



Electrochemical and electrophysiological characterization of neurotransmitter release from sympathetic nerves supplying rat mesenteric arteries

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- 1 Characteristic features of noradrenaline (NA) and adenosine 5'-triphosphate (ATP) release from postganglionic sympathetic nerves in rat small mesenteric arteries *in vitro* have been investigated on an impulse-by-impulse basis. NA release was measured using continuous amperometry and ATP release was monitored by intracellular recording of excitatory junction potentials (e.j.ps).
- 2 Electrical stimuli evoked transient increases in oxidation current. During trains of ten stimuli at 0.5–4 Hz there was a depression in the amplitude of oxidation currents evoked following the first stimulus in the train.
- 3 The neuronal NA uptake inhibitor, desmethylimipramine (1 μ M), increased the amplitude of the summed oxidation current evoked by ten stimuli at 1 Hz and slowed the decay of oxidation currents evoked by trains of ten stimuli at 1 and 10 Hz.
- 4 The α_2 -adrenoceptor antagonist, idazoxan (1 μ M), increased the amplitudes of the oxidation currents evoked during trains of ten stimuli at 0.5–10 Hz but had no effect on the oxidation currents evoked by the first stimulus in the train.
- 5 Idazoxan (1 μ M) increased the amplitude of all e.j.ps evoked during trains of stimuli at 0.5 and 1 Hz. In addition, the facilitatory effect of idazoxan on e.j.ps was significantly greater than that on oxidation currents.
- 6 The findings indicate that NA release from sympathetic nerves supplying small mesenteric arteries is regulated by activation of presynaptic α_2 -adrenoceptors and that clearance of released NA in this tissue depends, in part, upon neuronal uptake. The different effects of idazoxan on the oxidation currents and e.j.ps may indicate that the release of NA and ATP is differentially modulated.

Keywords: Noradrenaline release; ATP release; amperometry; rat mesenteric artery; excitatory junction potentials

Abbreviations: ATP, adenosine 5'-triphosphate; DMI, desmethylimipramine; e.j.ps, excitatory junction potentials; NA, noradrenaline

Introduction

The release of noradrenaline (NA) from perivascular sympathetic nerve endings has primarily been investigated using tissues labelled with tritiated NA (e.g. Ramme *et al.*, 1986; Cox *et al.*, 1996). In a smaller number of studies the release of endogenous NA has been measured by high-performance liquid chromatography (e.g. Buchholz & Duckles, 1992). Because of the relatively low sensitivity of these techniques, it has usually been necessary to measure the efflux of NA evoked by long trains of electrical stimuli. For this reason, at present, almost nothing is known about the characteristics of NA release from perivascular sympathetic nerves on an impulse-by-impulse basis.

To investigate this question, Gonon *et al.* (1993) introduced continuous amperometry to monitor in 'real' time NA release from sympathetic nerves innervating rat tail artery. This technique measures the stimulus evoked increase in NA concentration at the adventitial surface of the artery as an oxidation current, the amplitude of which is linearly related to concentration (Gonon *et al.*, 1993; Brock *et al.*, 1997). Using this electrochemical recording approach, it has been possible to monitor NA release in rat tail artery on an impulse-by-impulse

basis during low frequency trains of stimulation (see Msghina *et al.*, 1993; Brock *et al.*, 1997; Brock & Cunnane, 1999).

The data obtained using continuous amperometry have been compared with electrophysiological measures of adenosine 5'-triphosphate (ATP) release from the sympathetic nerves (Brock *et al.*, 1997; Brock & Cunnane, 1999). The electrophysiological studies have used intracellularly recorded excitatory junction potentials (e.j.ps) which provide an indirect measure of junctionally released ATP (see Brock & Cunnane, 1993). The results indicate that a variety of agents (e.g. β -adrenoceptor agonists and Ca^{2+} channel blockers) that are known to modify neurotransmitter release from sympathetic nerve terminals, similarly affect the oxidation currents and e.j.ps. Taken together these findings provide strong supporting evidence for the hypothesis that NA and ATP are co-released from the perivascular sympathetic nerve terminals in rat tail artery.

In the present study we have used continuous amperometry and intracellular recording of e.j.ps to monitor the release of NA and ATP evoked by short trains of stimuli from sympathetic nerves supplying rat mesenteric arteries with diameters $<250 \mu\text{m}$. Vessels of this diameter contribute significantly to the regulation of blood flow to the intestine (Christensen & Mulvany, 1993; Fenger-Gron *et al.*, 1997). In particular, this study has determined the basic characteristics

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of NA release on an impulse-by-impulse basis during short trains of electrical stimuli and has investigated various mechanisms that may contribute to the kinetics of NA clearance. In addition, the effects of blocking presynaptic α_2 -adrenoceptors on oxidation currents and e.j.ps were investigated.

