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Paired pulse analysis of ATP and noradrenaline release from sympathetic nerves of rat tail artery and mouse vas deferens: effects of K^+ channel blockers

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1 The paired pulse stimulus paradigm – two pulses of equal strength delivered at variable interpulse intervals – was used to study the release of ATP and noradrenaline (NA) from post ganglionic sympathetic nerves of rat tail artery and mouse vas deferens.

2 Excitatory junction currents (EJCs) were used to measure the release of ATP, and differential pulse amperometry to measure that of NA.

3 At interpulse intervals of 0.1–1 s paired pulse stimulation caused an increase in the size of the second EJC, both in rat tail artery and mouse vas deferens. As the interpulse interval was increased to 10 s or more, the two EJCs became of equal size.

4 In both preparations the K^+ channel blockers tetraethylammonium (TEA, 20 mM) and 4-aminopyridine (4-AP, 1 mM) prolonged the duration of the nerve terminal spike and greatly amplified the first EJC of the pair.

5 In the presence of TEA and 4-AP in rat tail artery paired pulse stimulation caused a dramatic depression of the second EJC without markedly affecting the nerve terminal spike. The depression of the second EJC decreased with increasing interpulse intervals, and also when external Ca^{2+} was reduced to 0.2 mM. In mouse vas deferens, TEA and 4-AP caused only a modest depression of the second EJC.

6 In rat tail artery in the presence of TEA and 4-AP paired pulse stimulation caused a depression of the NA oxidation current evoked by the second pulse, which was similar in magnitude and time course to that of the EJC. Similar TEA and 4-AP induced depression of the second pulse response was also observed when the purinergic and noradrenergic components of the contractile response were investigated.

7 The results show that in rat tail artery K^+ channel blockers cause a dramatic paired pulse depression of the release of ATP and NA. The similarity in the depression of the EJC, the NA oxidation current, and the purinergic and noradrenergic components of the contractile response is compatible with the hypothesis that ATP and NA are released in parallel from the same neuronal sources.

Keywords: ATP; Noradrenaline; potassium channel blockers; electrophysiology; amperometry; paired pulse

Introduction

In previous studies in the rat tail artery, excitatory junction currents (EJCs) and noradrenaline (NA) oxidation currents were found to vary in a similar fashion under a number of experimental conditions (Msghina *et al*., 1992; Msghina & Stjärne 1993; Brock *et al*., 1997). These results were thought to support the hypothesis that under normal conditions the sympathetic co-transmitters ATP and NA may be released in parallel from the same neuronal sites. However under other conditions, both in rat tail artery and other preparations, pharmacological agents were found to have differential effects on signals used to measure ATP and NA release (Msghina *et al*., 1992; Msghina & Stjärne 1993; Gonçalves *et al*., 1996). It was generally realized that the measured parameters may not always faithfully reflect the release of ATP or NA. Post release factors such as differences in the rate of clearance of released transmitters or desensitization of post junctional receptors may, especially during stimulation with a long train of pulses, cause dissociation of the measured signals. In the present study we have tried to minimize such errors by using the paired pulse stimulation paradigm. This approach, which consists in the application of two pulses of equal strength but variable interpulse interval, has been extensively used to characterize different forms of short-term synaptic plasticity such as facilitation and depression of transmitter release (Mallart & Martin, 1967; Betz, 1970).

Specifically, we have used this approach (i) to compare the release of ATP in rat tail artery and mouse vas deferens in the absence and presence of the K^+ channel blockers tetraethylammonium (TEA, 20 mM) and 4-aminopyridine (4-AP, 1 mM), and (ii) to compare in rat tail artery in the presence of TEA and 4-AP the release of ATP and NA by monitoring the EJCs, the NA oxidation current, and the purinergic and noradrenergic components of the neurogenic contractile response. It is known that K^+ channel blockers such as TEA and 4-AP prolong the duration of the action potential, allowing more calcium to enter the terminals which in turn is believed to increase the amount of transmitter released (Ito *et al*., 1980; Augustine, 1990).

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Methods

Male C_{57} mice (20–30 g) or Sprague-Dawley rats (180–300 g) were humanely sacrificed by a sudden stunning blow to the head, after which they were bled to death. The vasa deferentia or 2–4 cm of the proximal region of the central tail artery were dissected out, carefully cleaned of excessive connective tissue and pinned to the Sylgard layer covering the bottom of a 2–3 ml Perspex organ bath. The preparations were perifused at 1–2 ml min^{-1} with modified Tyrode's solution of the following composition (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.3, $MgCl_2$ 0.5, glucose 5.6, and Tris 20 (pH 7.4). In the electrophysiological and electrochemical experiments, prazosin (1 μM) was added to minimize neurogenic contractions, and nifedipine (10 μM) to block spontaneous contractions which sometimes occurred in the presence of TEA and 4-AP. The solution was gassed with 100% O_2, and bath temperature held at 35–37°C.

Nerve stimulation

In the electrophysiological and electrochemical experiments a close fitting suction electrode, into which the prostatic end of the vas deferens or the proximal part of the tail artery was drawn, was used for electrical stimulation of postganglionic sympathetic nerves, *via* an NL 800 constant-current (Neurolog) or DS2 constant-voltage (Digitimer) isolation unit. Rectangular pulses (0.3 ms, 0.1–0.4 mA, measured over a 1 kΩ resistance for the constant-voltage unit) were applied between chlorided silver wires inside and outside a polyethylene tubing. To avoid artifacts disturbing the electrochemical measurement of NA release, electrical stimulation was triggered by the 'Biopulse' and applied in the interval between measurements. For study of the contractile responses, field stimulation (0.5 ms, 50–65 V) was applied using a pair of platinum electrodes positioned on both sides along the length of the preparation.

