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# β-Adrenoceptor mediated facilitation of noradrenaline and adenosine 5′-triphosphate release from sympathetic nerves supplying the rat tail artery

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**1** The effects of prejunctional β-adrenoceptor activation on electrically evoked noradrenaline (NA) and adenosine 5′-triphosphate (ATP) were studied by use of continuous amperometry and conventional intracellular recording techniques. Excitatory junction potentials (e.j.ps) were used as a measure of ATP release, and NA-induced slow depolarizations and oxidation currents as measures of NA release, from postganglionic sympathetic nerves innervating the rat tail artery *in vitro*.

**2** Isoprenaline (0.1 μM) increased the amplitude of e.j.ps, slow depolarizations and oxidation currents evoked by short trains of stimuli at 1 to 4 Hz. The facilitatory effect of isoprenaline on e.j.ps and oxidation currents was most pronounced on responses evoked by the first stimulus in a train.

**3** Isoprenaline (0.1 μM) did not detectably alter the amplitude-frequency distribution of spontaneous e.j.ps.

**4** The facilitatory effect of isoprenaline on e.j.ps, slow depolarizations and oxidation currents was abolished by the β-adrenoceptor antagonist, propranolol (0.1 μM). Propranolol alone had no effect on e.j.ps, slow depolarizations or oxidation currents.

**5** Thus, activation of prejunctional β-adrenoceptors increases the release of both NA and ATP from postganglionic sympathetic nerves. The findings are consistent with the hypothesis that NA and ATP are released from the same population of nerve terminals and presumably from the same vesicles.

**Keywords:** Transmitter release; neuroeffector transmission; electrophysiology; electrochemistry; ATP; noradrenaline; prejunctional receptor; rat tail artery

## Introduction

Previous studies have demonstrated that activation of prejunctional β-adrenoceptors increases the stimulation-induced release of noradrenaline (NA) from sympathetic nerves (see Langer, 1981; Majewski, 1983). In contrast, activation of prejunctional β-adrenoceptors has been shown to decrease the electrically evoked release of endogenous adenosine 5′-triphosphate (ATP) from postganglionic sympathetic nerves innervating the guinea-pig vas deferens (Gonçalves *et al*., 1996; see also Driessen *et al*., 1996). Gonçalves *et al*. (1996) showed that the β-adrenoceptor agonist, isoprenaline, increased the overflow of [$^3$H]-NA from labelled tissues but decreased the overflow of endogenous ATP, findings which lend support to the idea that NA and ATP release can be differentially modulated by prejunctional receptor activation.

Clearly, Gonçalves *et al*. (1996) measured the stimulation-induced increases in ATP overflow originating from nerves but the question remains whether these measurements solely reflect the release of junctionally-active ATP (see Brock & Cunnane, 1996). In the present study, electrophysiological and electrochemical techniques were used to measure on an impulse-to-impulse basis the release of endogenous NA and ATP from the sympathetic nerves innervating the rat tail artery. Conventional intracellular recording techniques were used to monitor electrically evoked excitatory junction potentials (e.j.ps), which are a measure of ATP release from sympathetic nerves (Sneddon & Burnstock, 1984; McLaren *et al*., 1995). In the absence of stimulation, spontaneous excitatory junction potentials (s.e.j.ps) were recorded to provide a measure of the sensitivity of the postjunctional membrane to neuronally released ATP before and after the application of drugs. In addition to e.j.ps, nerve stimulation evokes a slow depolarization of the smooth muscle that results from the activation of postjunctional $\alpha_2$-adrenoceptors by neuronally released NA (see Cassell *et al*., 1988). The slow depolarization can be used as an indirect measurement of NA release. The release of endogenous NA was measured directly by continuous amperometry (Gonon *et al*., 1993). This latter technique provides a 'real time' measurement of endogenous NA release. By use of these various techniques, we conclude that prejunctional β-adrenoceptor activation produces a parallel increase in the stimulation-evoked release of both NA and ATP.

## Methods

Male rats (150–200 g) were killed by an overdose of pentobarbitone sodium (150 mg kg$^{-1}$, Nembutal) injected intraperitoneally. Sections of proximal tail artery approximately 10 mm in length were removed and individual preparations pinned to the Sylgard (Dow Corning) covered base of a 1 ml (intracellular recording) or 5 ml (electrochemical recording) recording chamber. The recording chambers were perfused continuously at 3–5 ml min$^{-1}$ with physiological saline of the following composition (mM): NaCl 118.4, $NaHCO_3$ 25.0, $NaH_2PO_4$ 1.13, $CaCl_2$ 2.4, KCl 4.7, $MgCl_2$ 1.3 and glucose 11.1. The physiological saline was gassed with a mixture of 95% $O_2$ and 5% $CO_2$ (to pH 7.4) and maintained at 35–36°C. In all experiments the competitive $\alpha_1$-adrenoceptor antagonist, prazosin (0.1 μM), was added to the physiological saline to inhibit neurally evoked contractions due to NA release. Desipramine (1 μM) was added to the physiological saline in some of the electrochemistry experiments to inhibit the clearance of released NA by uptake$_1$. The proximal end of the artery was drawn into a suction electrode and the perivascular nerves were excited by electrical field stimulation (1 ms pulse width, 3–20 V for the electrophysiological experiments and 0.3 ms, 20 V for the electrochemistry experiments) with trains of stimuli at 1–4 Hz.

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### *Electrochemical recording*

The release of endogenous NA was measured by continuous amperometry with a technique pioneered by Gonon *et al.* (1993). Recording electrodes were fabricated from a single carbon fibre (8 μm diameter) supported by a glass micropipette (see Gonon *et al.*, 1984). Briefly, a carbon fibre was passed along the barrel of a glass micropipette until its end lodged in the tip. The micropipette was then cut just proximal to the fibre's end, and the carbon fibre extended approximately 5 mm beyond the micropipette tip. The micropipette was then back filled with epoxy resin (RS components Ltd, Corby, NN17 9RS, U.K.) containing graphite powder (Aldrich Chemical Company, Castle Hill, NSW 2154, Australia), which was forced into the tip by inserting a copper wire. This procedure sealed the carbon fibre into the micropipette tip and provided electrical contact with the fibre. After the epoxy resin had set, the exposed length of carbon fibre was cut to approximately 50 μm.