Methods

Female outbred Wistar rats (150–200 g) were killed by an overdose of pentobarbitone sodium (100 mg kg⁻¹, i.p.). Segments of mesentery containing first and second order mesenteric arteries were dissected and pinned to the Sylgard (Dow-Corning) covered base of a 2 ml recording chamber. The chamber was perfused continuously at 3–5 ml min⁻¹ with physiological saline of the following composition (mM): NaCl 133.4, NaHCO₃ 16.3, NaH₂PO₄ 1.3, CaCl₂ 2.0, KCl 4.7, MgCl 1.2 and glucose 9.8. The physiological saline was gassed with a mixture of 95% O₂ and 5% CO₂ (to pH 7.4) and maintained at 35–36°C. The proximal end of the mesenteric artery and vein were cleared of fat and drawn into a suction electrode and the perivascular nerves excited by electrical field stimulation (0.5 ms, 20 V). Electrochemical and electrophysiological recordings were made from the surface of the second order arteries (Dunn & Gardiner, 1995) at a site 3–5 mm distal of the mouth of the suction stimulating electrode. At this site the connective tissue and fat cells were carefully removed to reveal the surface of the artery. Usually two tissues from the same animal were used, with the second being held in oxygenated physiological saline until required. No difference was detected in the properties or responses of the two preparations.

Electrochemical recording

The release of endogenous NA was measured by continuous amperometry using a technique similar to that previously described by Brock *et al.* (1997). The recording electrodes were constructed as previously described (Brock *et al.*, 1997) but with the exposed length of carbon fibre cut to about 200 μ m. Before use the electrode tips were dipped for 15–30 min in a 2.5% (w v⁻¹) solution of Nafion (Aldrich Chemical Company, Castle Hill, NSW, Australia) and then left to dry for at least 1 h. This treatment reduced the decline in electrode sensitivity observed during prolonged periods of recording (see Brock *et al.*, 1997).

The carbon fibre electrodes were mounted at about 30° to the horizontal and placed gently against the surface of the artery so that an approximately 100 μ m length of carbon fibre was in contact with the surface of the artery. 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 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.

After placement of the carbon fibre microelectrode, tissues were left for a 30 min period. Thereafter, in some experiments, a frequency-response curve was generated by stimulation with three trains of ten pulses (separated by 90 s) at frequencies between 0.5 and 10 Hz. In other experiments, tissues were stimulated with three trains of ten stimuli at 0.5, 1 and 10 Hz (each train separated by 90 s) before and 20 min following addition of experimental drugs to the superfusing solution. In control experiments in which no drug was added, the amplitude of the oxidation currents

did not change detectably between the recording periods ($n=6$, paired *t*-tests).

Electrophysiological recording

In separate experiments intracellular recordings were made from smooth muscle cells located near the adventitial-medial border of the mesenteric artery with borosilicate glass microelectrodes (80–160 M Ω) filled with 0.5 M KCl and connected to an Axoclamp bridge amplifier (Axon Instruments, Inc, Foster City, CA, U.S.A.). The criteria for accepting impalements were the same as those adopted by Brock and Van Helden (1995). Membrane potentials were determined upon withdrawal of the microelectrode. The effects of idazoxan on e.j.ps evoked by trains of ten stimuli at 0.5 and 1 Hz amplitude were determined in single cell experiments in which both control and test recordings were made during the same impalement. In control experiments, no significant change in e.j.p. amplitude was observed during recording periods lasting 20–30 min ($n=6$, paired *t*-test).

Data analysis

All data were digitized (sampling frequencies of 0.04–0.1 kHz) and collected with a MacLab recording system and the program Scope (ADIInstruments Pty Ltd, Castle Hill, NSW, Australia). Subsequent analysis was made with the computer program Igor Pro (Wavemetrics, Lake Oswego, OR, U.S.A.). The electrochemical and electrophysiological records for individual tissues under each condition were averaged before measurements were made. In some electrochemistry experiments, stimulation produced a positive- or negative-going artefact that lasted about 100 ms (revealed in Cd²⁺ or tetrodotoxin treated tissues). Therefore, the amplitudes of the oxidation currents evoked during trains of stimuli at 0.5 and 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 mono-exponential 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 the trains of stimuli at 0.5–10 Hz. Before integration of the electrochemical signals, the stimulus artefacts were blanked by setting the first 100 ms following each stimulus to zero.

Data are presented as mean \pm s.e.mean. The mono- and bi-exponential decay of the signals were fitted using the curve fitting functions in the computer program Igor Pro. Unless otherwise indicated, statistical comparisons were made using paired *t*-tests. In all tests *P* values <0.05 were considered to be significant. In all cases the number *n* refers to the number of tissues studied.

Drugs

Idazoxan, desmethylimipramine (DMI) prazosin and tetrodotoxin were supplied by Sigma Chemical Company (Castle Hill, NSW, Australia). Corticosterone was supplied by Aldrich Chemical Company (Castle Hill, NSW, Australia).

