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Selective modulation of noradrenaline release by α_2-adrenoceptor blockade in the rat-tail artery *in vitro*

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1 The effects of blocking α_2-adrenoceptors on noradrenaline (NA) and adenosine 5′-triphosphate (ATP) release from postganglionic sympathetic nerves have been investigated in rat-tail artery *in vitro*. Continuous amperometry was used to measure NA release and intracellularly recorded excitatory junction potentials (e.j.p.'s) were used to measure ATP release.

2 Application of the α_2-adrenoceptor antagonist, idazoxan (1 μM), increased the amplitude of NA-induced oxidation currents evoked by trains of 10 stimuli at 1 and 10 Hz.

3 In cells deep in the media, idazoxan (1 μM) had no effect on the amplitude of e.j.p.'s evoked by trains of 10 stimuli at 1 and 10 Hz. In cells close to the adventitial – medial border, idazoxan produced a small increase in the amplitude of e.j.p.'s evoked at the end of trains of 10 stimuli at 1 Hz.

4 In tissues pretreated with the neuronal NA uptake inhibitor, desmethylimpramine (0.3 μM), idazoxan (1 μM) markedly increased the amplitude of e.j.p.'s in cells deep in the media.

5 The α_2-adrenoceptor agonist, clonidine (0.5 μM), produced similar reductions in the amplitudes of both NA-induced oxidation currents and e.j.p.'s evoked by 10 stimuli at 1 Hz. These effects of clonidine were reversed by the subsequent addition of idazoxan (1 μM).

6 The release of both NA and ATP is inhibited to a similar extent by activation of prejunctional α_2-adrenoceptors by clonidine. In contrast, endogenously released NA more markedly inhibits NA release. These findings provide further support for the differential modulation of NA and ATP release.
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Abbreviations: ATP, Adenosine 5′-triphosphate; DMI, desmethylimpramine; e.j.p., excitatory junction potential; τe.j.p., excitatory junction potential time constant of decay; NA, noradrenaline; r.m.p., resting membrane potential

Introduction

Present evidence suggests that noradrenaline (NA) and the cotransmitter adenosine 5′-triphosphate (ATP) are costored in both the small and large synaptic vesicles present in postganglionic sympathetic nerve varicosities (Stjärne, 1989). Therefore, it has been assumed that NA and ATP are released together by presynaptic action potentials. For this reason, it might be expected that the release of both neurotransmitters would be modulated in parallel.

In a wide range of sympathetically innervated tissues, activation of prejunctional α_2-adrenoceptors by bath applied agonists inhibits the release of both NA and ATP (Driessen *et al*., 1993). Conversely, application of α_2-adrenoceptor antagonists to block the effects of endogenously released NA at prejunctional α_2-adrenoceptors usually increases the release of both neurotransmitters (Driessen *et al*., 1993). However, in tissues where transmitter overflow has been studied, blockade of prejunctional α_2-adrenoceptors produces a greater increase in the release of NA than of ATP (guinea-pig vas deferens: Driessen *et al*., 1993; Westfall *et al*., 1996; canine mesenteric artery and vein: Bobalova & Mutafova-Yambolieva, 2001). This finding is difficult to reconcile with the idea that the release of NA and ATP are modulated in parallel.

To investigate the effects of α_2-adrenoceptor agonists and antagonists on NA and ATP overflow, it has usually been necessary to use long trains of relatively high-frequency stimulation. To study neurotransmitter release during short trains of stimuli, we have used a combination of *in situ* amperometry and intracellular recording of purinergic e.j.p.'s to monitor, impulse-by-impulse, the release of NA and ATP respectively (Brock *et al*., 1997; Brock & Cunnane, 1999). In the present study, the effects of α_2-adrenoceptor agonists and antagonists on NA and ATP release from the rat-tail artery were investigated. This tissue was chosen because α_2-adrenoceptor blockade has been reported to increase NA release and the amplitude of extracellularly recorded e.j.p.'s during trains of 100 stimuli at 2 Hz to a similar extent (Msghina *et al*., 1992) but to selectively increase NA release during trains of 100 stimuli at 20 Hz (Msghina *et al*., 1999).

Methods

All experimental procedures conformed to the National Health and Medical Research Council of Australia guidelines and were approved by the University of New South Wales Animal Care and Ethics Committee.

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Female inbred Wistar rats (200–250 g) were exsanguinated under deep anaesthesia (pentobarbitone100 mg kg^{-1}, i.p.). The main ventral caudal artery was dissected from 10 to 40 mm distal to the base of the tail. Approximately 15 mm segments of artery were pinned to the Sylgard (Dow–Corning) coated base of a 1 ml recording chamber. The chamber was perfused continuously at 3–5 ml min^{-1} with physiological saline of the following composition (in mM): Na^+, 150.6; K^+, 4.7; Ca^{2+}, 2; Mg^{2+}, 1.2; Cl^-, 144.1; $H_2PO_4^-$, 1.3; HCO_3^-, 16.3; glucose, 7.8. This solution was gassed with 95% O_2/5% CO_2 (to pH 7.2) and warmed to 35–36°C. In all experiments, the physiological saline contained the α_1-adrenoceptor antagonist, prazosin (0.1 μM), to inhibit neurally evoked contraction due to NA release. In the experiments investigating the effects of idazoxan in tissues in which neuronal uptake of NA was blocked, the bathing solution also contained desmethylimpramine (0.3 μM). The proximal end of the artery was drawn into a suction electrode and the perivascular nerves were excited by electrical field stimulation (1 ms, 20 V). As increasing the stimulus voltage did not increase the size of the signals recorded, these stimulus parameters are assumed to be supramaximal. The electrochemical and electrophysiological recordings were made in separate tissues. For both techniques, recordings were made at sites 1–2 mm distal to the mouth of the stimulating electrode.