Recordings were made at sites 1–2 mm from the suction stimulating electrode. At the site of recording the superficial layer of connective tissue was carefully removed and a vertically mounted carbon fibre electrode was pushed gently against the surface of the artery making a slight indent. The electrode was connected to an AMU130 Nano-amperometer (Radiometer Analytical S.A., 69627 Villeurbanne CEDEX, France) and a potential difference of +0.3 V was applied between the recording electrode and a Ag-AgCl pellet placed in the recording chamber medium. The current required to maintain this voltage was monitored.

In control experiments the amplitude of the oxidation currents evoked by trains of 10 stimuli at 1, 2 and 4 Hz declined slowly during the period of recording. For this reason, comparisons were made between control and treated tissues. Tissues were given three cycles of stimulation ($S_1$, $S_2$ and $S_3$), with an interval of 15 min between cycles. During each cycle, tissues were stimulated with three trains of 10 stimuli at 1, 2 and 4 Hz, with an interval of 1 min between trains. Drugs were added immediately following $S_1$ and $S_2$. The responses obtained during $S_2$ and $S_3$ were expressed as the ratio of those obtained during $S_1$. Where determined, the % change in the stimulation-induced oxidation current in each tissues was calculated by dividing the $S_2/S_1$ ratio by the mean $S_2/S_1$ ratio for control experiments.

### *Intracellular recording*

Intracellular recordings were made from smooth muscle cells located near the adventitial-medial border of the tail artery with borosilicate glass microelectrodes connected to an Axoclamp bridge amplifier (Axon Instruments, Inc. Foster City, CA94404, U.S.A.). Microelectrodes were fabricated from glass capillaries (GC100F-15, Clark Electromedical Instruments, Pangbourne, RG8 7HU, U.K.) by a Flaming/Brown micropipette puller (Model P-87, Sutter Instruments Co., Novato, CA94949, U.S.A.) and had resistances of 80–160 MΩ when filled with 0.5 M KCl. Impalements were made within 1–2 mm of the suction stimulating electrode; criteria for accepting impalements were the same as those adopted by Cassell *et al.* (1988). Recordings were only made after the membrane po-

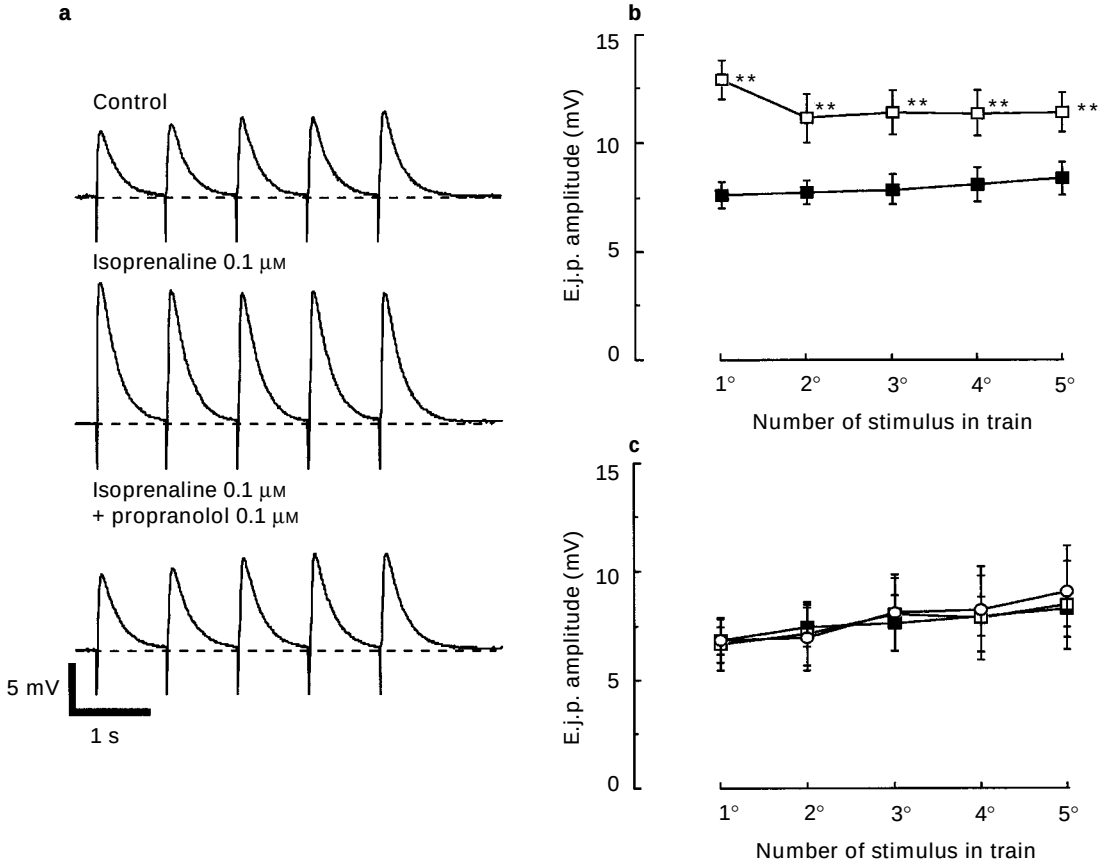


**Figure 1** Effects of isoprenaline (0.1 μM) and propranolol (0.1 μM) on excitatory junction potentials (e.j.ps) evoked by trains of 5 stimuli at 1 Hz. (a) Traces recorded before and during the sequential addition of isoprenaline followed by propranolol in a single preparation. (b) Graph showing the mean e.j.p. amplitude data ($n=7$ experiments) recorded before (■) and during application of isoprenaline (□). (c) Graph showing the mean e.j.p. amplitude data ($n=4$ experiments) recorded before (■) and during the sequential addition of propranolol (□) followed by isoprenaline (○). In (b) and (c) vertical lines show s.e.mean. Statistical comparisons between control and treated values were made by paired $t$ tests (**$P<0.01$).