Results

Electrochemical detection of released NA

Figure 1a shows typical responses evoked by a single stimulus and by trains of ten stimuli at 0.5–10 Hz. A single stimulus

produced a transient increase in oxidation current which peaked within 100 ms of the stimulus and then decayed monoexponentially within 2–3 s (decay time constant = 0.50 ± 0.11 s, $n=9$ experiments). During trains of ten pulses at frequencies of 0.5, 1, 2 and 4 Hz each stimulus during the train evoked a transient increase in oxidation current and the signals evoked by each successive stimulus summed with that remaining from the previous stimuli to produce a sustained increase in oxidation current. The first stimulus in the trains of stimuli at 0.5–4 Hz usually evoked the largest transient increase in oxidation current, the responses to subsequent stimuli being smaller in amplitude (Figure 1a and b, see also Figure 3a).

During trains of ten stimuli at 10 Hz, the stimulus artefact prevented the signals evoked by the individual stimuli being discerned, but summation of these signals produced large amplitude oxidation currents (Figure 1a). In six tissues the integrals of the oxidation currents evoked by the trains of ten stimuli at 0.5–10 Hz increased significantly with the frequency of stimulation (0.5 Hz, 5.1 ± 1.5 pA s $^{-1}$; 1 Hz, 5.9 ± 1.7 pA s $^{-1}$; 2 Hz, 7.4 ± 2.6 pA s $^{-1}$; 4 Hz, 10.1 ± 4.4 pA s $^{-1}$; 10 Hz 14.6 ± 6.5 pA s $^{-1}$; repeated measures ANOVA $P < 0.05$).

At the end of the trains of stimuli, the decay of the oxidation currents was best fitted by the sum of two exponentials. Table 1 shows the fast (τ_1) and slow (τ_2) time constants of decay for the oxidation currents evoked by ten pulses at 1, 2, 4 and 10 Hz. The value of τ_1 and τ_2 did not change over the range of stimulation frequencies used (repeated measures ANOVA).

Figure 1b shows a trace recorded during the sequential addition of 10, 30, 100 and 300 nM NA to the solution

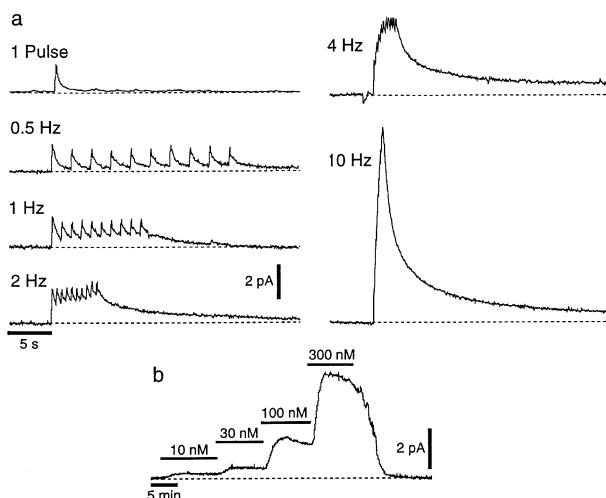


Figure 1 NA-induced oxidation currents recorded amperometrically with a carbon fibre electrode. (a) The responses evoked by a single electrical stimulus and by trains of ten electrical stimuli at 0.5, 1, 2, 4 and 10 Hz. (b) Trace recorded during the sequential addition of 10, 30, 100 and 300 nM NA to the physiological saline perfusing the recording chamber.

Table 1 Effects of stimulation frequency on the fast (τ_1) and slow (τ_2) time constants of decay of oxidation currents

Stimulus frequency (Hz)	τ_1 (s)	τ_2 (s)
1	0.50 ± 0.08	6.2 ± 0.3
2	0.62 ± 0.10	8.7 ± 1.8
4	0.73 ± 0.15	8.3 ± 2.4
10	0.63 ± 0.06	6.2 ± 0.4

perfusing the recording chamber. No electrochemical signals were evoked by electrical stimulation when the electrode potential was held at 0 V, a value below the oxidation potential for NA ($n=9$, Figure 2a). The oxidation currents evoked by electrical stimulation were abolished by tetrodotoxin (0.3 μ M, $n=6$, Figure 2b) and by the non-selective Ca^{2+} channel blocker, Cd^{2+} (0.1 mM, $n=18$, Figure 2c).

