.9 ± 2.5 ($n = 10$)
After phentolamine	56.6 ± 4.8 ($n = 10$)	32.5 ± 2.3 ($n = 10$)	28.0 ± 4.0 ($n = 10$)
After phentolamine + suramin	40.2 ± 5.0 ($n = 10$)	38.0 ± 3.9 ($n = 10$)	27.6 ± 3.5 ($n = 10$)
Group 2			
Control	97.8 ± 12.9 ($n = 7$)	40.0 ± 11.5 ($n = 7$)	45.3 ± 20.5 ($n = 6$)
After suramin	102.0 ± 2.9 ($n = 7$)	44.0 ± 6.5 ($n = 7$)	42.6 ± 4.4 ($n = 6$)
Group 3			
Control	90.0 ± 15.3 ($n = 9$)	41.0 ± 11.5 ($n = 9$)	43.2 ± 7.5 ($n = 5$)
After phentolamine	64.8 ± 5.0 ($n = 9$)	43.3 ± 5.8 ($n = 9$)	27.5 ± 3.7 ($n = 5$)

Values are means ± SE; n = no. of experiments. ABP, arterial blood pressure. Differences in baseline values for ABP, TVR, and FVR during control among *groups 1, 2,* and *3,* and between *groups 1* and *3* in the presence of phentolamine, were not significant.

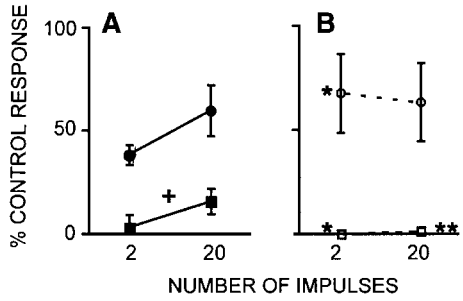

Fig. 5. Percentages of control responses evoked by couplets and trains remaining in the presence of phentolamine (*group 1*) or suramin (*group 2*). *A* and *B*: responses evoked from tail (solid lines and solid symbols; *A*) and hindlimb circulations (dashed lines and open symbols; *B*), respectively, by couplets and short trains in the presence of either phentolamine (squares) or suramin (circles). Significant differences were as follows: *$P < 0.05$, couplet TVR vs. couplet FVR in the presence of phentolamine or in the presence of suramin; **$P < 0.01$, train TVR vs. train FVR in the presence of phentolamine; +$P < 0.05$, couplet TVR vs. couplet FVR in the presence of phentolamine.

Responses Evoked After ATP Antagonism: Group 2

Suramin had no effect on the baseline values in *group 2* (Table 2). Before suramin, systemically injected ATP (2.2 mg/kg iv) produced a biphasic change in FVR (see Fig. 3): an initial reduction in FVR, indicating vasodilatation, was followed by recovery or an increase in FVR, indicating vasoconstriction. Concurrent with the second phase, there was an increase in TVR, indicating a predominant vasoconstriction in the tail. The initial decrease in FVR was largely unaffected by suramin, but the secondary increase in FVR no longer occurred, and the increase in TVR was reduced by 80–98% (mean = 88.8 ± 3.7%, $P < 0.001$, $n = 7$). Larger doses of suramin (up to 75 mg/kg) had no greater effect, suggesting suramin could not produce full antagonism of P2 receptors.

After suramin, the change evoked in TVR by the couplet was reduced (Figs. 3 and 4) to 38.0 ± 4.5% of the control (Fig. 5). Similarly, the change evoked in FVR by the couplet was reduced in individual experiments to 67.7 ± 11.2% of the control, but the reduction did not reach significance ($P = 0.079$, $n = 6$). The changes evoked in TVR and FVR by the train were significantly reduced (Figs. 3 and 4) to 59.4 ± 12.3% and 63.0 ± 14.3% of control, respectively (Fig. 5). In four additional experiments in *group 2*, α,β-meATP was used to densitize P2X receptors. The changes in TVR evoked by repeated boluses of α,β-meATP were generally abolished by the seventh minute of this protocol (see Fig. 6*B*). The blockade lasted for no more than 2 min; during this period, the changes evoked in TVR by the couplet and train were reduced to 56.9 ± 21.9% and 58.3 ± 29.1% of control, respectively ($n = 4$), as in most experiments with suramin.