Electrophysiological analysis of ATP release

The extracellularly recorded nerve terminal spike (NTS) and the excitatory junction current (EJC) were employed to monitor the nerve terminal action potential and the release of ATP. The recording electrode, a glass micropipette (tip diameter: 80–150 μm), was gently applied to the exposed surface of the preparation and continuously perfused at a rate of 0.1–0.5 μl min^{-1} using a peristaltic pump (Minipuls 3). A sintered Ag-AgCl pellet in the bath served as an indifferent electrode to the Ag-AgCl wire inside the recording electrode. The electrical activity was recorded through an a.c. amplifier (Neurolog NL 104, low frequency cut-off at 0.1 Hz), fed through a low-pass filter (Neurolog NL 125) with cut-off frequency at 1–5 kHz and stored on magnetic tape (Racal Store 4). A system based on an IBM AT personal computer and a data translation DT2801A analogue to digital converter card was used to digitize the data at 1.5–4 kHz, 512 points. The amplitude and duration of the EJCs was analysed by computer and selected traces were measured directly on the oscilloscope screen for verification of the computer measurements.

Negative- or positive-going EJCs are caused by ATP released from sites inside or outside the recording electrode, respectively (Brock & Cunnane, 1988; Stjärne & Stjärne, 1989). In most of the present experiments the recording electrode was filled with Tyrode's solution containing 1 μM tetrodotoxin (TTX) to block negative-going EJCs. When the purpose was to study the effect of K^+-channel blockers on the NTS, the recording electrode was filled with drug-free Tyrode's solution, and 10 μM α, β, methylene ATP added outside the electrode to block positive-going EJCs.

Electrochemical analysis of noradrenaline release

Differential pulse amperometry (Mermet *et al*., 1990) as modified by Gonon *et al*. (1993) was used to monitor NA release. Briefly, a conventional three electrode system consisting of an electrochemically treated carbon fibre electrode (working electrode), a stainless steel wire (auxiliary electrode) and a Ag/AgCl wire (reference electrode) was connected to an electrochemical detector ('Biopulse', Solea Tacussel). Before the start of each experiment the active part of the working electrode, a pyrolytic carbon fibre (50–100 μm in length and 8 or 12 μm in diameter), was electrochemically treated by applying an anodic potential of a triangular wave form (2.0 V, 70 Hz for 20 s).

Mechanical recording

The method used has been described by Bao *et al*. (1989). Ring segments (about 3 mm in length) from the proximal part of the tail artery were mounted between a pair of holders under a resting tension of 5 mN. Electrical field stimulation (50–65 V, 0.05–0.5 ms) was applied *via* a pair of platinum electrodes on either side of the preparation. The isometric contraction of the circular muscle was measured by Grass FTO3C force displacement transducer connected to a BBC Goerz Metrawatt SE 120 potentiometric recorder.

Experimental protocol for the electrophysiological experiments

1 μM tetrodotoxin inside (when recording positive-going EJCs), or 10 μM α, β, methylene ATP outside (when recording the NTS and negative-going EJCs) the recording electrode was added 15 min before the start of the experiment. The preparation was then stimulated with two pulses of identical strength, at interpulse intervals of 0.1–10 s. Stimulation was applied every 30–60 s, and five recordings were made at each interpulse interval.

Experimental protocol for the electrochemical experiments

In all these experiments a combination of 20 mM TEA and 1 mM 4-AP 1 mM was added to the bath 15 min before the start of the experiment. Nerve stimulation was applied alternatingly with a single pulse and two pulses (interpulse intervals 0.1–10 s) every 30–60 s. The average of the single pulse response before and after a two pulse response was taken as control. The contribution of the second pulse to the cumulative response was derived by subtracting the amplitude of a single pulse response from the amplitude of the twin pulse response.

Experimental protocol for the mechanical recordings

To study the purinergic component of the contractile response, the noradrenergic component was eliminated by a combination of α_1- and α_2-adrenoceptor antagonists prazosin (1 μM) and yohimbine (1 μM). To study the noradrenergic component, the purinergic component was blocked by adding α, β, methylene ATP (10 μM) to the bath. Nerve stimulation was

applied alternatingly with a single pulse and twin pulses (interpulse intervals 0.1 – 10 s), every 60 s. Similarly as in the electrochemical experiments, the contribution of the second pulse to the total contractile response was derived by subtracting the response to a single pulse from that to the twin pulse.

Drugs

4-aminopyridine, α, β, methylene ATP, nifedipine, tetraethylammonium, tetrodotoxin, yohimbine (all Sigma), and prazosin (Pfizer). All substances were prepared as concentrated stock solution in distilled water (except for nifedipine which was dissolved in ethanol, and prazosin dissolved in 50% ethanol and 50% distilled water) and diluted in Tyrode's solution as required.

Results

Single pulse electrophysiological responses

Under the current experimental conditions, both in rat tail artery and mouse vas deferens stimulation with a series of single pulses triggered a multiphasic nerve terminal spike (NTS), and a non-fluctuating excitatory junction current (EJC, Figure 1A). Addition of tetraethylammonium (TEA, 20 mM) and 4-aminopyridine (4-AP, 1 mM) to the medium perfusing the recording electrode caused the NTS to acquire a late negative component (LNC), and greatly increased the amplitude and duration of the EJCs (Figure 1B). Further addition of α,β-methylene ATP (10 μM) inside the recording electrode abolished the EJCs, without any marked effect on the NTS or the LNC (Figure 1C).