Effects of idazoxan on electrochemical signals

In six experiments, application of the α_2 -adrenoceptor antagonist, idazoxan (1 μ M), had no effect on the oxidation currents evoked by the first stimulus in the trains of ten pulses at 0.5 and 1 Hz, but those evoked by subsequent stimuli were significantly increased in amplitude (Figure 3a, b and d). The amplitude of the summed oxidation current measured at the end of the trains of stimuli at 0.5 and 1 Hz was increased by $165 \pm 45\%$ ($P < 0.01$) and $223 \pm 37\%$ ($P < 0.01$) respectively. Idazoxan also increased the amplitude of the oxidation currents evoked by ten pulses at 10 Hz by $63 \pm 10\%$ (Figure 3c, $n=6$, $P < 0.01$). The idazoxan-induced increase in amplitude at 1 and 10 Hz occurred without any significant change in values of τ_1 or τ_2 for the bi-exponential fits for the

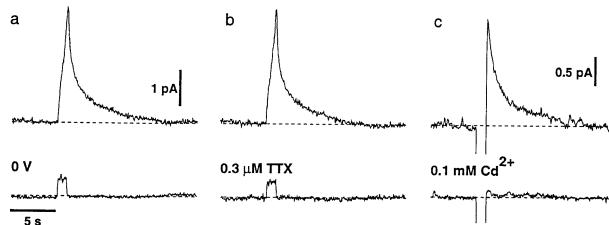


Figure 2 Effects of changing the electrode potential and of tetrodotoxin (0.3 μ M, TTX) and Cd^{2+} (0.1 mM) on NA-induced oxidation currents evoked by trains of ten stimuli at 10 Hz. (a and b) Responses recorded in a single tissue under control conditions (upper traces) and when the electrode potential was held at 0 V (a, lower trace) and during application of TTX (b, lower trace). (c) Responses in another tissue before (upper trace) and during application of Cd^{2+} (lower trace). The ordinate scale bar in (a) also applies in (b) and the abscissa scale bar in (a) applies in (b) and (c).

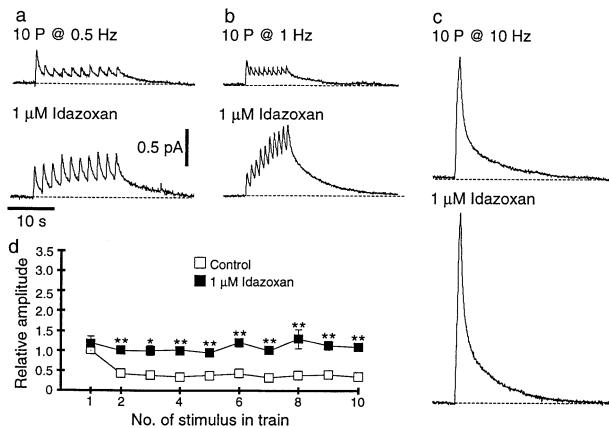


Figure 3 Effects of the α_2 -adrenoceptor antagonist, idazoxan (1 μ M), on NA induced oxidation currents. (a–c) NA-induced oxidation current evoked by ten stimuli at 0.5 (a), 1 (b) and 10 Hz (c) before (upper traces) and 20 min following addition of idazoxan (lower traces). The scale bars in (a) also apply in (b) and (c). (d) Graph showing the effect of idazoxan on the amplitude of oxidation currents evoked during trains of ten stimuli at 1 Hz ($n=6$) * $P < 0.05$, ** $P < 0.01$.

decay phase of the oxidation currents (1 Hz control $\tau_1=0.47\pm 0.08$ s, $\tau_2=10.2\pm 2.4$ s, 1 Hz idazoxan $\tau_1=0.8\pm 0.16$ s, $\tau_2=9.5\pm 1.4$ s; 10 Hz Control $\tau_1=0.77\pm 0.08$ s, $\tau_2=7.9\pm 1.0$ s, 10 Hz idazoxan $\tau_1=0.77\pm 0.07$ s, $\tau_2=8.7\pm 1.0$ s).

In idazoxan treated tissues ($n=5$), application of the α_1 -adrenoceptor antagonist prazosin (0.1 μ M) did not change the amplitude or the time course of the electrochemical signals evoked by ten pulses at 1 and 10 Hz (1 Hz percentage change in amplitude = $-7\pm 4\%$, in $\tau_1=-5\pm 21\%$ and in $\tau_2=-5\pm 5\%$; 10 Hz percentage change in amplitude = $-2\pm 6\%$, in $\tau_1=-2\pm 2\%$ and in $\tau_2=-5\pm 2\%$).

Effect of DMI on electrochemical signals

Application of the neuronal NA uptake ($uptake_1$) blocker, DMI (1 μ M), did not significantly change the amplitude of oxidation currents evoked by single stimuli (Figure 4a and b, percentage change = $-11\pm 11\%$, $n=7$) or by ten stimuli at 10 Hz (Figure 4e and f, percentage change = $+11\pm 7$, $n=9$) but significantly increased the amplitude of the summed oxidation current measured at the end of the trains of stimuli at 1 Hz by $39\pm 12\%$ (Figure 4c and d, $P<0.05$, $n=6$). In tissues treated with idazoxan to block α -adrenoceptor mediated autoinhibition, DMI still had no effect on the amplitude of responses to ten stimuli at 10 Hz (percentage change = $-1\pm 8\%$, $n=5$) but significantly increased the amplitude of the summed oxidation current evoked by ten stimuli at 1 Hz (percentage increase = $20\pm 8\%$, $P<0.05$, $n=5$). Thus the failure to detect an effect of DMI on the amplitude of responses evoked by ten stimuli at 10 Hz cannot be attributed to an increase in α -adrenoceptor-mediated autoinhibition.