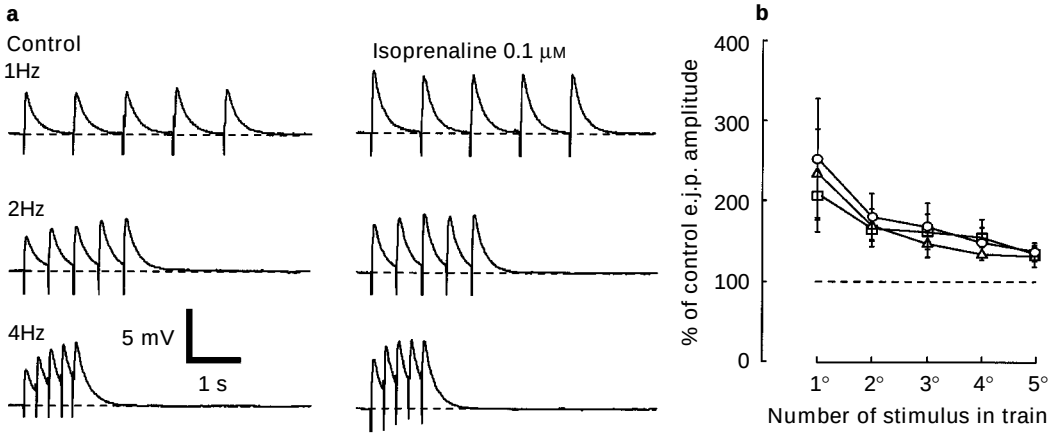


**Figure 2** Effects of isoprenaline (0.1 $\mu$M) on excitatory junction potentials (e.j.ps) evoked by trains of 5 stimuli at 1, 2 and 4 Hz. (a) Traces recorded before and during application of isoprenaline in a single preparation. (b) Graph showing the mean % change in e.j.p. amplitudes ($n=4$ experiments) produced by isoprenaline during trains at 1 (□), 2 (○) and 4 Hz (△). Vertical lines show s.e.mean. At all frequencies of stimulation, e.j.p. amplitudes were significantly (paired $t$ tests, $P<0.05$) increased compared to control values.

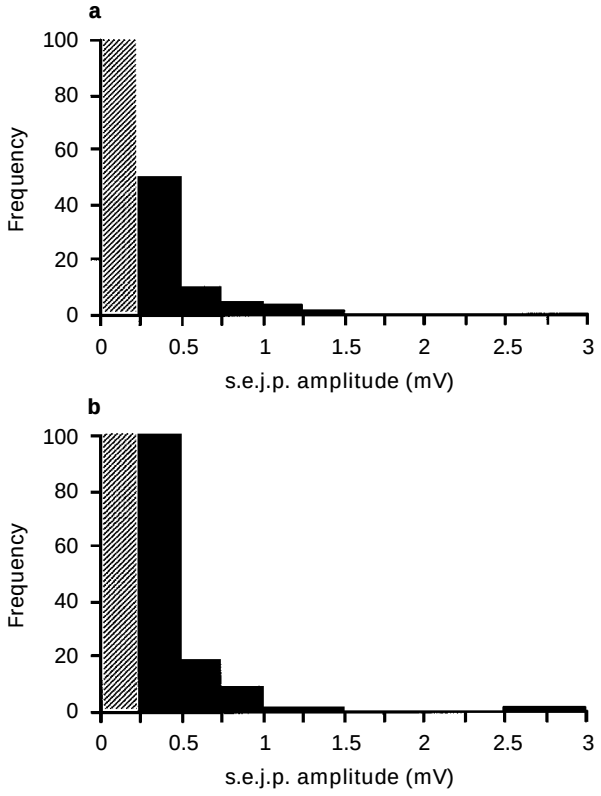


**Figure 3** Effects of isoprenaline (0.1 $\mu$M) on spontaneous excitatory junction potentials (s.e.j.ps) recorded in a single impalement. (a) and (b) The amplitude distributions of s.e.j.ps recorded during a 14 min period (a) before ($n=67$ s.e.j.ps) and (b) during the application of isoprenaline ($n=131$ s.e.j.ps). The hatched area represents twice the background noise level and is the level at which the presence or absence of individual events could not be detected with any degree of confidence. Isoprenaline had no apparent effect on the amplitude distribution of s.e.j.ps but, in the presence of this agent, the frequency of s.e.j.p. occurrence was increased.

tential had been stable for $\geqslant 5$ min. To facilitate comparisons between different preparations, the stimulus intensity was increased until e.j.ps evoked by single stimuli were approximately 10 mV in amplitude.

The effects of drugs on e.j.ps and s.e.j.ps were determined in single cell experiments in which both control and test recordings were made during the same impalement. To parallel the experiments in which NA was detected electrochemically (see above), tissues were given two or three cycles of stimulation, each separated by an interval of 15 min. During each stimulation cycle, tissues were excited by three trains of 5 stimuli at 1, 2 and 4 Hz, with an interval of 1 min between trains. Drugs were added immediately following the first and second cycles of stimulation. In control recordings, no significant difference in e.j.p. amplitudes was detected between the first, second and third cycles of stimulation. Therefore, responses recorded in the presence of drugs were compared with those recorded in the same experiment before the addition of drugs.