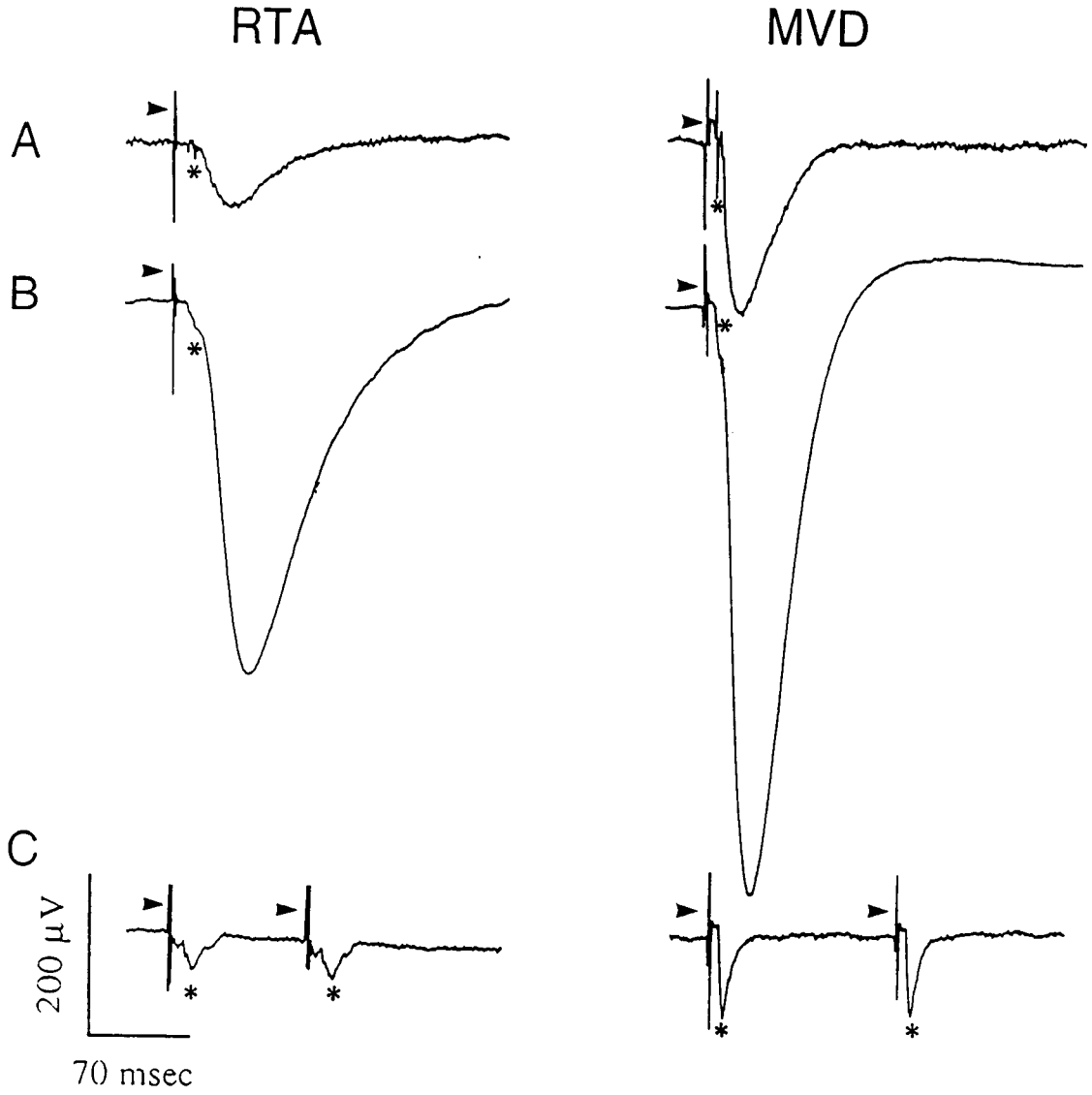

Figure 1 Averages of ten consecutive EJCs and the associated nerve terminal spike (NTS) evoked by nerve stimulation at 0.1 Hz. (A) in controls, and (B) after addition of 20 mM TEA and 1 mM 4-AP inside the recording electrode. (C) Shows the NTS evoked by paired pulse stimulation (interpulse interval: 0.1 s). In the presence of TEA and 4-AP the NTS acquired a late negative component, which is shown here isolated by further addition of α, β, methylene ATP (10 μM) inside and outside the recording electrode to block the EJC. RTA: rat tail artery; MVD: mouse vas deferens. In each tracing the arrowhead indicates the stimulus artifact, and * indicates the NTS, which in 'A' and 'B' is followed by an EJC.

Paired pulse electrophysiological responses in the absence of drugs

Both in rat tail artery and mouse vas deferens, the amplitude of the second EJC was greater than that of the first EJC ('facilitation') when stimulation with two identical pulses of supramaximal strength with varying interpulse intervals (0.1–1 s) was applied. In both preparations maximal facilitation, by $25.2 \pm 8.8\%$ in the rat tail artery ($n=6$) and $33.6 \pm 7.2\%$ in the mouse vas deferens ($n=5$), was observed when the interpulse interval was 0.1 s (Figures 2A,B, and 3A,B). As the interval was increased to 10 s, the two EJCs became of equal size (not shown)

Paired pulse stimulation in the presence of K^+-channel blockers

The effects of TEA and 4-AP on the EJCs caused by paired pulse stimulation at varying interpulse intervals are shown in Figure 2C,D, and 3A,B. In rat tail artery, the second EJC was dramatically depressed when the interpulse interval was 0.2 s or less. In the mouse vas deferens the amplitude of the second EJC was only modestly reduced at an interpulse interval of 0.1 s (by $15.3 \pm 27.9\%$, $n=5$), and even slightly enhanced at intervals of 0.3–1 s.