However, it was of note that in one α,β-meATP experiment (see Fig. 6*B*) changes evoked in TVR by couplets and trains were potentiated (by 105% and 149%, respectively). This potentiation disappeared as desensitization of the P2X receptors wore off (see Fig. 6*B*). Similarly, in one experiment, suramin potentiated all responses evoked by LSC stimulation (the changes evoked in TVR and FVR by the couplet and train ranging from 30 to 300%; see Fig. 6*A*). Baseline values for ABP, TVR, and FVR were similar to those of other experiments. However, the TVR response to exogenous ATP was reduced to only 45% of control, and so these data were not included in statistical analyses.

In the one experiment in which the P2X receptor antagonist PPADS was used, it blocked the effects of exogenous ATP so that 20% of the control TVR response remained. As in most experiments with suramin or α,β-meATP, the TVR responses to a couplet and train decreased (to 54% and 49%, respectively).

Phentolamine was given after suramin to five animals in *group 2*. In each case, this caused such a dramatic reduction in arterial pressure (to <30 mmHg) that the experiment could not be continued.

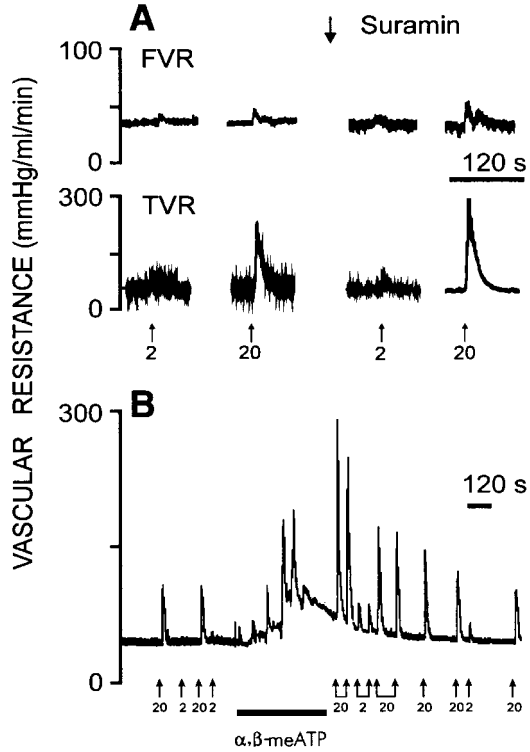

Fig. 6. Examples of potentiation of responses evoked by couplet and train stimuli in the presence of purinergic blockade. *A*: on one occasion, the responses evoked in FVR (*top trace*) and TVR (*bottom trace*) by couplet and train stimulation in the presence of suramin were greater than those seen during control. Baseline values for arterial blood pressure and vascular resistances (not shown) were similar to other *group 2* preparations, although suramin blocked the response to ATP (given intravenously) to only 45% of control (not shown). These data were not included in statistical analyses. *B*: responses evoked by couplet and train stimuli are shown before and after desensitization of P2X receptors with α,β-methylene ATP (α,β-meATP) (seven consecutive doses, 0.05–0.5 mg/kg, indicated by the black bar, which is equivalent to 7 min). The responses evoked by both stimuli were potentiated immediately after the desensitization, which wore off progressively over the next 20 min. FVR was not recorded on this occasion.

Responses Evoked by the Natural Pattern of LSC Stimulation: Group 3

Figure 7 shows original traces in which simultaneous recordings were made of TBF and computed TVR while the activity of a single sympathetic fiber on the adventitia of the tail artery was being recorded. During periods of high-frequency bursts or periods of prolonged stimulation, large increases in TVR were seen, and there was an obvious qualitative relationship between the changes in TVR and the changes in nerve activity. This period of activity was used in the following experiments as a natural pattern of impulses.