DMI had no significant effect on time course of decay of the oxidation currents evoked by single stimuli (Figure 4b) but slowed the decay of the signals evoked by trains of ten stimuli at 1 and 10 Hz (Figure 4d and f). As with the control signals,

the decay phase of the oxidation currents evoked by single stimuli in the presence of DMI was best fitted by a single exponential while that of the signals evoked by trains at 1 and 10 Hz was best fitted by the sum of two exponentials. In comparison with the control values, DMI had no significant effect on τ_1 for the oxidation currents evoked by trains at 1 and 10 Hz but increased τ_2 for both responses (Figure 4d and f).

Effects of corticosterone on electrochemical signals

In tissues treated with DMI (1 μ M) and idazoxan (1 μ M), the addition of the uptake₂ blocker, corticosterone (10 μ M), did not significantly change the amplitude or time course of the electrochemical signals evoked by ten pulses at 1 and 10 Hz (1 Hz percentage change in amplitude = $-7\pm 5\%$, in $\tau_1=8\pm 18\%$ and in $\tau_2=8\pm 5\%$, $n=5$; 10 Hz percentage change in amplitude = $-5\pm 5\%$, in $\tau_1=-2\pm 5\%$ and in $\tau_2=-2\pm 5\%$, $n=5$), indicating that under the conditions of the experiment uptake₂ does not contribute significantly to the clearance of NA in rat mesenteric arteries. This finding is similar to that reported for the rat tail artery (Stjärne *et al.*, 1994).

Effects of idazoxan on e.j.ps

The effects of idazoxan (1 μ M) on e.j.ps evoked by trains of ten stimuli at 0.5 and 1 Hz were also investigated. Under control conditions the e.j.ps evoked by the first five or so stimuli at 0.5 and 1 Hz initially increased in amplitude and then decreased to plateau level which was maintained for the duration of the train of stimuli (Figure 5a–c). Application of the α_2 -adrenoceptor antagonist, idazoxan (1 μ M), produced a pronounced increase in the amplitudes of e.j.ps evoked during the trains of stimuli at 0.5 and 1 Hz (Figure 5a and b). In contrast to the electrochemical signals, idazoxan also significantly increased the amplitude of the e.j.ps evoked by the first stimulus in the trains of stimuli at 0.5 and 1 Hz (Figure 5a–c). However, as with the electrochemical signals, the magnitude of the idazoxan-induced facilitation of e.j.p

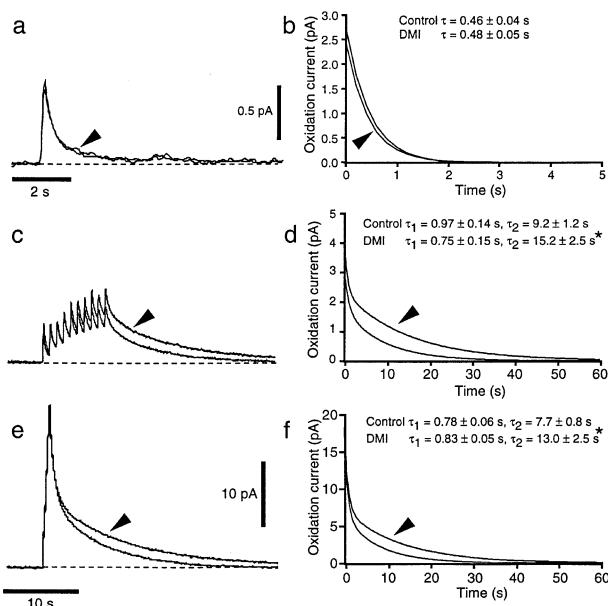


Figure 4 Effects of the NA uptake inhibitor desmethylimipramine (1 μ M, DMI) on NA-induced oxidation currents evoked by a single stimulus (a and b) and by trains of ten stimuli at 1 (c and d) and 10 Hz (e and f). (a, c and e) Traces recorded before and 20 min following addition of DMI (indicated by the arrowhead). The scale bars in (e) also apply in (c). (b, d and f) Graphs showing the mean effects of DMI (indicated by the arrowhead) on the decay of the electrochemical signals ($n=7$ in (b), 6 in (d) and 9 in (f)). * $P<0.05$.