To rule out the possibility that the depression of the second EJC was due to action potential failure we examined, in the presence of TEA and 4-AP, the NTS evoked by paired pulse stimulation ($n=4$). As shown in Figure 1C, the NTS caused by the second pulse was similar in size and shape to the first NTS, even at interpulse intervals ($\leqslant 0.1$ s) that greatly depressed the amplitude of the second EJC.

Ca^{2+}-dependency of TEA and 4-AP induced paired pulse depression

The Ca^{2+} dependency of the TEA and 4-AP induced paired pulse depression was studied in the rat tail artery in five experiments. As shown in Figure 4A,B, the depression of the second EJC could to a great extent be alleviated by lowering the external Ca^{2+} level to 0.2 mM. However, even at this low Ca^{2+} concentration the second EJC was still significantly depressed at interpulse intervals of 0.1 s (by $33.1 \pm 7.2\%$).

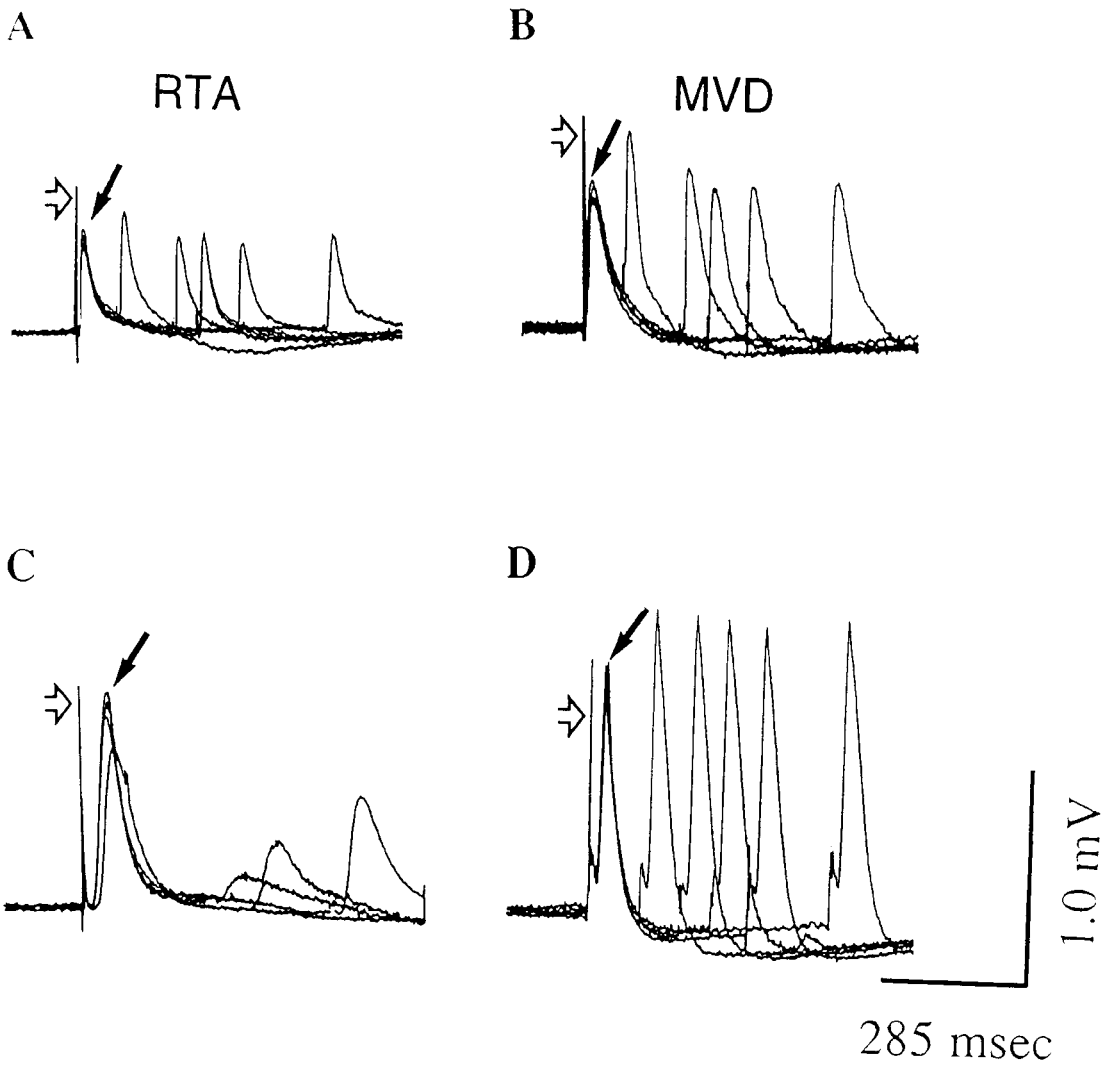

Figure 2 Original paired pulse recording of EJCs in controls (A–B), and with TEA and 4-AP present outside the recording electrode (C–D). (A,C) are in rat tail artery, and (B,D) in mouse vas deferens. The first EJC of the pair is indicated by closed arrows, and the second EJC was evoked by a pulse delivered 0.10, 0.20, 0.25, 0.33, and 0.50 s after the first pulse. Open arrows indicate the stimulus artefact, which for the sake of clarity are shown only for the first pulse.