The baseline values in *group 3* were not different from those of the other groups (see Table 2). When the natural pattern of activity was used to stimulate the LSCs, the evoked changes in TVR were qualitatively similar to those seen in the original experiments (cf Figs. 7 and 8*A*). Furthermore, the evoked changes in FVR were qualitatively similar to those in TVR (Fig. 8*B*). In particular, it may be noted that both TVR and

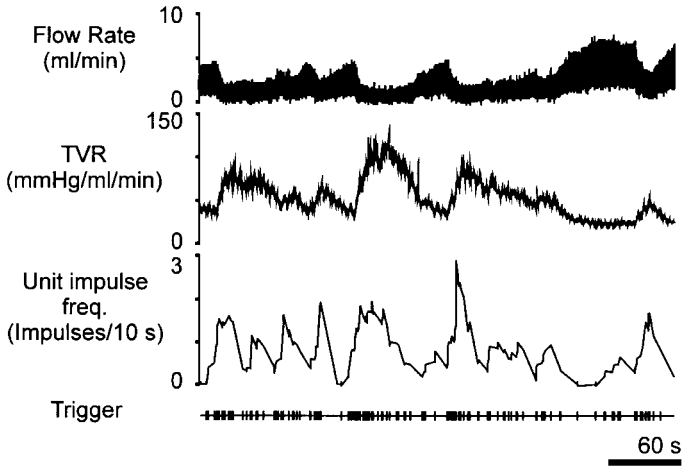

Fig. 7. Original traces of sympathetic nerve firing (triggers) and vascular responses recorded from the caudal ventral artery (CVA). Original traces are shown of simultaneous recordings of blood flow in the CVA (and computed TVR) and activity of a single sympathetic fiber on the adventitia of the CVA. During periods of high-frequency bursts or periods of prolonged stimulation, large increases in TVR are seen, and there is an obvious qualitative relationship between the changes in TVR and the changes in nerve activity. Freq, frequency.

FVR increased in response to the first few impulses in the pattern. TVR remained above baseline throughout the whole period of stimulation despite gaps between impulses of up to 25 s. FVR remained above baseline for the vast majority of the stimulation period. High-frequency bursts or sustained periods of impulse delivery resulted in large increases in TVR and FVR, whereas singlets or couplets evoked transient changes (Figs. 8 and 9).

Overall, the increase in TVR evoked by the natural pattern of stimulation over the 7 min was greater than the change evoked in FVR (Figs. 7 and 8). Phentolamine markedly reduced the change evoked in both TVR and FVR (to 14.0 ± 14.5% and 6.6 ± 11.1% of control, respectively; see Figs. 8 and 9).

In Fig. 8, the two arrows indicate the responses evoked by low-frequency (a couplet at 0.5 Hz) and high-frequency (5 pulses at 5–40 Hz) components of the recordings that were chosen for analysis (see METHODS) before and after phentolamine. For both TVR and FVR, the response to the low-frequency component was decreased (Fig. 9) to 11.1 ± 3.6% and 8.1 ± 3.9% of control, respectively, as was the response to the high-

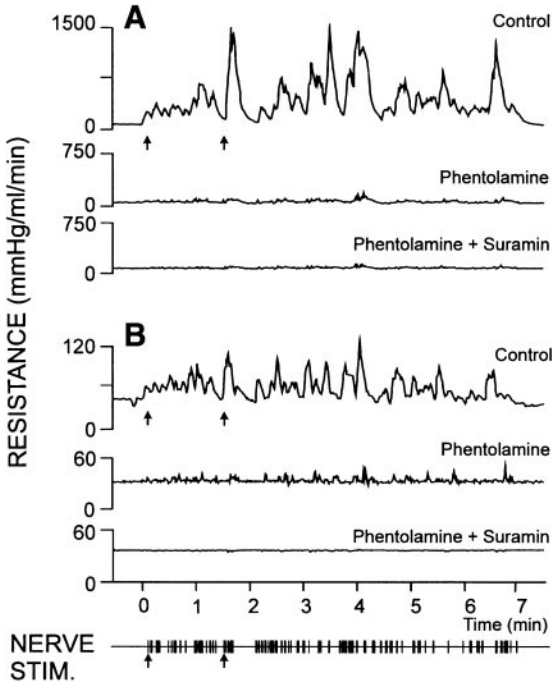

Fig. 8. Responses evoked in FVR and TVR by the natural pattern of stimulation (Stim) in the presence and absence of the actions of NE (*group 3*). Data shown are TVR (*A*) and FVR (*B*) responses (recorded simultaneously) evoked by the natural pattern of stimulation during control, in the presence of phentolamine, and in the additional presence of suramin. The arrows indicate the sections of the responses that were analyzed in more detail (see text for details); the first and second arrows corresponding to low- and high-frequency stimulation, respectively.