### *Data analysis*

All data were digitized (sampling frequencies of 0.04–0.2 kHz) and collected with a Maclab recording system and the program Chart (ADInstruments Pty Ltd, Castle Hill, NSW 2154, Australia). Subsequent analysis was made with the computer programme Igor Pro (Wavemetrics Inc, Lake Oswego, OR 97035, U.S.A.). To assess the effects of drug treatments, the electrophysiological or electrochemical records for individual tissues under each condition were averaged before comparison. The amplitudes of e.j.ps, s.e.j.ps and NA-induced slow depolarizations were determined at their peak. In some electrochemistry experiments, stimulation produced a positive-going artefact which lasted about 100 ms (revealed in tetrodotoxin treated tissues). Therefore, the amplitudes of the oxidation currents (evoked by trains of stimuli at 1 Hz) were determined by averaging the period 100–200 ms following each stimulus. In cases where the individual responses summed during the train of stimuli, the time constant of decay of the signals was estimated and used to correct the baseline value. Comparisons were also made between the integrals of the oxidation currents evoked by trains of 10 stimuli at 1, 2 and 4 Hz. Signals recorded in the absence or presence of desipramine were integrated respectively over periods of 20 or 50 s from the start of stimulation. Stimulus artefacts were blanked before integration of electrochemical signals by setting the first 100 ms following each stimulus to zero.

Data are presented as mean ± s.e.mean. Statistical comparisons of the amplitudes of e.j.ps and of NA-induced slow depolarizations were made by paired $t$ tests. Chi squared tests were used to determine whether the normalized distributions of s.e.j.p. amplitudes recorded in isoprenaline differed from those recorded under control conditions. The electrochemical data were compared by unpaired $t$ tests or one-way analysis of variance followed by Tukey tests. In all tests $P$ values $<0.05$ were considered to be significant.

### Drugs

Desipramine HCl, (−)-isoprenaline HCl, (−)-noradrenaline bitartrate, pargyline HCl, prazosin HCl, (±)-propranolol HCl and tetrodotoxin were supplied by Sigma Chemical Company (Castle Hill, NSW 2154, Australia). ω-Conotoxin GVIA was supplied by Alomone Labs (Jerusalem, Israel). Prazosin was prepared as 1 mM solution in 10% (v/v) N,N-dimetylacetamide in water. The remaining drugs were prepared as concentrated stock solutions in distilled water. The drug solutions were serially diluted in physiological saline to the required final bath concentration. Drugs were delivered to the preparation by replacing the solution perfusing the recording chamber with one containing the drug.

## Results

### Effects of isoprenaline and propranolol on resting membrane potential

In control preparations the resting membrane potential was $-65 \pm 0.9$ mV ($n=11$ tissues, range $-61$ to $-69$ mV). Application of isoprenaline (0.1 μM) and the subsequent addition of propranolol (0.1 μM) had no significant effect on the resting membrane potential (control $= 64.1 \pm 1.5$ mV, isoprenaline $= -64.9 \pm 1.3$ mV and isoprenaline + propranolol $= -63.7 \pm 1.7$ mV, $n=4$ tissues; in each tissue, the resting membrane potential was measured from 4 cells under each condition).

### Effects of isoprenaline and propranolol on e.j.ps evoked by short trains of stimuli

Under control conditions, e.j.ps evoked by 5 stimuli at 1 Hz increased in amplitude (i.e. facilitated) slightly during the train (see Figure 1a, b and c). Bath application of the non-selective β-adrenoceptor agonist, isoprenaline (0.1 μM, $n=7$ experiments), significantly increased the amplitude of all e.j.ps in a train (see Figure 1a and b), the first e.j.p. in the train being most markedly enhanced, subsequent e.j.ps being significantly smaller ($P<0.05$) in amplitude than the first. The increase in e.j.p. amplitude was not associated with any change in the time constant of decay of the e.j.p. (control $= 228 \pm 25$ ms, isoprenaline $= 229 \pm 24$ ms, $n=7$ experiments). Thus, changes in the passive electrical properties of the vascular smooth muscle cannot account for the increase in e.j.p. amplitude produced by isoprenaline (see Cassell *et al*., 1988). The addition of the non-selective β-adrenoceptor antagonist, propranolol (0.1 μM, $n=4$ experiments), abolished the effects of isoprenaline on e.j.p. amplitude (see Figure 1a). Application of propranolol (0.1 μM, $n=4$ experiments) alone was without significant effect on e.j.p. amplitude (Figure 1c). Furthermore, in the presence of propranolol, isoprenaline (0.1 μM) failed to produce a significant effect on e.j.p. amplitude (see Figure 1c).

In 4 experiments, the effects of isoprenaline on e.j.ps evoked by trains of 5 stimuli at 1, 2 and 4 Hz were investigated (e.g. see Figure 2a). At all stimulation frequencies, isoprenaline (0.1 μM) significantly increased ($P<0.05$) the amplitude of every e.j.p. in a train. Comparison between the percentage increase in e.j.p. amplitude evoked by each stimulus during the trains at 1, 2 and 4 Hz revealed no significant difference (see Figure 2b). However, at all frequencies of stimulation the magnitude of the isoprenaline-induced increase in e.j.p. amplitude tended to decline during the train.

### Effects of isoprenaline on s.e.j.ps

To determine whether isoprenaline changed the sensitivity of the vascular smooth muscle to neuronally released ATP, the effect of isoprenaline (0.1 μM) on s.e.j.p. amplitude was investigated. As previously found for the rat tail artery, s.e.j.ps occur at low frequencies (0.1 – 0.01 Hz) under control conditions (Jobling & McLachlan, 1992). In 4 out of 6 experiments the frequency of s.e.j.p. occurrence increased in the presence of isoprenaline (e.g. see Figure 3). This effect varied considerably from preparation to preparation (the mean % change in s.e.j.p. frequency was $145 \pm 36$%, range 66 – 263%, $n=6$ experiments) and was not statistically significant. In all 6 experiments isoprenaline had no significant effect on the distribution of s.e.j.p. amplitudes (e.g. see Figure 3).