NA oxidation current during paired pulse stimulation in the presence of K^+ channel blockers

The NA oxidation current caused by a single pulse in the absence of drugs was fluctuating and could not always be reliably resolved from the background noise (Msghina *et al*., 1993). The paired pulse analysis of NA release in the rat tail artery was therefore performed in the presence of TEA and 4-AP, which greatly amplified the single pulse response. As can be seen from Figures 5A and 6A, the amplitude of the NA oxidation current caused by two pulses was significantly larger than that caused by a single pulse, when the interpulse interval was $\geqslant 0.2$ s. However, even at these intervals the twin pulse response was not twice as large as the single pulse response, as would be expected if the two pulses released equal amounts of NA. The contribution of the second pulse declined with decreasing interpulse interval, and at intervals of $\leqslant 0.1$ s it was barely detectable.

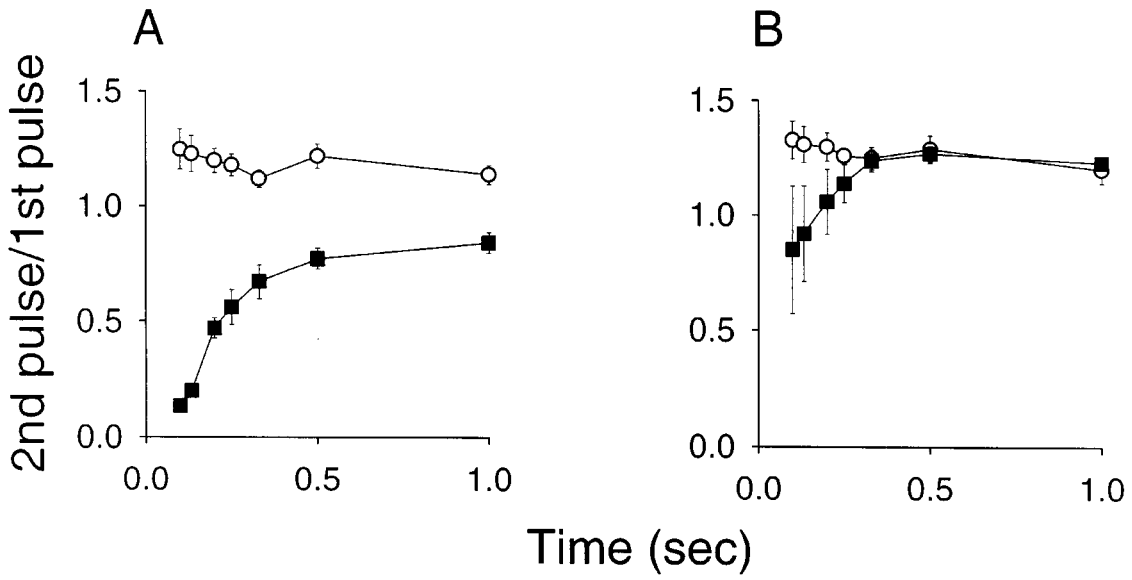

Figure 3 Ratio of second EJC over the first, plotted against the interpulse interval in controls (○) and in the presence of TEA and 4-AP (■), in rat tail artery (A) and mouse vas deferens (B). Both (A) and (B) show pooled data (mean±s.e.mean) from five experiments similar to those shown in Figure 2.