Electrochemical recording

The release of endogenous NA was monitored by continuous amperometry using a technique similar to that described by Dunn *et al*. (1999). This technique measures the stimulus-evoked increase in NA at the adventitial surface of the artery as an oxidation current, the amplitude of which is linearly related to the change in NA concentration. Briefly, Nafion-coated carbon fibre electrodes (see Brock *et al*., 1997; Dunn *et al*., 1999) were mounted at about 30° to horizontal and placed gently against the surface of the artery so that an approximately 100 μm length of carbon fibre was in contact with the adventitia. The electrode was connected to an AMU130 Nano-amperometer (Radiometer-Analytical S.A., Villeurbanne Cedex, France) and a potential difference of +0.3 V was applied between the recording electrode and an Ag–AgCl pellet placed in the recording chamber medium. The current required to maintain this voltage was monitored. After placement of the carbon fibre electrode, the preparation was left for 20–30 min before starting the experiment.

Electrophysiological recording

Intracellular recordings were made using glass microelectrodes (120–200 MΩ) filled with 0.5 M. KCl and connected to an Axoclamp bridge amplifier (Axon Instruments, Inc. Foster City, CA, U.S.A.). To avoid e.j.p.'s with an early fast component recorded in cells close to the adventitial–medial border (superficial cells; see Cassell *et al*., 1988), most recordings were made from cells three or more cells deep in the media (deep cells) in which e.j.p.'s decayed monoexponentially. Criteria for accepting impalements were the same as those adopted by Brock & Van Helden (1995). In all electrophysiology experiments, the data were obtained in single impalements during which recordings were made in control and test solutions. Membrane potentials were determined upon withdrawal of the microelectrode.

Experimental protocols

In all experiments, except those investigating the effects of idazoxan on e.j.p.'s recorded in superficial cells, the tissues were stimulated alternately at 1 min intervals with 10 stimuli at 1 and 10 Hz. In the experiments determining the effects of idazoxan on e.j.p.'s recorded in superficial cells, the tissues were stimulated at 1 min intervals with trains of 10 stimuli at 1 Hz. In the electrophysiology and electrochemistry experiments, drugs were added to the superfusing solution following the 10th and 20th trains of stimuli, respectively, and left in contact with the tissue for 16–20 trains of stimuli. In the experiments with clonidine, idazoxan was subsequently added to confirm that the inhibitory effects of this agent were due to activation of α_2-adrenoceptors by recovery of response amplitude. At the end of the electrochemistry experiments, the Ca^{2+} channel blocker, Cd^{2+} (0.1 mM), was added to verify that the signals recorded are due to the Ca^{2+}-dependent release of NA. To assess the effects of idazoxan and clonidine, comparisons were made with data obtained in control experiments where neither drug was added.

Data analysis

All data were digitized (sampling frequencies of 0.1 kHz) and collected with a PowerLab recording system and the program Scope (ADInstruments, Castle Hill, N.S.W., Australia). Subsequent analysis was made using the computer program Igor Pro (Wavemetrics, Lake Oswego, OR, U.S.A.). Three electrochemical and electrophysiological responses to 10 stimuli at 1 and 10 Hz recorded just before (T_1) and 11–16 min following addition of the drug (T_2) were averaged before measurements were made. To account for time-dependent changes in the amplitude of the signals, responses recorded in control experiments at the same time points (T_1 and T_2) were averaged. In the experiments where the addition of clonidine was followed by that of idazoxan, three traces recorded 11–16 min following the addition of idazoxan were also averaged (T_3). In the electrochemistry experiments, stimulation produced a positive or negative-going artefact (revealed in Cd^{2+}) that lasted up to 100 ms. Therefore, the amplitude of the electrochemical responses was determined by taking the average value recorded between 100 and 200 ms following the first (1 Hz only) and 10th stimulus (1 and 10 Hz) in the train. In the electrophysiology experiments, the peak amplitude of each e.j.p. evoked during the trains of stimuli at 1 Hz was determined. The depolarization evoked by 10 stimuli at 10 Hz peaked during the train of stimuli and then declined in amplitude before the cessation of stimulation. For these signals, both the peak amplitude and the amplitude immediately following the last stimulus in the train was measured.

Data are presented as mean ± s.e. mean. For both the control and drug-treated tissues, the amplitude of the responses at T_2 and T_3 is expressed as a ratio of that at T_1. The exponential decay of e.j.p.'s was fitted using the curve-fitting functions in the computer program Igor pro. Statistical comparisons were made using repeated measures ANOVA and Student's unpaired and paired two-tailed *t*-tests with $P<0.05$ taken as a significant difference. When multiple pairwise

comparisons were made, *P*-values were corrected using the Dunn – Šidák method. In all cases, *n* refers to the number of tissues studied.