### Effects of isoprenaline and propranolol on the NA-induced slow depolarization

Stimulation with short trains of stimuli (5 pulses at 1 Hz) evoked a slow depolarzation which peaked 15 – 20 seconds following the start of the train and had an overall duration of about 1 min (e.g. see Figure 4a). This slow depolarization is generated by the activation by postjunctional $\alpha_2$-adrenoceptors by neuronally released NA (see Cassell *et al*., 1988). Under control conditions the peak amplitude of the slow depolarization varied considerably between preparations (range 0.4 –

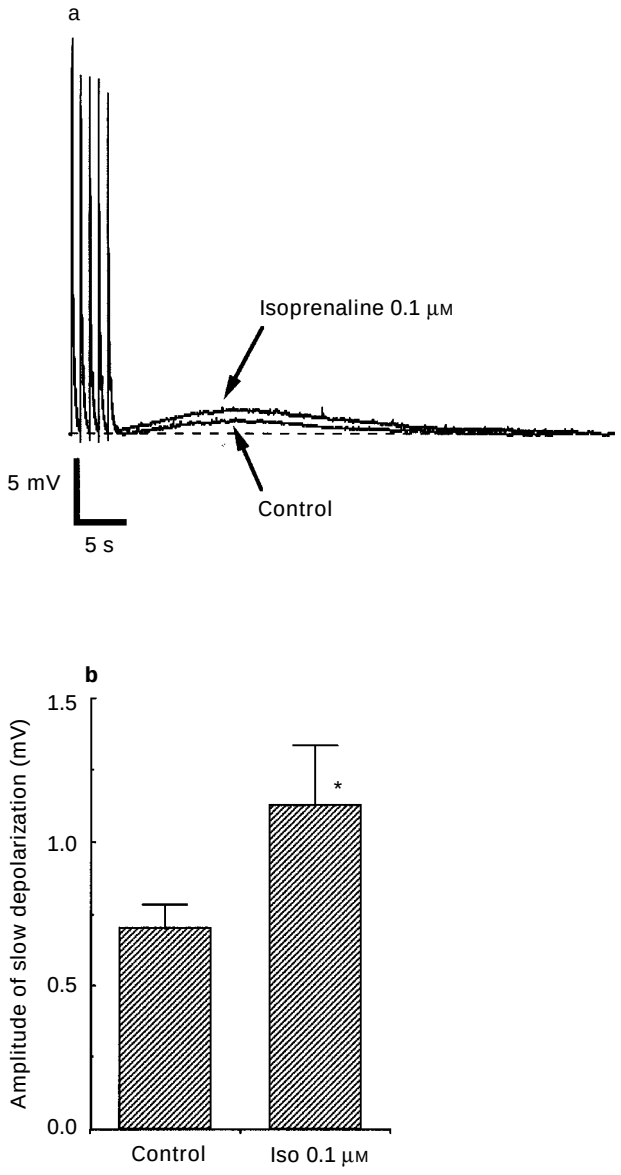


**Figure 4** Effects of isoprenaline (0.1 μM) on the noradrenaline (NA)-induced depolarization. (a) Overlaid traces recorded before and during application of isoprenaline. (b) Graph showing the mean effect of isoprenaline on the amplitude of the NA-induced slow depolarization. Statistical comparisons between control and treated values were made by paired *t* tests (*$P<0.05$).

2 mV, $n=7$ experiments). Isoprenaline (0.1 μM, $n=7$ experiments) significantly increased the amplitude of the slow depolarization evoked by a train of 5 stimuli at 1 Hz (see Figure 4a and b). In contrast, the combined application of isoprenaline (0.1 μM) and propranolol (0.1 μM) had no effect on the slow depolarization (control $=0.7\pm0.1$ mV, isoprenaline + propranolol $=0.7\pm0.2$ mV, $n=6$ experiments).

### *Electrochemical detection of released NA*

The signals recorded by the carbon fibre electrodes were similar to those previously obtained by Gonon *et al*. (1993). Under control conditions a single stimulus evoked a transient increase in the oxidation current that peaked within 100 ms of the stimulus and then decayed within 2 s. During trains of stimuli $\geqslant 1$ Hz, signals evoked by successive stimuli summated with the previous response (see Figure 5a). The signals evoked by trains of 10 stimuli at 1, 2 and 4 Hz were abolished by the voltage-dependent $Na^+$ channel blocker, tetrodotoxin (0.3 μM, $n=2$ experiments), and by the N-type $Ca^{2+}$ channel blocker, ω-conotoxin GVIA (0.1 μM, $n=6$ experiments), consistent with their being due to action potential evoked, $Ca^{2+}$-dependent neurotransmitter release. (It is noteworthy that e.j.ps recorded in the rat tail artery are also markedly reduced in amplitude by ω-conotoxin GIVA, Brock *et al*., 1995). The neuronal uptake inhibitor, desipramine (1 μM, $n=14$ experiments), markedly increased the amplitude of oxidation currents evoked by single stimuli but only slightly increased the amplitude of the summed responses evoked by trains of 10 stimuli at 1, 2 and 4 Hz (see also Stjärne *et al*., 1994). In the presence of desipramine, the decay of all signals was markedly prolonged. The monoamine oxidase inhibitor, pargyline (100 μM, $n=2$ experiments), did not detectably alter electrically-evoked oxidation currents. Finally, perfusing the bath with 25, 50 and 100 nM NA produced an oxidation current that increased linearly with concentration (Figure 5b). These findings support the conclusion (see Gonon *et al*., 1993; Msghina *et al*., 1993, Stjärne *et al*., 1994) that the stimulation-induced oxidation currents result from an increase in NA concentration at the surface of the recording electrode following its release from postganglionic sympathetic nerves.

### *Effects of isoprenaline and propranolol on NA release*

The addition of isoprenaline (0.1 μM), but not propranolol (0.1 μM), to the solution perfusing the recording chamber produced a small background oxidation current. This effect did not change the sensitivity of the carbon fibre electrode to neuronally released NA as, in the presence of propranolol, isoprenaline was without effect on the stimulation-induced oxidation currents (see below).