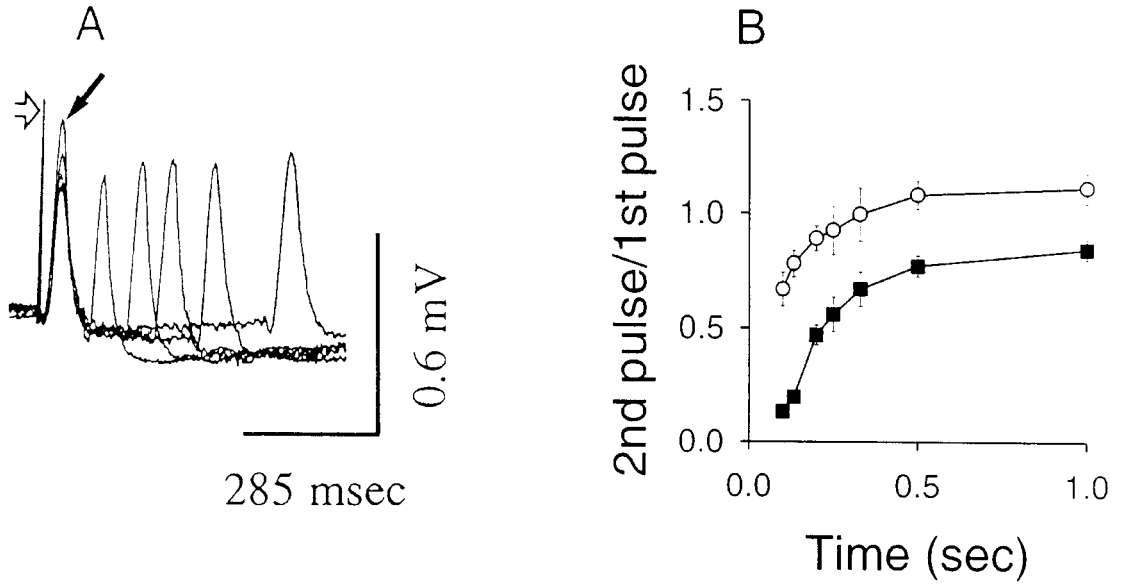

Figure 4 Ca^{2+} dependency of the TEA and 4-AP induced paired pulse depression of EJC in rat tail artery. (A) Original paired pulse recording of EJCs at an external Ca^{2+} concentration of 0.2 mM. The first EJC of the pair is indicated by the closed arrow, and the second EJC was evoked at 0.10, 0.20, 0.25, 0.33, and 0.50 s after the first EJC. Open arrow indicates the stimulus artifact which for the sake of clarity is shown only for the first pulse. (B) Pooled data (mean±s.e.mean) from five experiments similar to that shown in Figure 4A (○). For comparison the TEA and 4-AP induced paired pulse depression at 1.3 mM external Ca^{2+} is also shown (■).

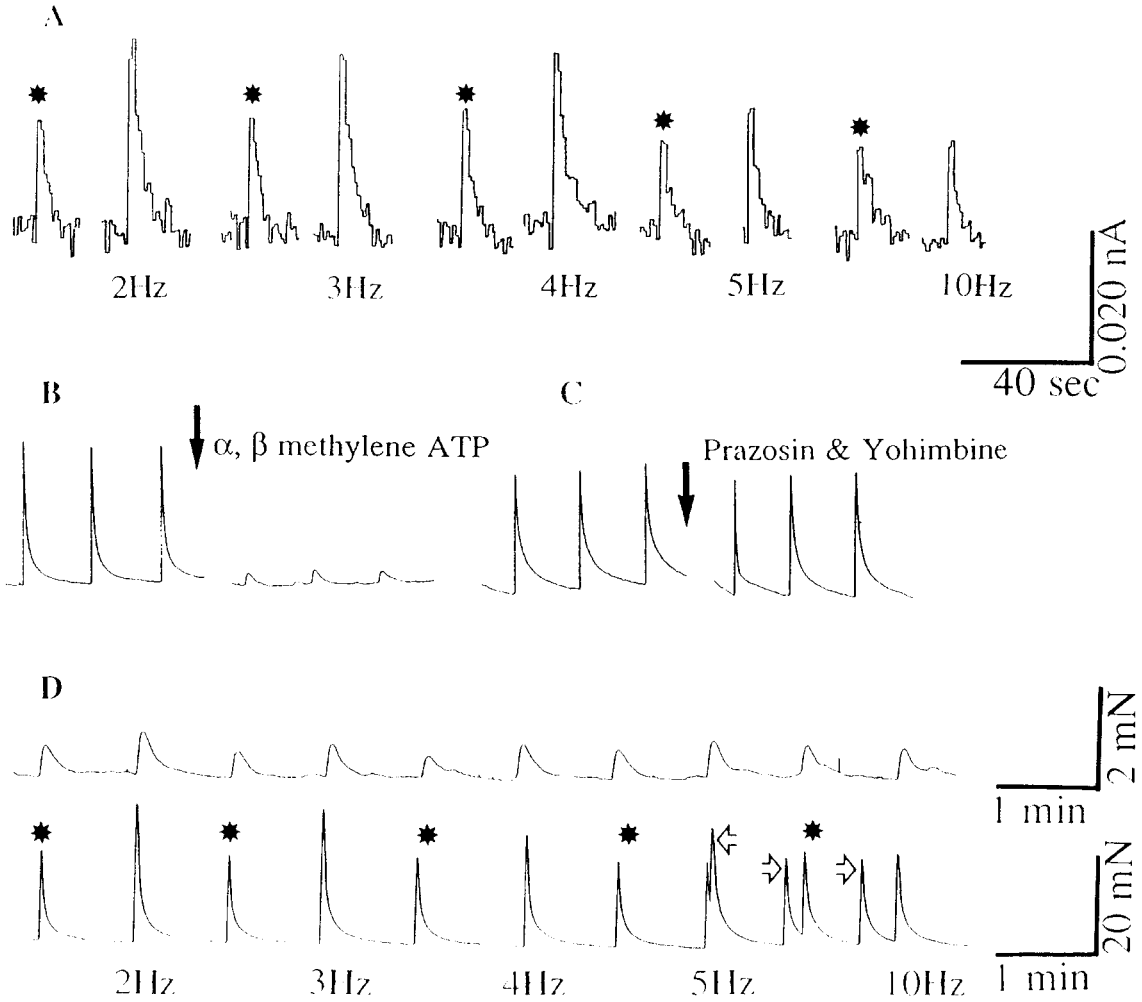

Figure 5 Representative amperometric (A) and mechanical recordings (B–D) in rat tail artery in the presence of TEA and 4-AP. (A) NA oxidation currents evoked by alternate nerve stimulation with a single pulse (*) and two pulses at different frequencies, as indicated. At interpulse interval of 0.1 s (10 Hz), the second pulse barely increased the amplitude of the single pulse response. (B–C) Noradrenergic (B) and purinergic (C) components of the contractile response isolated by the P_{2x}-purinoceptor desensitizing agent α, β, methylene ATP (10 μM), and the α_1- and α_2-adrenoceptor antagonists prazosin and yohimbine (both at 1 μM), respectively. (D) Noradrenergic (upper trace) and purinergic (lower trace) components of the contractile response evoked by alternate nerve stimulation with a single (*) and twin pulses at different frequencies, as indicated. The open arrows in the lower trace indicate spontaneous contractions which often occurred when TEA and 4-AP were present in the bath.