Drugs

Clonidine HCl, desmethylimpramine HCl (DMI), idazoxan HCl and prazosin HCl were supplied by Sigma Chemical Company (Castle Hill, N.S.W., Australia). Prazosin was prepared as a 1 mM stock solution in 10% ($w\,v^{-1}$) dimethylsulphoxide in water. The other drugs were prepared as 1 mM stock solutions in water.

Results

Electrochemistry

Under control conditions, each stimulus during a train at 1 Hz evoked a transient increase in oxidation current and the signals evoked by each successive stimulus summed with that remaining from the previous stimulus to produce a sustained increase in oxidation current (Figure 1a). At 10 Hz, the stimulus artefact prevented the signals evoked by individual stimuli from being discerned, but summation of these signals produced large amplitude oxidation currents (Figure 1b). In the control experiments ($n=6$), the oxidation currents recorded at T_2 were slightly smaller than those recorded at T_1 (Figure 1c and d).

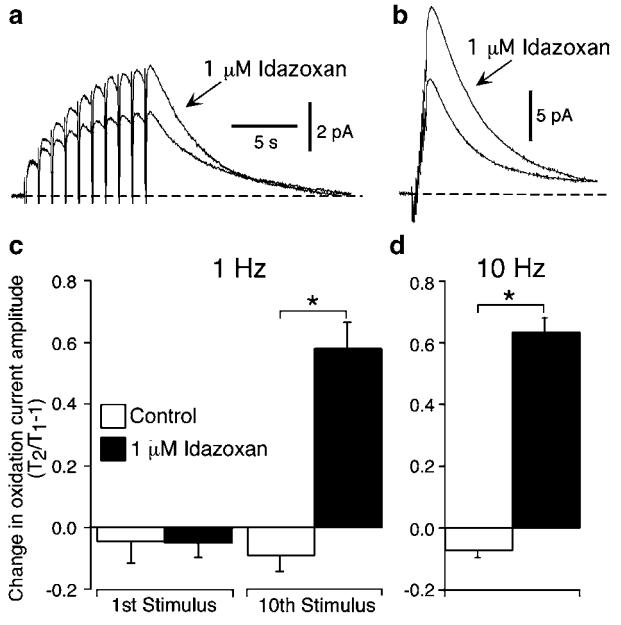

Figure 1 Effects of idazoxan (1 μM) on NA-induced oxidation currents. (a,b) Overlaid averaged traces of oxidation currents evoked by 10 stimuli at 1 (a) and 10 Hz (b) recorded before and during application of idazoxan. (c,d) Histograms showing the effects of idazoxan on the amplitude of the oxidation currents evoked by trains of 10 stimuli at 1 (c) and 10 Hz (d) ($n=6$). In (c), the amplitude of the oxidation current was measured following the first and last (10th) stimulus of the train of stimuli. Statistical comparisons were made between the T_2/T_1 ratios in drug-treated and untreated control tissues ($n=6$) (unpaired *t*-tests, $^*P<0.01$). Idazoxan increased the amplitude of the NA-induced oxidation currents measured at end of the trains of stimuli.

Effects of idazoxan on NA-induced oxidation currents

Figure 1a and b shows responses to trains of 10 stimuli at 1 and 10 Hz before and during application of the α_2-adrenoceptor antagonist, idazoxan (1 μM). In comparison with the control experiments, idazoxan ($n=6$) had no effect on the amplitude of the oxidation currents evoked by the first stimulus in the train at 1 Hz (Figure 1a and c), but increased the amplitude of the summed oxidation currents measured at the end of the trains of stimuli at 1 and 10 Hz (Figure 1a–d). In comparison with control tissues, idazoxan increased the T_2/T_1 ratio at the end of the trains at 1 and 10 Hz by 73 ± 9 and 76 ± 5%, respectively.

Effects of clonidine on NA-induced oxidation currents

Previous studies have demonstrated that the α_2-adrenoceptor agonist, clonidine, at 1 μM produces a near maximal inhibition of NA-induced oxidation currents (Msghina *et al*., 1992). In the present study, application of 0.5 μM clonidine ($n=6$) reduced the amplitude of the oxidation currents evoked by the first stimulus in the train at 1 Hz and that of the summed oxidation currents measured at the end of the trains of stimuli at 1 and 10 Hz (Figure 2a–d). In comparison with the control tissues, clonidine reduced the T_2/T_1 ratios for the oxidation currents measured following the first and the last stimulus in the train at 1 Hz by 55 ± 4 and 38 ± 6%, respectively. These