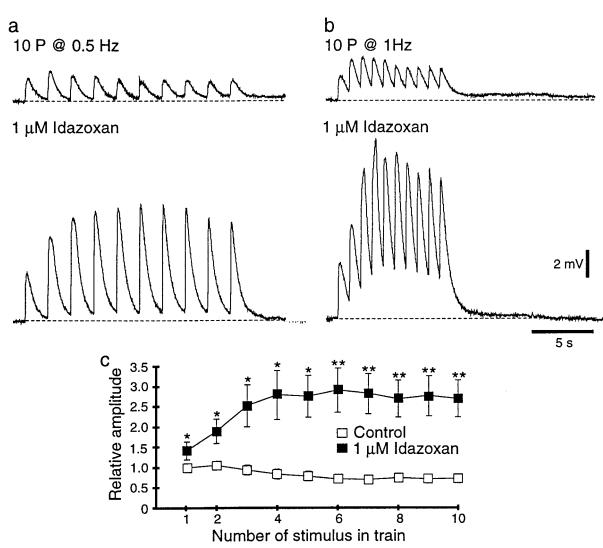


Figure 5 Effects of the α_2 -adrenoceptor antagonist, idazoxan (1 μ M), on intracellularly recorded excitatory junction potentials (e.j.ps). (a and b) E.j.ps evoked by ten stimuli at 0.5 (a) and 1 Hz (b) before (upper trace) and 20 min following addition of idazoxan (lower traces). The scale bars in (b) also apply in (a). (c) Graph showing the effect of idazoxan on the amplitude of e.j.ps evoked during trains of ten stimuli at 1 Hz ($n=6$). * $P<0.05$, ** $P<0.01$.

amplitude increased during the trains of stimuli (Figure 6). Application of idazoxan did not change the smooth muscle resting membrane potential (control = -62 ± 1 mV; idazoxan = -61 ± 1 mV; $n=6$) or the e.j.p time constant of decay (control = 435 ± 48 ms; idazoxan = 428 ± 39 ms; $n=6$). Therefore none of the observed effects of idazoxan on e.j.ps can be attributed to changes in the electrical properties of the vascular smooth muscle.

The findings with idazoxan indicate that in the rat mesenteric artery both NA and ATP release are modulated by α -adrenoceptor-mediated autoinhibition. However, in this tissue, the facilitatory effect of idazoxan on e.j.ps was significantly greater than that on NA-induced oxidation currents (Figure 6, unpaired *t*-test $P<0.05$).

Discussion

Despite indirect evidence from functional studies implicating NA as the principal sympathetic neurotransmitter in small mesenteric arteries isolated from rats (Nilsson *et al.*, 1986; Angus *et al.*, 1988; Stephens *et al.*, 1991), the present study represents the first direct demonstration of the release of endogenous NA from the sympathetic nerves supplying these resistance vessels. Electrical field stimulation of these arteries produced amperometric signals which were abolished in the presence of the voltage-dependent Na^+ channel blocker, tetrodotoxin, or the Ca^{2+} channel blocker, Cd^{2+} , consistent with their being due to action potential evoked, Ca^{2+} -dependent neurotransmitter release. Furthermore, the amperometric signals were only observed when the voltage of the recording electrode was held above the oxidation potential for NA (i.e. +0.3 V) and not at potentials below this value (e.g. 0 V). Similar observations have previously been made for NA-induced electrochemical signals recorded at the adventitial surface of the rat tail artery (Mermet *et al.*, 1990; Gonon *et al.*, 1993; Brock *et al.*, 1997). Further support that the electrically evoked amperometric signals measured in the small mesenteric arteries represent neurally released NA is provided by the effects of the uptake₁ inhibitor, DMI, and the α_2 -adrenoceptor antagonist, idazoxan (see below).

The NA-induced signals recorded using this electrochemical technique are measured at the adventitial surface of the artery and not at the sites of NA release. It would be expected that at the sites of release the concentration of NA declines very rapidly and that diffusion, even over a few μm , results in a pronounced slowing in the time course of signals recorded at the adventitial surface (see Crank, 1967). In addition, the

concentration of NA detected at the recording electrode is determined by the amount of NA released minus the amount of NA that is removed by uptake (see Stjärne, 1995). For these reasons the electrochemical technique cannot be used to estimate the concentration of NA at junctional receptors. The electrochemical signals can, however, provide a rough estimate of the NA concentration that might be 'seen' by extrajunctional receptors located at some distance from the sites of NA release. The mean peak current evoked by trains of ten stimuli at 10 Hz was about 20 pA and electrodes of similar dimensions to those used in this study produced a current of 9.1 ± 2.9 pA ($n=4$) when exposed to a solution containing $0.3 \mu\text{M}$ NA. This gives a value of about $0.5 \mu\text{M}$ for the peak concentration of NA detected. However, as it cannot be assumed that the stimulation induced change in NA concentration at the electrode surface is uniform (i.e. the concentration will be higher at the point of contact with the adventitia), this estimate should be considered to be a minimum value. The EC_{50} value for NA at α_1 -adrenoceptors in rat small mesenteric arteries is about $0.8 \mu\text{M}$ (Chen *et al.*, 1996). Therefore, even at some distance from the sites of transmitter release, there is potential for NA released during these short trains of stimuli at 10 Hz to activate the vascular smooth muscle.