Noradrenergic and purinergic contractile responses

Neurogenic contractile response caused by a series of single shocks in the presence of TEA and 4-AP were recored in the rat tail artery (Figure 5B,C). This contractile response consisted of a smaller noradrenergic component, and a larger purinergic component. Therefore, pharmacological agents were used to isolate the two components. The P_{2x}-purinoceptor desensitizing agent α, β, methylene ATP (10 μM) greatly reduced the size of the contraction, isolating a smaller noradrenergic component. A combination of α_1- and α_2-adrenoceptor antagonists, prazosin (1 μM) and yohimbine (1 μM), was used to isolate the larger purinergic component. The effects of paired pulse stimulation on these responses are shown in Figure 5D, and are summarized in Figure 6B. For both the noradrenergic and purinergic components, the contribution to the total contractile response by the second pulse declined with decreasing interpulse interval, and at interpulse intervals $\leqslant$0.1 s was largely insignificant.

Discussion

The aims of the present paper were: (i) to compare in rat tail artery and mouse vas deferens the facilitation and depression of ATP release in controls, and when release is amplified by K^+ channel blockers, and (ii) to compare in rat tail artery whether short-term synaptic plasticities – facilitation or depression of ATP and NA release – are similarly or differentially altered by K^+ channel blockers.

In previous studies where the release of ATP and NA was compared in post-ganglionic sympathetic nerves of rat tail artery, the release of the two co-transmitters was found to vary in a fashion consistent with the hypothesis that ATP and NA may be released in parallel (Msghina *et al*., 1992; Msghina & Stjärne 1993, Brock *et al*., 1997). However, it was also found that under some conditions, both in rat tail artery and other preparations, pharmacological agents had differential effects on the ATP and NA signals (Msghina *et al*., 1992; Msghina & Stjärne, 1993; Gonçalves *et al*., 1996). Two possible explanations were considered: (i) dissociation caused by differential

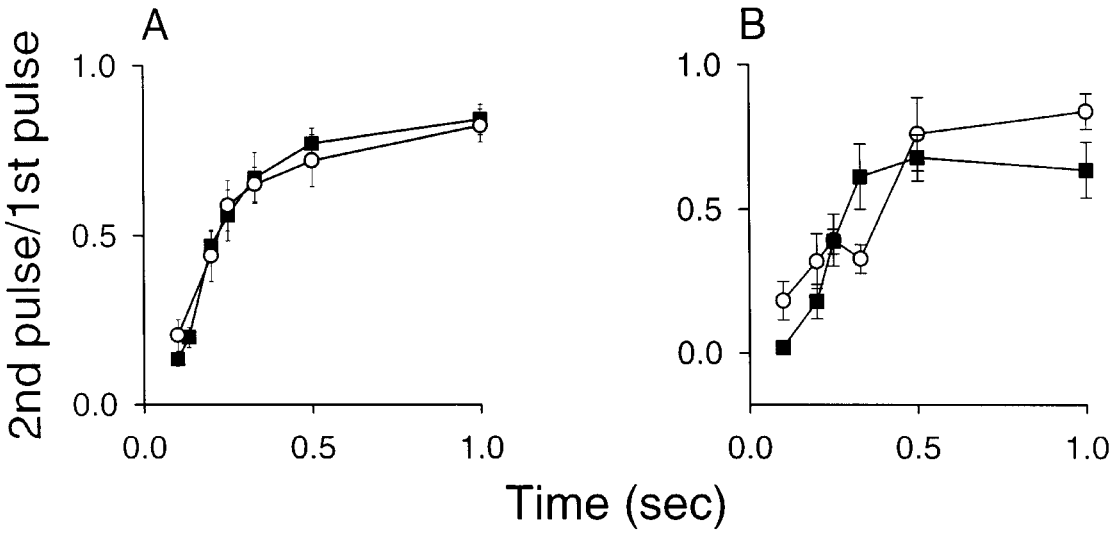

Figure 6 Ratio of the NA oxidation current evoked by the second pulse over the first pulse response, plotted against the interpulse interval (○, $n=6$). For comparison the EJC data is also included (■). Note that in both cases the contribution of the second pulse decreased with decreasing interpulse interval. (B) Ratio of the contractile response to the second pulse over the first pulse response, vs interpulse interval. ○: noradrenergic component ($n=4$); ■: purinergic component ($n=4$). All experiments were done in rat tail artery in the presence of TEA and 4-AP. Shown are means ± s.e.mean.

release of ATP and NA, and (ii) dissociation caused by post-release factors, such as difference in the clearance of the released transmitters.

In the present study, we address this issue under conditions which minimize the error caused by post-release factors by using paired pulse stimulation. This approach made it in some cases necessary to amplify the single pulse response by adding the K^+ channel blockers, TEA and 4-AP. Based on earlier experiences (Stjärne *et al*., 1991), we added both drugs at fixed concentrations, 20 mM TEA, and 1 mM 4-AP, which have previously been found to 'supramaximally' amplify the EJCs and the NA oxidation current.