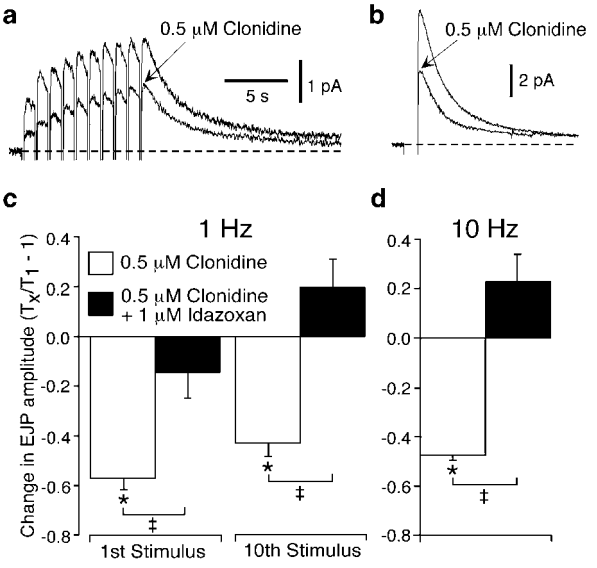

Figure 2 Effects of clonidine (0.5 μM) on NA-induced oxidation currents. (a,b) Overlaid averaged traces of oxidation currents evoked by 10 stimuli at 1 (a) and 10 Hz (b) recorded before and during application of clonidine. (c,d) Histograms showing the effects of clonidine alone (T_2/T_1) and clonidine plus idazoxan (1 μM, T_3/T_1) on the amplitude of the oxidation currents evoked by trains of 10 stimuli at 1 (c) and 10 Hz (d) ($n=6$). In (c), the amplitude of the oxidation current was measured following the first and last (10th) stimulus of the train of stimuli. For clonidine, statistical comparisons were made between the T_2/T_1 ratios in drug treated and untreated control tissues (see Figure 1c and d) using unpaired *t*-tests ($^*P<0.01$). For the effects of adding idazoxan, the T_3/T_1 ratios in the presence of clonidine plus idazoxan was compared with the T_2/T_1 ratios in the presence of clonidine alone (paired *t*-test, $\ddagger P<0.01$). Clonidine reduced the amplitude of the NA-induced oxidation currents and this effect was reversed by idazoxan.

inhibitory effects of clonidine were substantially reversed by the subsequent addition of idazoxan (1 μM, Figure 2c and d).

Idazoxan increased the amplitude of all oxidation currents in the presence of clonidine but, in the absence of this agent, the facilitatory effects of idazoxan were stimulus-dependent; that is, idazoxan had no effect on the oxidation current evoked by the first stimulus in the train at 1 Hz when the extracellular concentration of NA would be expected to be low. Therefore, it is likely that the facilitatory action of idazoxan, both in the presence and absence of clonidine, is due to blockade of α_2-adrenoceptors.

Electrophysiology

Each stimulus during the trains of 10 stimuli at 1 Hz evoked an e.j.p. that lasted about 1 s and successive e.j.p.'s increased in amplitude to reach a plateau level (Figures 3a and 4a). In control experiments, the e.j.p.'s evoked by 1 Hz stimulation in both deep cells ($n=6$) and superficial cells ($n=7$) at T_2 were smaller in amplitude than those recorded at T_1 (paired t-test $P<0.01$; Figures 3c and 4b). During the trains of 10 stimuli at 10 Hz, the e.j.p.'s evoked by each successive stimulus in deep cells summed to produce a large amplitude depolarization that peaked then waned (Figure 3b). At T_1 and T_2, the amplitude of the depolarization measured at the peak and at the end of the trains of stimuli at 10 Hz did not differ significantly (paired t-tests, Figure 3d).

Stimulation at 1 and 10 Hz also produced a slow depolarization that peaked 15–20 s following the first stimulus in the train and lasted about 1 min (Figure 3a and b).

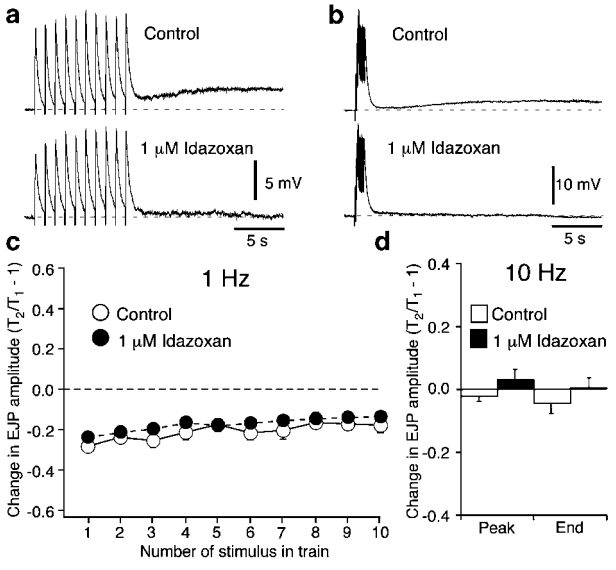

Figure 3 Effects of idazoxan (1 μM) on electrical responses to nerve stimulation recorded in deep cells. (a,b) Averaged traces of e.j.p.'s and slow depolarizations evoked by 10 stimuli at 1 (a) and 10 Hz (b) recorded before and during application of idazoxan. (c) Graph showing the change in amplitude of e.j.p.'s evoked by 10 stimuli at 1 Hz in control ($n=6$) and idazoxan-($n=6$) treated tissues. (d) Histogram showing the effect of idazoxan on the peak amplitude and the amplitude measured immediately following the last stimulus (end) of the train of 10 stimuli at 10 Hz. Idazoxan had no effect on e.j.p. amplitude but abolished the neurally evoked slow depolarization.