Isoprenaline (0.1 μM) added before $S_2$ increased the amplitude of the NA-induced oxidation currents evoked by trains of 10 stimuli at 1, 2 and 4 Hz (Figure 5a). In comparison with control tissues ($n=10$), isoprenaline ($n=17$ tissues) significantly increased the $S_2/S_1$ ratios for the integrated oxidation currents at all frequencies of stimulation (see Table 1). Isoprenaline (0.1 μM) also significantly increased the $S_2/S_1$ ratios for the integrated oxidation currents in tissues pretreated with desipramine (1 μM, $n=5$ control and 9 treated tissues, see Table 1).

To make comparisons with the e.j.p. experiments, the amplitudes of the oxidation currents evoked by the first 5 stimuli at 1 Hz were determined. Figure 5c shows the effects of isoprenaline on the $S_2/S_1$ ratios for the oxidation currents evoked by each stimulus during the train. In comparison with control values, isoprenaline significantly increased the $S_2/S_1$ ratios for the responses evoked by the first and the second stimulus in the train, but the $S_2/S_1$ ratios for the responses evoked by the subsequent stimuli were not significantly affected. Figure 5d shows the % change in $S_2/S_1$ ratios in isoprenaline-treated tissues compared to the mean $S_2/S_1$ ratios for control tissues.

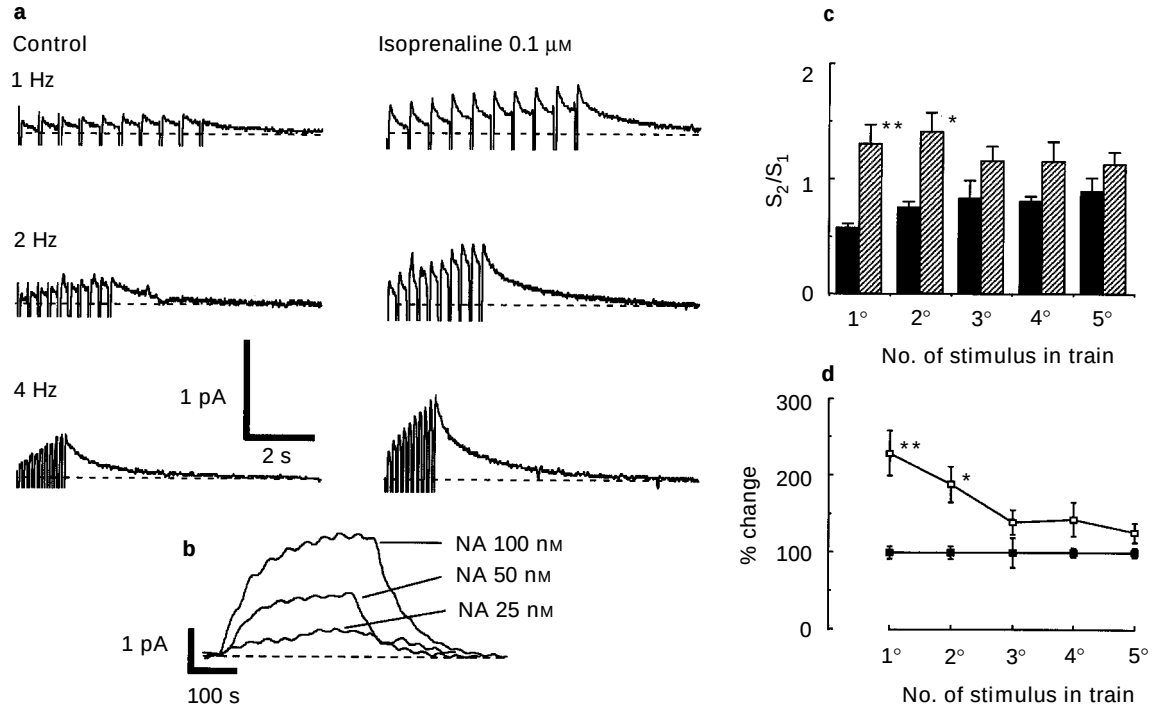


**Figure 5** Effects of isoprenaline (0.1 μM) on stimulation-induced oxidation currents. (a) Traces recorded during trains of 10 stimuli at 1, 2 and 4 Hz before and during application of isoprenaline in a single preparation. (b) Traces recorded during perfusion of the recording chamber with physiological saline containing 25, 50 and 100 nM noradrenaline (NA). (c) The mean $S_2/S_1$ ratios for each of the first 5 stimuli of trains at 1 Hz in control (solid columns, $n=9$) and isoprenaline treated (hatched columns, $n=16$) tissues. (d) The mean % change in $S_2/S_1$ ratio for the first 5 stimuli of trains at 1 Hz in control (■) and isoprenaline treated (□) tissues. Statistical comparisons were made by unpaired $t$ tests (*$P<0.05$, **$P<0.01$).

**Table 1** The $S_2/S_1$ ratios for the integrated oxidation currents evoked in the presence and absence of desipramine (DMI, 1 μM) by trains of 10 stimuli at 1, 2, and 4 Hz in control and isoprenaline (0.1 μM) treated tissues

| | *Control* ($n=9$) $S_2/S_1$ | *Isoprenaline* ($n=17$) $S_2/S_1$ | *DMI* ($n=5$) $S_2/S_1$ | *Isoprenaline + DMI* ($n=9$) $S_2/S_1$ |
|---|---|---|---|---|
| 1 Hz | 0.79±0.04 | 1.64±0.16** | 0.77±0.06 | 2.17±0.15** |
| 2 Hz | 0.86±0.03 | 1.51±0.10** | 0.90±0.02 | 1.99±0.18** |
| 4 Hz | 0.94±0.05 | 1.43±0.06** | 0.86±0.03 | 1.81±0.19** |

Data shown are means±s.e.mean. Pairs of values were compared by unpaired $t$ tests. **$P<0.01$.