ATP release in rat tail artery and mouse vas deferens

In previous work we found that the EJCs in rat tail artery and mouse vas deferens behaved differently during nerve stimulation with long trains at high frequency (20 Hz, 100–700 pulses). The EJCs in rat tail artery became profoundly depressed after an initial modest facilitation, while those in mouse vas deferens showed a more stronger build up of facilitation which gradually returned to control levels as stimulation progressed (Msghina & Stjärne, 1993). In the present study the paired pulse facilitation of EJCs in the absence of drugs was largely similar in rat tail artery and mouse vas deferens. In both tissues the EJCs were modestly facilitated, by 25.2 ± 8.8% in the rat tail artery and 33.6 ± 7.2% in the mouse vas deferens, with maximal facilitation in both cases observed at an interpulse interval of 0.1 s. It seems then that the build up of facilitation in the rat tail artery during long trains at high frequencies was masked by parallel inhibitory processes.

When the release of ATP was amplified by addition of TEA and 4-AP, paired pulse stimulation revealed marked differences between rat tail artery and mouse vas deferens. In rat tail artery, the second pulse often failed to cause an EJC when the interpulse interval was 0.1 s or less. On average, the amplitude of the second EJC at these intervals was depressed by more than 90%. The magnitude of the depression declined as the interpulse interval was increased. Reduction of the external Ca^{2+} concentration from 1.3 to 0.2 mM greatly reduced the magnitude of depression. In the mouse vas deferens the depression of the second pulse caused by TEA and 4-AP was much smaller than in rat tail artery. Even at an interpulse interval of 0.1 s, the second EJC was only moderately depressed, and occasionally even slightly facilitated as can be seen from the example shown in Figure 2D. These results demonstrate that transmitter release from postganglionic sympathetic nerve terminals in mouse vas deferens is more fatigue resistant than that in rat tail artery.

Causes of the TEA and 4-AP induced paired pulse depression

One possible cause for the failure of the second pulse to evoke an EJC in the rat tail artery at short interpulse intervals may be action potential failure. This was investigated by studying the nerve terminal spike (NTS). When K^+ channel blockers are present inside the recording electrode, the NTS acquires a late negative component (LNC). The LNC directly reflects the increased duration of the nerve terminal action potential, and indirectly also the increased influx of Ca^{2+} into the terminals (Åstrand & Stjärne, 1991; Stjärne *et al*., 1991; Brock & Cunnane, 1995). The fact that the NTS caused by the second pulse was similar in size and shape to that caused by the first pulse, even at interpulse intervals ($\leqslant 0.1$ s) that greatly depressed the second EJC, shows that action potential failure or reduced Ca^{2+} influx into the terminals were not the cause of the depression. These results are in agreement with previous findings in *Aplysia* (Gingrich & Byrne, 1985). In this paper it was reported that in the presence of 100 mM TEA, the second pulse failed to trigger an excitatory postsynaptic potential (EPSP) when the interpulse interval was $\leqslant 0.3$ s, while the intracellularly recorded action potential was essentially unchanged. The authors concluded that the depression of the second EPSP was not due to conduction failure or reduced Ca^{2+} influx into the terminals, but due to an incomplete replenishment of the transmitter vesicles which had released their content in response to the first pulse. Our results are compatible with such an interpretation.

Depression of ATP and NA release in rat tail artery

To further investigate the issue we compared in the rat tail artery the release of ATP with that of its co-transmitter NA, by recording EJCs, NA oxidation current, and the purinergic and noradrenergic components of the contractile response in the presence of TEA and 4-AP. In contrast to the EJCs which allow a pulse by pulse determination of ATP release at frequencies up to 20 Hz, the NA or ATP induced electrochemical and mechanical signals fuse into a cumulative response when stimulation is applied at frequencies $\geqslant$1 Hz. Thus, in these later cases the amplitude of the response caused by the second pulse was derived by subtracting the response to a single pulse from the response to two pulses. Under these conditions, it was found that the contribution of the second pulse to the twin pulse NA oxidation current response was extremely small when the interpulse interval was $\leqslant$0.2 s, indicating that at these intervals the NA release caused by the second pulse was much smaller than that caused by the first pulse. The purinergic and noradrenergic components of the neurogenic contraction in response to the second pulse were also similarly depressed, and were barely detectable at interpulse interval of 0.1 s or less.

As was earlier discussed for the EJCs, the fact that the second NTS was of equal size and duration as the first NTS makes it unlikely that the depression of the NA oxidation current, or the purinergic and adrenergic components of the contractile response caused by the second pulse was due to action potential failure. In theory the depression of the second EJC can be thought to be due to desensitization of post junctional P_{2x}-purinoceptors, or due to depolarization of post junctional membrane leading to decreased driving force for the ATP evoked EJCs. However, these mechanisms cannot explain the depression of the amplitude of NA oxidation current caused by the second pulse, as this signal is measured independent of post junctional receptors or post junctional membrane potential. Thus, four independent lines of evidence show that the paired pulse depression of ATP and NA release in rat tail artery in the presence of K^+ channel blockers is similar in magnitude and time course. These findings support the notion that ATP and NA, at least under the present conditions, are released in parallel from the same neuronal sources.

Conclusion

From these results we can conclude, (i) in controls the release of ATP from the sympathetic nerves of rat tail artery and mouse vas deferens is equally and modestly facilitated, (ii) in the presence of K^+ channel blockers the release of ATP exhibits a dramatic paired pulse depression in rat tail artery, but not in mouse vas deferens, (iii) the depression of release does not seem to be caused by action potential failure, or reduced Ca^{2+} influx into the nerve terminals, and (iv) the close similarity in the depression of the signals caused by the release of ATP and NA is compatible with the hypothesis that the two co-transmitters may be released in parallel from the same neuronal sources.

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