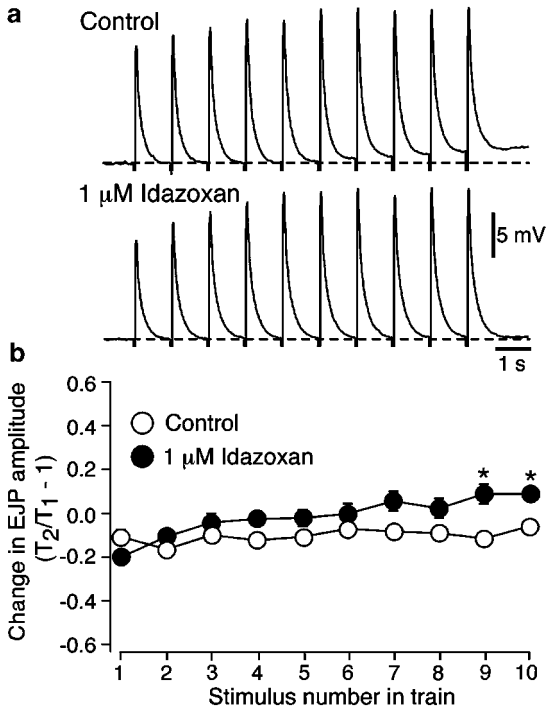

Figure 4 Effects of idazoxan (1 μM) on e.j.p.'s recorded in superficial cells. (a) Averaged traces of e.j.p.'s evoked by 10 stimuli at 1 Hz recorded before and during application of idazoxan. (b) Graph showing the change in amplitude of e.j.p.'s evoked by 10 stimuli at 1 Hz in control ($n=6$) and idazoxan-($n=6$) treated tissues. Statistical comparisons were made between the T_2/T_1 ratios in drug-treated and untreated control tissues (unpaired t-tests, $^*P<0.05$). Idazoxan produced a small increase in e.j.p. amplitude at the end of the trains of stimuli at 1 Hz.

Effects of idazoxan on e.j.p.'s

In deep cells ($n=6$), idazoxan (1 μM) had no effect on the amplitudes of e.j.p.'s evoked by trains of stimuli at 1 Hz (repeated measures ANOVA, between groups $P=0.16$, groups $\times$ stimulus interaction $P=0.45$; Figure 3a and c). In these cells, idazoxan also did not change the peak amplitude of the depolarization evoked by 10 stimuli at 10 Hz or amplitude of the depolarization measured at the end of these trains of stimuli (Figure 3b and d).

In superficial cells ($n=6$), idazoxan produced a small increase in the amplitude of e.j.p.'s evoked at the end of the trains of 10 stimuli at 1 Hz (repeated measures ANOVA, between groups $P<0.05$, groups $\times$ stimulus interaction $P<0.01$; Figure 4a and b). In comparison with recordings from superficial cells in control tissues, idazoxan increased the T_2/T_1 ratio for the last e.j.p. in the train by $14\pm2\%$.

In all cases, idazoxan abolished the slow depolarization (Figure 3a and b), consistent with its being due to activation of postjunctional α_2-adrenoceptors by neurally released NA (Itoh *et al*., 1983; Cassell *et al*., 1988).

Effects of idazoxan on e.j.p.'s in the presence of DMI

In tissues pretreated with DMI ($n=6$), e.j.p.'s recorded from deep cells declined in amplitude during trains of 10 stimuli at 1 Hz (Figure 5a) and, in the control experiments ($n=6$), the

amplitude of e.j.p.'s at T_2 was smaller than that at T_1 (Figure 5c). In tissues treated with idazoxan (1 μM, $n=6$), the T_2/T_1 ratio for e.j.p.'s evoked by 10 stimuli at 1 Hz was increased (repeated measures ANOVA, between groups $P<0.001$, groups $\times$ stimulus interaction $P<0.001$; Figure 5a and c). This facilitatory effect was observed for the first e.j.p. in the train of stimuli and increased in magnitude during the train. In contrast, in the presence of DMI, the peak amplitude of the depolarization to 10 stimuli at 10 Hz was unaffected by idazoxan but this agent did increase the T_2/T_1 ratio for the depolarization measured at the end of the trains of stimuli (Figure 5b and d). In control experiments, there was no significant difference in either measure of the depolarization to 10 stimuli at 10 Hz between T_1 and T_2 (Figure 5d).

Effects of clonidine on e.j.p.'s

In deep cells, clonidine (0.5 μM, $n=6$) produced a similar reduction in the amplitude of all e.j.p.'s evoked during the trains of 10 stimuli at 1 Hz (repeated measures ANOVA, between groups $P<0.001$, groups $\times$ stimulus interaction $P=0.11$; Figure 6a and b) and abolished the slow depolarization (Figure 6a). In comparison with the control tissues, clonidine reduced the T_2/T_1 ratios for the e.j.p.'s evoked by the first and the last stimulus by 55 ± 5 and $46\pm5\%$ respectively. The magnitude of these inhibitory effects of clonidine on e.j.p.'s did not differ significantly from those observed for this agent on the NA-induced oxidation currents (see above).