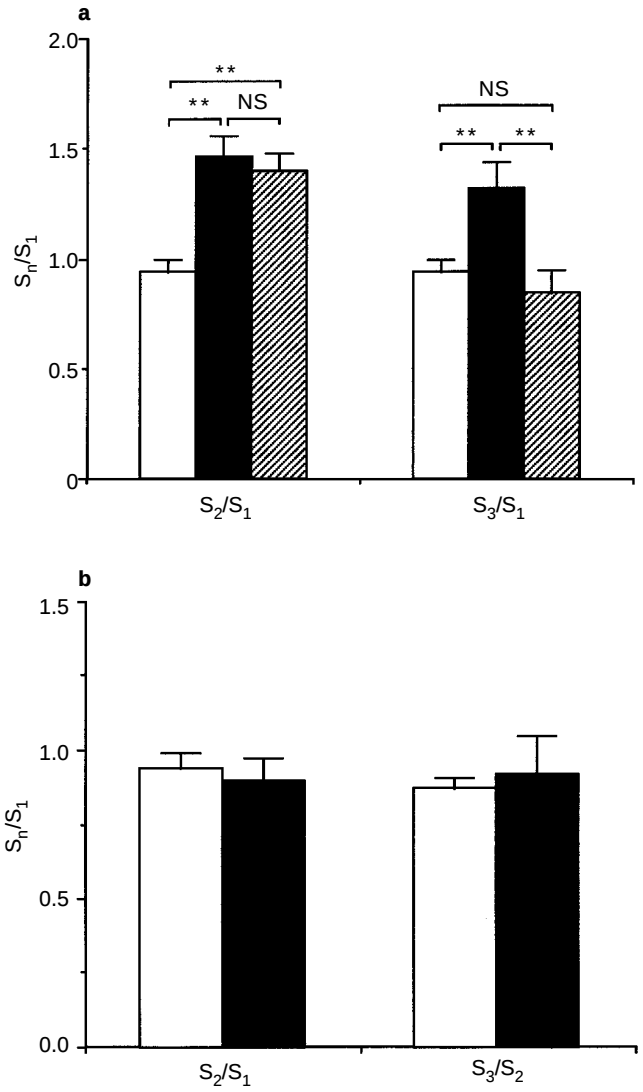


**Figure 6** Effects of isoprenaline (0.1 μM) and propranolol (0.1 μM) on integrated oxidation currents evoked by trains of 10 stimuli at 4 Hz. (a) The $S_2/S_1$ and $S_3/S_1$ ratios for control tissues (open columns, $n=10$) and for tissues treated with isoprenaline only (i.e. at $S_2$ and $S_3$, solid columns, $n=7$) and isoprenaline (at $S_2$) followed by propranolol (at $S_3$, hatched columns, $n=5$). Statistical comparisons were made by one-way analysis of variance followed by Tukey tests (*$P<0.05$, **$P<0.01$, NS = not significant). (b) The $S_2/S_1$ and $S_3/S_1$ ratios for control tissues (open columns, $n=10$) and for tissues treated with propranolol (at $S_2$) followed by isoprenaline (at $S_3$, solid columns, $n=5$). Statistical comparisons revealed no significant difference.

As with e.j.ps, the potentiating effect of isoprenaline on the stimulation-induced oxidation currents declined during the train.

At all frequencies of stimulation the effects of isoprenaline (0.1 μM) on the stimulation-induced oxidation currents were significantly inhibited when propranolol (0.1 μM, $n=5$ tissues) was added before $S_3$. Figure 6a shows the effects of isoprenaline alone (i.e. present during $S_2$ and $S_3$) and for isoprenaline (at $S_2$) followed by propranolol (at $S_3$) on the $S_2/S_1$ and $S_3/S_1$ ratios for the integrated responses evoked by 10 stimuli at 4 Hz. In separate experiments, propranolol (0.1 μM, $n=5$ tissues) added before $S_2$ had no significant effect on the responses evoked by trains of 10 stimuli at 1, 2 and 4 Hz and, in the presence of this agent, isoprenaline (0.1 μM, $n=5$ tissues), added before $S_3$, was also without effect. Figure 6b shows the effects of propranolol (at $S_2$) followed by isoprenaline (at $S_3$) on the $S_2/S_1$ and $S_3/S_1$ ratios for the integrated responses evoked by 10 stimuli at 4 Hz.

### *A comparison of the effects of isoprenaline on e.j.ps and on NA-induced oxidation currents*

Because of the different temporal characteristics of the e.j.ps and the oxidation currents, only the responses evoked by 5 stimuli at 1 Hz have been compared. The % change produced by isoprenaline in e.j.p. amplitudes and in $S_2/S_1$ ratios was significantly greater (unpaired $t$ test, $P<0.05$) for responses evoked by the first stimulus (e.j.ps = 195±25%, $n=7$; $S_2/S_1$ ratio = 229±29%, $n=16$) than for responses evoked by the fifth stimulus in the train (e.j.ps = 139±5%, $n=7$; $S_2/S_1$ ratio = 126±12%, $n=16$). However, comparisons between the % change in e.j.p. amplitudes and in $S_2/S_1$ ratios evoked by the first stimulus or the fifth stimulus revealed no significant difference. When the effects of non-linear summation on e.j.p. amplitude were estimated (assuming a reversal potential of 0 mV for the current underlying the e.j.p.; see Brock & Cunnane, 1992), there was only a very small increase in the % change in e.j.p. amplitudes in isoprenaline-treated tissues (results not shown).