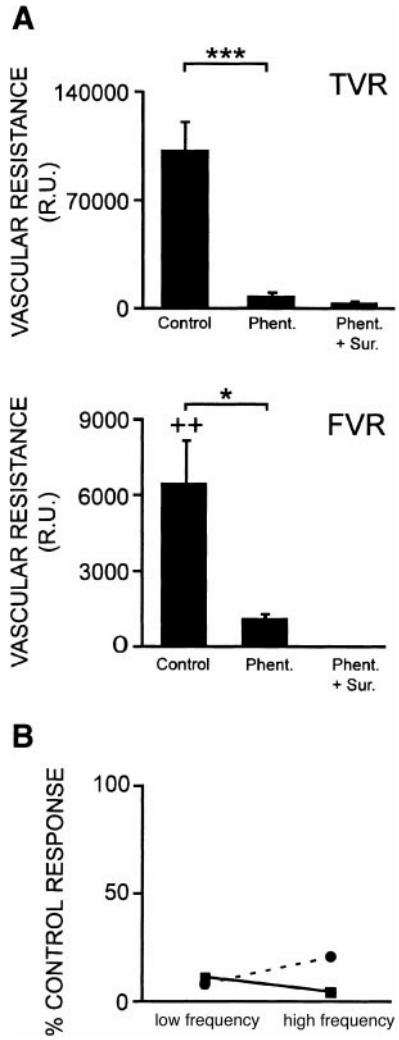

Fig. 9. Mean responses evoked in TVR (*A*, *top*) and FVR (*A*, *bottom*) by the natural pattern of stimulation in the presence and absence of the actions of NE (*group 3*). *A*: absolute values; *B*: percentages of control responses. Square, TVR; circle, FVR. $^{*}P < 0.05$ and $^{***}P < 0.001$, control vs. phentolamine; $++P < 0.01$, TVR vs. FVR.

frequency component (to 4.3 ± 1.4% and 20.9 ± 3.0%, respectively).

When suramin was given after phentolamine, a small increase in TVR was evoked in only two of five experiments while the FVR response was abolished (Figs. 8 and 9).

Suramin was given as the first antagonist in two experiments; TVR was recorded in both experiments and FVR was recorded in one experiment. The TVR response was increased in both, by 105% and 136%, and the FVR response was increased by 112%.

DISCUSSION

The findings of the present in vivo study provide new insight into the influence that three different patterns of sympathetic impulses can have on vascular resistance in two main vascular beds (skin and skeletal muscle, represented by TVR and FVR, respectively). They also provide new insight into the roles of two sympathetic cotransmitters, NE and ATP, in vivo. In discussing our results, we made comparisons where possible with the results and conclusions of in vitro studies on single arteries.

Responses Evoked Under Control Conditions

The finding that just two pulses delivered to the LSC in vivo can evoke an increase in TVR and usually in FVR is novel and indicates that the arterial/arteriolar vessels that contribute to gross vascular resistance in these tissues are very sensitive to changes in sympathetic nerve activity. Similar sensitivity has been suggested in vitro (5, 21) but not in vivo (Ref. 26; see below). The relatively long-lasting changes in TVR and FVR evoked by couplets (11–39 s) and trains (44–66 s) suggest that a low level of intermittent activity can maintain a tonic level of vasoconstriction in both beds. Accordingly, when the natural pattern of nerve activity was applied to the LSC, TVR and FVR remained above the original baseline even when there were intervals of 10–25 s between bursts of activity.

The increases in TVR evoked by the three types of stimuli were 16–50 times greater than the increases in FVR, probably reflecting the high density of the sympathetic noradrenergic supply to the tail arteries (2, 12, 33) and the relatively weak supply to the muscle vasculature (24). Furthermore, the density of P2X purinoceptors that mediate vasoconstriction to ATP is about three times greater in the tail artery than in the femoral artery (7). It is also likely that the influence of sympathetic activity on FVR was offset by vasodilator metabolites produced by the decrease in blood flow (see Refs. 8 and 24) and that this effect was less marked in the tail.