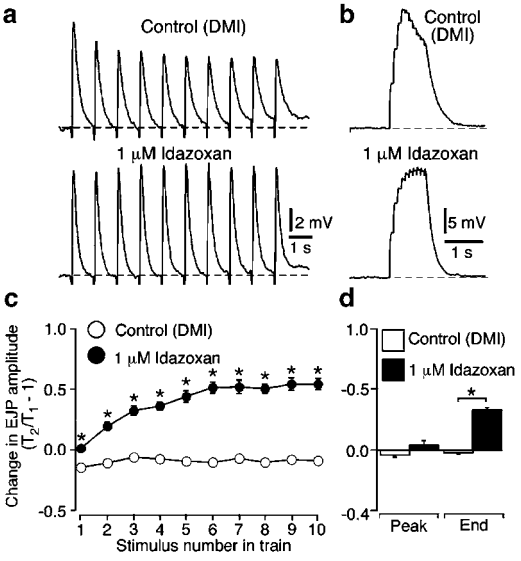

Figure 5 Effects of idazoxan (1 μM) on e.j.p.'s recorded in deep cells in tissues pretreated with DMI (0.3 μM). (a,b) Averaged traces of e.j.p.'s evoked by 10 stimuli at 1 (a) and 10 Hz (b) recorded before and during application of idazoxan. (c) Graph showing the change in amplitude of e.j.p.'s evoked by ten stimuli at 1 Hz in control ($n=6$) and idazoxan-($n=6$) treated tissues. (d) Histogram showing the effect of idazoxan on the peak amplitude and the amplitude measured immediately following the last stimulus (end) of the train of 10 stimuli at 10 Hz. Statistical comparisons were made between the T_2/T_1 ratios in the idazoxan and control (DMI treated) tissues (unpaired t-tests, $^*P<0.05$). In the presence of DMI, idazoxan increased the amplitude of e.j.p.'s evoked by stimulation at 1 and 10 Hz.

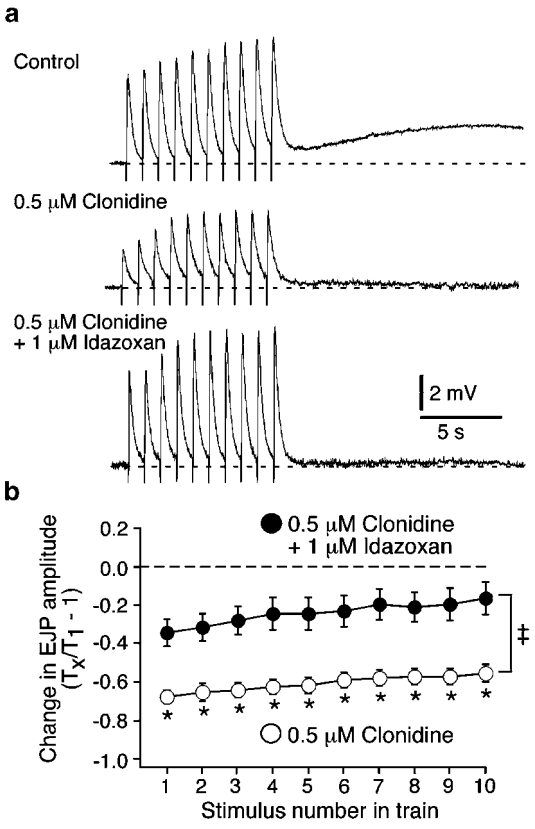

Figure 6 Effects of clonidine (0.5 μM) on electrical responses evoked by 10 stimuli at 1 Hz in deep cells. (a) Averaged traces of e.j.p.'s and slow depolarizations recorded before and during application of clonidine followed by idazoxan (1 μM). (b) Graph showing the effects of clonidine alone (T_2/T_1) and clonidine plus idazoxan (T_3/T_1) on the amplitudes of e.j.p.'s evoked by the trains of stimuli at 1 Hz ($n=6$). For clonidine, statistical comparisons were made between the T_2/T_1 ratios in control (see Figure 3c) and drug-treated tissues (unpaired t-tests, $^*P<0.01$). For the effects of adding idazoxan, the mean T_3/T_1 ratio in the presence clonidine plus idazoxan was compared with the mean T_2/T_1 ratio in the presence of clonidine alone (paired t-tests, $\ddagger P<0.01$). Clonidine reduced the amplitude of e.j.p.'s and this effect was reversed by idazoxan.

The amplitude of depolarization measured at the peak and at the end of the trains of 10 stimuli at 10 Hz was also reduced by clonidine (peak $T_2/T_1=0.73\pm0.04$, end $T_2/T_1=0.76\pm0.05$, unpaired t-tests $P<0.01$ for both comparisons with control data).

The inhibitory effect of clonidine on e.j.p.'s was reduced by the subsequent application of idazoxan (1 μM, Figure 6a and b). In contrast, idazoxan did not reverse the inhibitory effect of clonidine on the slow depolarization (Figure 6a).

Effects of idazoxan, clonidine and DMI on passive membrane properties

In deep cells, idazoxan did not detectably change the resting membrane potential (r.m.p.; control -63 ± 1 mV; idazoxan -63 ± 1 mV) or the e.j.p. time constant of decay (τe.j.p.; control 281 ± 16 ms; idazoxan 296 ± 11 ms). Similarly, in superficial cells, application of idazoxan did not change the r.m.p. (control -68 ± 1; idazoxan -68 ± 1), but the r.m.p. was more negative than in deep cells (unpaired t-test, $P<0.05$). The

decay of e.j.p.'s in superficial cells was biphasic with an initial fast component (see Cassell *et al*., 1988) and it was not possible to obtain fits to either a single or biexponential curve.