## Discussion

E.j.ps in the rat tail artery are most probably caused by neuronally released adenosine 5′-triphosphate (ATP) acting at $P_{2X}$-purinoceptors (Sneddon & Burnstock, 1984; McLaren *et al.*, 1995). The results of the present study therefore indicate that ATP release is enhanced by activating prejunctional β-adrenoceptors. A similar facilitatory effect of isoprenaline on e.j.ps has been shown in guinea-pig mesenteric arteries (Kuriyama & Makita, 1984; Ishikawa & Sperelakis, 1989; Nozaki & Sperelakis, 1991). In the present study this facilitatory effect cannot be accounted for by changes in the passive electrical properties of the vascular smooth muscle as this agent had no effect on the resting membrane potential or the time course of the e.j.ps. Similarly, in guinea-pig mesenteric arteries the isoprenaline-induced increase in e.j.p. amplitude could not be attributed to changes in the passive electrical properties of the vascular smooth muscle (Ishikawa & Sperekalis, 1989; Nozaki & Sperelakis, 1991). In addition, in rat tail artery, it is unlikely that isoprenaline alters the sensitivity of the vascular smooth muscle to neuronally released ATP, as the distributions of s.e.j.p. amplitudes were not detectably altered.

The present study shows that continuous amperometry can be used to monitor on an impulse-by-impulse basis the release of NA (see also Gonon *et al.*, 1993). However, it should be noted that this technique does not measure the amount of NA released, but the rise in concentration of endogenous NA at the surface of the carbon electrode following activation of sympathetic nerves (i.e. NA release minus NA clearance by reuptake and diffusion, see Mermet *et al.*, 1990). A previous study has shown that released NA in the tail artery is cleared primarily by neuronal uptake (uptake$_1$) and diffusion (Stjärne

*et al*., 1994), blockade of extraneuronal uptake of NA (uptake$_2$) having a negligible effect on signals recorded electrochemically. Importantly, inhibiting neuronal uptake with desipramine did not inhibit the facilitatory effect of isoprenaline on the stimulus-induced oxidation current (see Table 1). Thus activation of prejunctional β-adrenoceptors most probably increases the amount of NA released from sympathetic nerve terminals. This conclusion is also supported by the finding that isoprenaline increased the amplitude of the nerve stimulation evoked slow depolarization.

During short trains of stimuli at 1 Hz, the facilitatory effects of isoprenaline on e.j.ps and on the stimulation-induced oxidation currents declined during the train. Furthermore, comparison between the % change produced by isoprenaline in e.j.p. amplitudes and in the $S_2/S_1$ ratios evoked by the first or the fifth stimulus of a train at 1 Hz revealed no significant difference. These findings suggest that the release of NA and ATP are modulated in parallel when prejunctional β-adrenoceptors are activated by isoprenaline. An inverse relationship between train length and the facilitatory effects of isoprenaline on the nerve stimulation-induced overflow of [$^3$H]-NA from labelled rat tail artery has previously been found (Medgett *et al*., 1980). One possible explanation is that activation of prejunctional $\alpha_2$-adrenoceptors by neuronally released NA limits the facilitatory effects produced when prejunctional β-adrenoceptors are activated. Consistent with this suggestion, blockade of prejunctional $\alpha_2$-adrenoceptors has been shown to increase the facilitatory effect of isoprenaline on nerve stimulation-induced [$^3$H]-NA overflow from a number of tissues including rat atria (Medgett *et al*., 1980; Majewski & Rand, 1981) and the rat anococcygeus muscle (Li *et al*., 1988).

The results of the present study differ from those of Gonçalves *et al*. (1996), who showed that activation of prejunctional β-adrenoceptors in guinea-pig vas deferens increased [$^3$H]-NA overflow but decreased that of endogenous ATP. In the experiments of Gonçalves *et al*. (1996), it is unlikely that the stimulation-induced overflow of ATP originated from the smooth muscle, as the postjunctional effects of the released transmitters were blocked. However, it remains to be established whether the stimulation-induced overflow of ATP represents only that which is released from the sympathetic nerves at neuromuscular junctions. It should be noted that, in contrast with the present study, the tissues in the study of Gonçalves *et al*. (1996) were stimulated with relatively long trains of stimuli (210 pulses, 7 Hz) and that under these conditions the differential effects of activating prejunctional β-adrenoceptors on NA and ATP release may have been revealed. Two additional findings, at first sight, appear to support the view that β-adrenoceptor activation inhibits purinergic transmission in the guinea-pig vas deferens: (1) isoprenaline selectivity inhibited the first phase of the neurally evoked contraction which is due to purinoceptor activation (Gonçalves *et al*., 1996; Driessen *et al*., 1996); (2) the amplitudes of e.j.ps recorded by the sucrose gap technique were reduced by isoprenaline (Sjöstrand, 1973). However, the effect of isoprenaline on e.j.p. amplitude was associated with a hyperpolarization of the smooth muscle membrane and may have resulted from an increase in membrane conductance. Furthermore, in tissues treated with isoprenaline, muscle action potentials triggered by summation of e.j.ps were substantially reduced in amplitude (Sjöstrand, 1973). This latter effect may explain the selective inhibitory action of isoprenaline on the first phase of contraction, as this component is dependent on voltage-dependent $Ca^{2+}$ entry (see Blakeley *et al*., 1981). Thus, the existing evidence does not necessarily indicate that release of ATP at sympathetic neuroeffector junctions in the guinea-pig vas deferens is inhibited by activating prejunctional β-adrenoceptors. Isoprenaline has also been shown to reduce the amplitude of e.j.ps in rabbit mesenteric artery (Kuriyama & Makita, 1984), but its effect on NA release in this tissue is unknown.

In conclusion, activation of prejunctional β-adrenoceptors affects in a parallel manner the release of NA and ATP from the sympthetic nerves innervating the rat tail artery. Previous studies of the inhibitory effects of activating prejunctional $\alpha_2$-adrenoceptors on neurotransmitter release in rat tail artery also suggest that the release of these two co-transmitters is modulated in parallel (Msghina *et al*., 1992). Taken together, these findings are consistent with the hypothesis that NA and ATP are released from the same population of nerve terminals and presumably from the same vesicles.

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