Contributions of NE and ATP to Responses Evoked by Couplets and Trains

We used the nonselective α_1/α_2-adrenoceptor antagonist phentolamine to block the postjunctional effects of NE, because NE acts via both receptor subtypes in the tail vasculature (3). Presynaptic α_2-adrenoceptors can inhibit the release of NE and ATP in the tail artery in vitro (3). This raises the possibility that autoinhibition via these receptors limited the effect of nerve-released NE and ATP under control conditions and that phentolamine facilitated the ATP component. Any such effects were probably small because presynaptic inhibition becomes more important with greater numbers of impulses in the train, up to 150 (37).

When phentolamine was given as the first antagonist, the TVR and FVR responses evoked by the couplet were almost, or completely, abolished. This contrasts markedly with in vitro findings that responses evoked in the rat tail artery by a single impulse or a couplet at 20 Hz were not affected by the α_1-adrenoceptor antagonist prazosin (3), whereas those evoked in rat mesenteric arteries by a couplet at 20 Hz were reduced by only 20% (34). Indeed, in both studies (3, 34), it was concluded that NE makes little contribution to the responses evoked by a few impulses.

In vitro, when the number of stimuli applied in the train was increased, the reduction in the evoked response caused by α_1-adrenoceptor blockade became larger; in the rat tail artery (4) and in both rat mesenteric and rabbit ear arteries (21, 34), the response to 10 pulses and 20 pulses, respectively, at 20 Hz was reduced by 80%. It was therefore suggested that NE becomes more important when the train length or impulse frequency is increased. This trend was obviously not apparent in our study (see Fig. 5): increases in TVR and FVR evoked by both the couplet and the train were almost or completely abolished by NE.

Were these large effects of phentolamine on the evoked responses related to its effect on baseline ABP? In vitro and in vivo studies (6, 29a, 32) have shown that the magnitude of constrictor responses to agonists and sympathetic nerve stimulation can change depending on the resting tone of the blood vessels and the perfusion pressure, or passive stretch. These relationships are complicated and the conclusions drawn vary (cf Refs. 6, 29a, and 32). In a preparation of rat hindquarters, Rodionov et al. (32) found a parabolic relationship between the size of the sympathetically evoked responses and initial resistance at constant perfusion pressure; the maximum constrictor response was substantially increased when perfusion pressure was reduced from 80 to 40 mmHg. This was similar to the fall in ABP induced by phentolamine in our experiments, and, in agreement with that study, the rats with the lowest ABP after phentolamine tended to show the largest TVR responses to the couplet and the train (see RESULTS). On this basis, it seems reasonable to propose that NE did make the major contribution to the response to the couplet and the train in both the tail and hindlimb, even though caution is required in drawing quantitative conclusions about the NE contribution.

The results obtained when a P2 receptor antagonist was given as the first drug, and there was no fall in ABP, lead to a similar proposal. TVR and FVR responses to the couplet and the short train were reduced to 40–60% of control, indicating that as much as 40–60% of both these responses were due to the actions of

NE. This proposal also contrasts markedly with in vitro findings that purinergic antagonists had a large effect on the constrictor response to a few impulses and a small effect on that evoked by a train (21, 34).

If we now consider together the effects of phentolamine and P2 receptor antagonists, it seems that ATP contributed as a primary transmitter and facilitated the action of NE during both types of stimulus. If the portion of the response remaining after phentolamine represented the purinergic component, and the response remaining after suramin represented the NE component, then the sum of these two components was less than the size of the original response (see Fig. 5). With the proviso that the size of responses left after phentolamine may have been affected by the fall in perfusion pressure (see above), this implies considerable synergy between the actions of neurally released NE and ATP. Synergism of NE and ATP have been reported in in vitro studies (31, 35) and attributed to pre- and postjunctional mechanisms.

Although the dominant effect of suramin or α,β-meATP given as the first drug was to inhibit evoked responses, occasionally they were potentiated. Similar potentiating effects have been reported as an occasional rather than a routine effect in vivo (10) and in vitro in several different blood vessels (9, 22, 27), including the rat tail artery (5). Because neither suramin nor α,β-meATP affected the release of NE evoked by field stimulation, it was concluded that nerve-released ATP can either inhibit or facilitate NE-evoked contraction, by acting postjunctionally (5).