In deep cells, clonidine produced a small depolarization of the r.m.p. (5 ± 1 mV, paired *t*-test $P<0.01$) and slowed the τe.j.p. (control 284 ± 7 ms; clonidine 349 ± 12 ms, paired *t*-test $P<0.01$). As slowing of the τe.j.p. indicates an increase in membrane resistance (Cassell *et al*., 1988), the clonidine-induced depolarization is most likely due to a decrease in the K^+ conductance of the muscle cell membrane. The subsequent addition of idazoxan returned the r.m.p. to pretreatment control values (control -64 ± 2 mV; clonidine + idazoxan -63 ± 2 mV) and reversed the effects of clonidine on τe.j.p. (306 ± 13 ms).

The r.m.p. for deep cells in tissues treated with DMI (-66 ± 2 mV) or DMI plus idazoxan (-64 ± 2 mV) did not differ from that of control untreated tissues (-63 ± 1 mV) but the τe.j.p. for both groups of DMI treated tissues was significantly smaller (control τe.j.p. $=292\pm11$; DMI τe.j.p. $=246\pm14$ ms; DMI + idazoxan τe.j.p. $=233\pm22$ ms). There were no significant differences in the r.m.p. and τe.j.p. between the DMI and DMI plus idazoxan treated tissues.

Discussion

Activation of prejunctional α_2-adrenoceptors by clonidine produced a similar reduction in the amplitude of both the NA-induced oxidation currents and e.j.p.'s. This finding indicates that the release of both NA and ATP is inhibited by activation of prejunctional α_2-adrenoceptors. In contrast, blocking the actions of endogenously released NA at α_2-adrenoceptors much more markedly increased the amplitudes of the NA-induced oxidation currents. The effects of idazoxan are consistent with the idea that NA release is autoregulated in the rat-tail artery, as this agent did not change the oxidation current evoked by the first stimulus of the train at 1 Hz (Figure 1a and c) when the concentration of NA at the prejunctional α_2-adrenoceptors would be expected to be low.

In the electrophysiological studies investigating the effects of clonidine, this agent abolished the NA-mediated slow depolarization evoked by 10 stimuli at 1 Hz. The slow depolarization evoked by this stimulus is 2–3 mV in amplitude and is due to activation of postjunctional α_2-adrenoceptors (Itoh *et al*., 1983; Cassell *et al*., 1988). In the present study, clonidine (0.5 μM) depolarized the smooth muscle by 5 mV, so the marked reduction in the amplitude of the neurally evoked slow depolarization produced by clonidine can probably be accounted for both by a reduction in NA release and by 'occlusion' of the depolarizing action of neurally released NA at the postjunctional α_2-adrenoceptors.

In the rat-tail artery, only cells close to the adventitial–medial border are directly innervated (Luff *et al*., 1995) and e.j.p.'s recorded from cells deep in the media result from the electrotonic spread of potential, via gap junctions, from these superficial cells (Henery *et al*., 2002). Therefore, it would be predicted that changes in e.j.p. amplitude in superficial cells would be reflected in changes in e.j.p. amplitude in cells deep in the media. However, in the present study, idazoxan produced a small increase in the amplitude of e.j.p.'s at the end of the trains of stimuli at 1 Hz in the superficial cells but this change was not detected in deep cells. In superficial cells, the e.j.p.'s have an early fast component that is thought to reflect the local junctional action of released ATP (Jobling & Mclachlan, 1992) and changes in this component of the signal may provide a more sensitive measure of small changes in ATP release. The e.j.p.'s recorded from cells deep in the media are smaller in amplitude and do not have the early fast component, consistent with marked dissipation of the synaptic current as it spreads in a radial direction through the media.

In previous studies, we have compared the electrochemical signals with e.j.p.'s recorded from cells deep in the media. These studies have shown that the amplitudes of both NA-induced oxidation currents and e.j.p.'s have a similar dependence on extracellular Ca^{2+} and a similar sensitivity to a range of Ca^{2+} channel blocking agents (Brock & Cunnane, 1999). It has also been demonstrated that application of the β-adrenoceptor agonist, isoprenaline, produces a similar augmentation in the amplitudes of both the NA-induced oxidation currents and e.j.p.'s (Brock *et al*., 1997). These findings, together with those for clonidine in the present study, indicate that controlling Ca^{2+} entry and activating prejunctional receptors with bath applied agents modulates the release of NA and ATP in parallel. Only blocking the actions of endogenously released NA at prejunctional α_2-adrenoceptors appears to differentially affect NA-induced oxidation currents and e.j.p.'s. A similar conclusion has been made by Stjärne and his colleagues using a combination of NA-induced oxidation currents and extracellularly recorded e.j.p.'s (Msghina *et al*., 1999). However, they observed that blocking α_2-adrenoceptors increased the amplitude of e.j.p.'s evoked during trains of 100 stimuli at 2 Hz (Msghina *et al*., 1992), but did not effect e.j.p.'s evoked by 100 stimuli at 20 Hz (Msghina *et al*., 1999). These findings suggest that the effect of endogenously released NA on ATP release may depend on the stimulation conditions.