The effects of DMI on the electrochemical signals are consistent with blockade of uptake₁. However, the effects of this agent differed depending on the stimulation parameters. The peak amplitude of responses to single stimuli and to trains of ten stimuli at 10 Hz was not significantly altered but the amplitude of the summed response, measured at the end of a train of ten stimuli at 1 Hz, was increased. The finding that the peak concentration of NA measured following a brief period of nerve activation (i.e. one pulse and ten pulses at 10 Hz) is not altered by blockade of neuronal uptake can be explained if clearance of NA from its site of release is primarily by diffusion and not by uptake (see Eccles & Jaeger, 1958; Clements, 1996). Perhaps in the rat mesenteric artery the sites of NA uptake are spatially separated from the sites of NA release. This arrangement has been suggested in rat brains for the uptake of dopamine released in nucleus accumbens (Garris *et al.*, 1994) and 5-hydroxytryptamine released in dorsal raphae and substantia nigra (Bunin & Wrightman, 1998). In these brain regions, as in rat mesenteric artery, the electrochemically measured increase in amine concentration evoked by single stimuli and by short trains of high frequency stimuli was not changed by blockade of neuronal uptake. The findings of the present study do, however, differ from those of Stjärne *et al.* (1994) who investigated the effects of blocking uptake₁ on electrochemical signals in rat tail artery. In this tissue blockade of uptake₁ increased the peak amplitude oxidation currents evoked by single stimuli and by trains of 2–100 stimuli at 20 Hz. This may indicate that in rat tail artery the sites of NA uptake are located close to the sites of NA release (see Stjärne *et al.*, 1994).

The slow (τ_2) but not the fast (τ_1) time constant of decay for the signals evoked by ten pulses at 1 and 10 Hz was increased by DMI. This DMI-induced slowing of the decay phase would be expected to increase summation of NA signals recorded during trains of stimuli lasting several seconds (see Garris *et al.*, 1994) and presumably explains the increased amplitude of the summed response to stimulation at 1 Hz.

If it is assumed that NA molecules diffuse freely within the adventitia (see Bevan & Su, 1973) and that the adventitia is about $10 \mu\text{m}$ in thickness (Lee *et al.*, 1987), then the overall rate of decay of NA concentration at the surface of the electrode following the trains of stimuli would be expected to be much faster than that measured in the present experiments

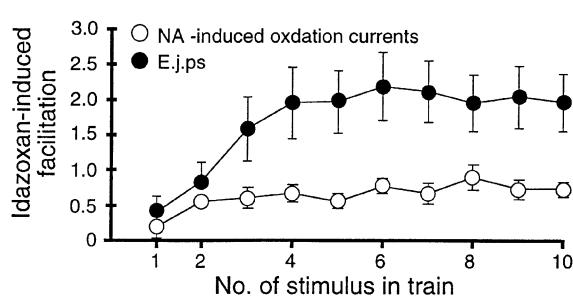


Figure 6 Graph showing the idazoxan-induced facilitation of NA-induced oxidation currents and excitatory junction potentials (e.j.ps) evoked during trains of ten stimuli at 1 Hz. Idazoxan-induced facilitation was calculated by dividing the amplitude of the idazoxan responses by that of the control responses and subtracting 1 ($n=6$ for both NA-induced oxidation currents and e.j.ps).

(see Crank, 1967). This slowing of NA clearance can be explained if the diffusion of NA is impeded either by binding to tissue elements (e.g. receptors and transporters) or by the presence of extracellular diffusion barriers. At the skeletal neuromuscular junction clearance of acetylcholine from the junctional cleft is believed to be slowed by binding to nicotinic receptors (see Katz & Miledi, 1973). In the present experiments blockade of α_1 - and α_2 -adrenoceptors with competitive antagonists did not change τ_1 or τ_2 and in the presence of DMI the decay phase was slowed. These findings may indicate that there are other binding sites or tissue structures (e.g. the external elastic lamina) which slow NA diffusion (see also Stjärne *et al.*, 1994). Interestingly, Stjärne & Stjärne (1995) have proposed a low affinity intrajunctional NA binding site that does not play a role in activation of the smooth muscle but slows NA diffusion and thereby maintains the NA concentration at the smooth muscle receptors. Their results with the rat tail artery suggest that binding of NA at this site is unaffected by idazoxan and prazosin but is strongly inhibited by phenoxybenzamine. The effects of the latter agent was not tested in the present study.