Contributions of NE and ATP to Responses Evoked by Natural Patterns

In vivo, sympathetic activity is irregular, composed of singlets, couplets, and short bursts with intraburst frequencies of 20–50 Hz (see Ref. 17). The pattern of natural activity we used to stimulate the LSCs was recorded when the unit activity to the tail was low, during hyperthermia. Because it contained pulses occurring at a wide range of frequencies and burst lengths, it was likely to reveal elements of both purinergic and noradrenergic transmission.

Our study is the first in which sympathetic nerve activity recorded from a particular artery has been "played back" to that same artery in vivo. Previously, activity recorded from the peroneal or median nerve of human subjects was applied to a single mesenteric artery or vein of the rat in vitro (34, 35) or to dog skeletal muscle or pig spleen in vivo (30). Our results show that the effects of phentolamine and suramin on responses evoked in the tail vasculature by a natural pattern of activity were comparable with those evoked by the modelled patterns. The full response and the elements evoked by low- and high-frequency components of nerve activity were almost abolished by phentolamine and further attenuated by suramin. Moreover, in two experiments, suramin given first facilitated responses to the natural pattern. Again, it seems reasonable to propose that NE plays a major role in constrictor responses evoked by both low- and high-frequency components of natural sympathetic activity to tail vasculature in vivo, with ATP acting as a primary transmitter that facilitates the influence of NE, but also having an inhibitory influence (see above).

We did not record single unit activity from a skeletal muscle vessel and "replay" it to the hindlimb. However, such activity does contain high- and low-frequency components like those in tail fibers (C. D. Johnson, unpublished observations). Because the results obtained in the hindlimb were qualitatively similar to those in the tail, we tentatively propose that NE and ATP play similar roles in responses evoked by natural activity in the hindlimb as in the tail.

Differences in Contributions of NE and ATP to Responses Evoked in Vitro and in Vivo

The present study indicates that ATP makes a much smaller contribution to tail and hindlimb responses evoked by a couplet than expected from in vitro studies (4, 21, 35). Does this disparity reflect a real difference between in vitro and in vivo preparations?

Cervical sympathetic stimulation with a single pulse or short trains at low frequencies (<2 Hz) failed to constrict small ear arteries of the guinea pig in vivo, even though similar stimuli, applied to the same arteries by field stimulation in vitro, produced membrane depolarizations (26). Furthermore, suramin had no effect on constrictions evoked in small ear arteries in vivo by stimulation with frequencies of up to 10 Hz, even though ATP was responsible for the excitatory junction potentials (EJPs) evoked in these arteries in vitro by field stimulation at frequencies of up to 20 Hz (26). Thus field stimulation in vitro might be more effective in releasing ATP than stimulation of the sympathetic chain in vivo. This is unlikely, for our study indicates that, in the rat tail and hindlimb vasculatures at least, ATP is released by LSC stimulation and contributes to the evoked constriction.

However, in small ear arteries in vitro, EJPs evoked by trains of pulses showed summation but not the active membrane responses required for contraction (27). In contrast, similar stimuli applied to main ear artery in vitro not only evoked active membrane responses, but also contractions that were partially blocked by suramin (26, 27). Thus ATP apparently played a larger direct role as a transmitter in the large arteries than in the more distal ones (see Ref. 26). If this is also the case in the tail and hindlimb, then the smaller role of ATP as a direct transmitter in our study may simply reflect the fact that changes in gross TVR and FVR are dominated by the behavior of the smaller arteries and arterioles.

In summary, the present in vivo study on the rat showed for the first time that the tail and hindlimb vasculatures both respond with constriction to just two impulses delivered to the LSCs, this sensitivity being especially marked in the tail. The effects of α-adrenoceptor and P2 receptor antagonists suggest that NE plays a major role in the constrictor responses to both

a couplet and a short train at 20 Hz in both vascular beds. They also indicate that ATP is coreleased and that it makes a smaller contribution as a primary transmitter in the response to the couplet and the train, but also that it acts synergistically with NE, in both vasculatures. In addition, our study is novel in describing the responses evoked when the neural activity recorded from a sympathetic fiber supplying the tail artery was used to stimulate the sympathetic supply to the tail: NE and ATP apparently played similar roles to those deduced for the couplet and train. Our results contrast with those of in vitro studies particularly because the response to a couplet was not mainly attributable to ATP. This emphasizes the need for in vivo studies to fully assess the physiological significance of results obtained in vitro.

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