In many tissues studied so far, α_2-adrenoceptor blockade has a facilitatory effect on purinergic e.j.p.'s that develops during short trains of stimuli at 1 Hz (e.g. guinea-pig and mouse vas deferens: Bennett & Middleton, 1975; Blakeley *et al*., 1981; rat, rabbit, guinea-pig and canine mesenteric artery: Dunn *et al*., 1999; Mishima *et al*., 1984; Nagao & Suzuki, 1987; Suzuki, 1984; guinea-pig ear arteries: Morris *et al*., 1998; guinea pig submucosal arterioles: Evans & Surprenant, 1992). This finding is consistent with the idea that endogenously released NA acts prejunctionally to inhibit ATP release. Therefore, in rat-tail artery, the failure to demonstrate a facilitatory effect of α_2-adrenoceptor blockade on e.j.p.'s recorded in deep cells is unusual. To our knowledge, the only other tissue in which α_2-adrenoceptor antagonists have been reported to be without a facilitatory effect on e.j.p. amplitude is the rabbit ear artery (Suzuki & Kou, 1983).

As both NA and ATP release is inhibited by activating prejunctional α_2-adrenoceptors, it is difficult to explain the relatively selective action of idazoxan on the NA-induced oxidation currents if the electrochemical and electrophysiological techniques both measure neurotransmitter secretion from the same release sites. The simplest explanation for the observed effects of α_2-adrenoceptor blockade is if two populations of varicosities exist, one of which preferentially releases NA and the other ATP. While all the varicosities have α_2-adrenoceptors, only those that preferentially release NA are subject to α-adrenoceptor-mediated autoinhibition. The idea that NA and ATP may be differentially stored and released has previously been suggested to explain differences in the

presynaptic action of drugs on NA and ATP release (e.g. Burnstock, 1990; Gonçalves *et al*., 1996; Westfall *et al*., 1996).

However it remains possible that NA and ATP are coreleased together at all release sites. A potential problem with using the e.j.p. to monitor ATP release is that this signal only measures neurotransmitter released at close neuromuscular junctions where the synaptic delay is about 1 ms. In the rat-tail artery, as in other arterial vessels, the probability of quantal neurotransmitter release at close neuromuscular junctions is known to be very low ($P<0.02$, Astrand & Stjärne, 1989). Therefore, if NA and ATP are released together, the intervals between the secretory events are likely to be too long for the concentration of NA to accumulate locally at prejunctional α_2-adrenoceptors. In the guinea-pig vas deferens, where the probability of quantal release is also very low (Brock & Cunnane, 1993; Brain *et al*., 2002), electrophysiological studies of neurotransmitter release from small populations of varicosities have failed to demonstrate that the occurrence of a release event reduces the probability of subsequent release events (i.e. there is no evidence for local feedback regulation of release; Blakeley *et al*., 1982; Brock & Cunnane, 1991). However, in the guinea-pig vas deferens, blockade of α_2-adrenoceptors increases the probability of quantal release (Brock *et al*., 1990). To explain these observations, it was proposed that NA diffuses to act at release sites remote from its own site of release (lateral inhibition; see Brock & Cunnane, 1991). In the rat-tail artery, NA diffusing in the adventitia appears to have only a minimal effect on the release of ATP. Therefore, it remains possible that at close neuromuscular junctions, NA and ATP are released together but that the intervals between the secretory events are too long for autoregulation to operate.

In accord with the idea of lateral inhibition, idazoxan did increase the amplitude of e.j.p.'s in deep cells when neuronal uptake of released NA was blocked by DMI. Under these conditions, idazoxan increased the amplitude of the first e.j.p. in the train of stimuli at 1 Hz. This finding suggests the basal extracellular concentration of NA is increased by DMI. However, during the train of stimuli at 1 Hz, the facilitatory action of idazoxan increased (Figure 5b), consistent with this agent antagonizing the presynaptic actions of neurally released NA. Presumably, following blockade of neuronal uptake, NA diffuses from its sites of release to activate α_2-adrenoceptors that regulate the release of ATP at close neuroeffector junctions.

In the rat-tail artery, less than 50% of the sympathetic nerve varicosities make close contacts with the vascular smooth muscle (Luff *et al*., 1995). At noncontacting varicosities, NA and ATP may be released with a higher probability and at these sites released NA may act locally to inhibit the release of both neurotransmitters. If this were the case, the increased release of ATP produced by idazoxan would not be detected using the e.j.p. As ATP released from the noncontacting varicosities does not appear to have a neurotransmitter function in the rat-tail artery, it would still be the case that purinergic transmission is only regulated to a minimal extent by endogenously released NA.

In conclusion, in the rat-tail artery, there is a substantial body of evidence that regulation of Ca^{2+} entry and bath application of agonists to activate prejunctional receptors produce similar changes in the NA-induced oxidation currents and e.j.p.'s. These findings support the view that the release of NA and ATP is modulated in parallel. In contrast, the effects of α_2-adrenoceptor blockade suggest that the release of these neurotransmitters may be differentially modulated. This finding accords with the previous demonstration that α_2-adrenoceptor antagonists produce a greater increase in the overflow of NA than of ATP in guinea-pig vas deferens and canine mesenteric artery and vein (Driessen *et al*., 1993; Westfall *et al*., 1996; Bobalova & Mutafova-Yambolieva, 2001). While it is possible that the apparent difference in the modulation of NA and ATP release reflects the different methods used to monitor the release of these neurotransmitters, the findings with α_2-adrenoceptor blockade are most readily explained if NA and ATP are released separately and from different release sites.

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