The idazoxan-induced increase in the amplitude of the oxidation currents evoked by trains of ten stimuli at 0.5, 1 and 10 Hz indicates that, under control conditions, NA released during these short trains of stimuli acts at presynaptic α_2 -adrenoceptors to inhibit subsequent NA release (i.e. α -autoinhibition; Starke, 1987). In support of this suggestion, idazoxan had no effect on the oxidation currents evoked by first stimulus in the trains of stimuli at 0.5 and 1 Hz, presumably because at this time the NA concentration at the α_2 -adrenoceptors was too low to activate them. Furthermore, the effects of idazoxan could not be accounted for by changes in NA clearance, as this agent had no effect on the decay time constants τ_1 or τ_2 for the signals evoked by ten stimuli at 1 and 10 Hz. The finding that responses to trains of 10 stimuli at 10 Hz were increased by idazoxan, indicates that the time required for the effects of α_2 -adrenoceptor activation to be manifested is <0.9 s.

The amplitude of intracellularly recorded e.j.ps was also increased by idazoxan. However, comparison of the e.j.p data with that for the electrochemical signals revealed a number of differences. Firstly, under control conditions, the decline in e.j.p amplitude during the trains of stimuli at 0.5 and 1 Hz occurred more slowly than for the electrochemical signals, which were markedly depressed following the first stimulus in the train. Secondly, idazoxan significantly increased the amplitude of e.j.ps evoked by first stimulus in the trains of stimuli at 0.5 and 1 Hz. Holman & Surprenant (1980) made a similar observation for e.j.ps recorded in rat tail artery and rabbit mesenteric artery following application of α_2 -adrenoceptor antagonists and questioned whether the effects of these agents on transmitter release could be accounted for by a blockade of α -autoinhibition. However, in the present experiments the magnitude of the idazoxan-induced facilitation of e.j.p amplitude increased during the trains of stimuli, consistent with α -autoinhibition. Thirdly, in the presence of idazoxan, e.j.ps evoked by the first five or so stimuli in the trains at 0.5 and 1 Hz increased amplitude (facilitated), while the amplitude of the electrochemical signals remained fairly constant throughout the trains of stimuli. Lastly, the overall magnitude of the idazoxan-induced increase in e.j.p amplitude

was significantly greater than that for the electrochemical signals.

These findings are perhaps most readily explained if α_2 -adrenoceptor mediated autoinhibition differentially effects the release of NA and ATP. A similar suggestion has been made for the guinea-pig vas deferens in which α_2 -adrenoceptor antagonists have been reported to differentially effect NA and ATP overflow (Driessens *et al.*, 1993; Todorov *et al.*, 1996). In both these studies the overflow of NA and ATP was increased by α_2 -adrenoceptor antagonists but the effect on NA overflow was greater than that on ATP overflow. In contrast, in rat tail artery where the release of NA and ATP was monitored using *in situ* amperometry and electrophysiological recording techniques respectively, α_2 -adrenoceptor antagonists similarly affected the release of both substances during trains of stimuli at 2 Hz (Msghina *et al.*, 1992) but selectively increased NA release during long trains of stimuli at 20 Hz (Msghina *et al.*, 1999).

One potential problem in making comparisons between the NA-induced electrochemical signals and e.j.ps is that they may not be measuring neurotransmitter release from identical sites. Firstly, electrophysiological studies indicate that e.j.ps result from the action of ATP released at close neuroeffector junctions (Hirst *et al.*, 1996), where the synaptic delay is similar to that observed at skeletal neuromuscular junctions (about 1 ms; Brock & Cunnane, 1988). However, while it is known that some sympathetic nerve varicosities make well defined neuromuscular junctions in rat mesenteric arteries where the neuromuscular cleft width is <100 nm (Luff & McLachlan, 1989), many sympathetic varicosities in this tissue do not form close neuroeffector contacts (S.E. Luff, unpublished observations). Secondly, while it is believed that NA and ATP are co-stored in both the 'small' and the 'large' dense cored vesicles present in sympathetic nerve varicosities, e.j.ps are believed to result only from ATP released from 'small' dense cored vesicles (Stjärne, 1995), whereas the electrochemically measured NA may originate from both 'small' and 'large' dense cored vesicles. It is therefore possible that the differences observed in this study result from differential modulation of neurotransmitter release from 'close' and 'distant' varicosities and/or 'small' and 'large' dense cored vesicles.

In conclusion, the findings of this study indicate that the release of NA from the sympathetic nerves supplying rat small mesenteric arteries is tightly regulated by activation of presynaptic α_2 -adrenoceptors. The study also suggests that released NA is cleared both by diffusion and by uptake₁. Finally, comparison between the effects of blocking α -adrenoceptor mediated autoinhibition on NA-induced oxidation currents and e.j.ps, indicates that the release of NA may be separately modulated from that of ATP. However, it remains possible that this apparent difference in the modulation of NA and ATP release is a reflection of the different methods used to monitor NA and ATP secretion.

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