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# Inhibition of purinergic transmission by prostaglandin $E_1$ and $E_2$ in the guinea-pig vas deferens: an electrophysiological study

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1 The effects of prostaglandin $E_1$ ($PGE_1$) and $E_2$ ($PGE_2$) on postjunctional electrical activity in the guinea-pig vas deferens evoked by sympathetic nerve stimulation were investigated using both intracellular and focal extracellular recording techniques *in vitro*.

2 Bath application of $PGE_1$ (1-100 nM) or $PGE_2$ (0.1-100 nM) concentration-dependently inhibited the amplitudes of all excitatory junction potentials (e.j.ps) evoked during short trains of stimuli (10 stimuli at 1 Hz). Increasing the duration of nerve stimulation (100 stimuli at 1 Hz) did not overcome this inhibitory effect. At these concentrations $PGE_1$ and $PGE_2$ were without any apparent inhibitory effect on the amplitudes of spontaneous e.j.ps.

3 Local application of $PGE_1$ (10-100 nM) or $PGE_2$ (10-30 nM) markedly reduced the frequency of occurrence of excitatory junction currents (e.j.cs) evoked by trains of 20-100 stimuli at 1 to 4 Hz without changing the amplitudes of spontaneous e.j.cs or the configuration of the nerve terminal impulse.

4 In the presence of $PGE_1$ or $PGE_2$, raising the frequency of stimulation (from 1 to 4 Hz), increased the likelihood of e.j.c. occurrence.

5 The postjunctional electrical activity recorded in the guinea-pig vas deferens is believed to be due to ATP released from the sympathetic nerve endings. Thus the present study demonstrates that both $PGE_1$ and $PGE_2$ powerfully inhibit quantal ATP release in the guinea-pig vas deferens.

**Keywords:** prostaglandin E1, prostaglandin E2, electrophysiology; ATP; sympathetic nerve terminals; neuroeffector transmission; prejunctional inhibition; nerve terminal impulse; vas deferens.

## Introduction

Previous studies have demonstrated in a range of sympathetically innervated tissues that prostaglandin $E_1$ ($PGE_1$) and $E_2$ ($PGE_2$) inhibit the release of noradrenaline (NA) (Hedqvist, 1977; Malik & Sehic, 1990). However, in guinea-pig vas deferens while contractions evoked by short trains of low frequency stimuli are potently inhibited by $PGE_2$ (10 pulses at 2 Hz), those evoked by longer trains of stimuli (50-120 pulses at 2 Hz) are little affected or potentiated (Ambache & Zar, 1971; Ellis & Burnstock, 1990). Ellis & Burnstock (1990) reported that $PGE_2$ inhibited the stimulation (120 pulses at 2 Hz)-induced increase in overflow of tritium from tissues labelled with [$^3$H]-NA but potentiated the stimulation induced overflow of endogenous ATP. As the stimulation-induced overflow of endogenous ATP in the guinea-pig vas deferens was thought to originate solely from the sympathetic nerves (Kirkpatrick & Burnstock, 1987; Lew & White, 1987), they proposed that $PGE_2$ differentially modulated the release of the co-transmitters NA and ATP. This suggestion might in part account for the failure to detect an inhibitory effect of this agent on contractions evoked by long trains of low frequency stimuli; i.e. because $PGE_2$ inhibits noradrenergic transmission but potentiates purinergic transmission. However, $PGE_2$ in addition increases contractions of the guinea-pig vas deferens evoked by exogenously applied NA or ATP and this may also contribute to the failure to detect an inhibitory effect (Ellis & Burnstock, 1990).

More recently it has been demonstrated that as much as 80% of the stimulation-induced overflow of ATP from the guinea-pig vas deferens has a non-neural origin (see Driessen *et al.*, 1993). For this reason we have investigated electrophysiologically the effects of $PGE_1$ and $PGE_2$ on purinergic transmission in the guinea-pig vas deferens using both intracellular and focal extracellular recording techniques. Neurally released ATP acts postjunctionally at $P_{2X}$-purinoceptors to generate an excitatory junction potential (e.j.p.) measured intracellularly or an excitatory junction current (e.j.c.) measured extracellularly (see Brock & Cunnane, 1992a). In the absence of stimulation, spontaneous excitatory junction potentials (s.e.j.ps) or currents (s.e.j.cs) are recorded which result from the spontaneous quantal release of ATP. The peak amplitude of these spontaneous events provides a measure of the sensitivity of the postjunctional membrane to neurally released ATP. A previous study has demonstrated in guinea-pig vas deferens that $PGE_1$ and $PGE_2$ inhibit e.j.ps evoked by short trains of low frequency stimuli (0.2-3 Hz, Ito & Tajima, 1979) without changing the amplitude frequency distribution of s.e.j.ps, a finding that indicates these agents, at least during short trains of stimuli, inhibit the release of ATP. In the present study we have re-investigated the effects of $PGE_1$ and $PGE_2$ on electrical activity evoked by nerve stimulation and in particular the effects on e.j.ps and e.j.cs evoked by longer trains of stimuli which are similar to those used to measure biochemically the release of NA and ATP.

## Methods

Male guinea-pigs (200-350 g) were killed by a blow to the head and bled. Vasa deferentia and associated hypogastric nerve trunks were removed and individual preparations pinned to the Sylgard (Dow Corning) covered base of a 1 ml (intracellular recording) or 3 ml (extracellular recording) recording chamber. The recording chamber was perfused continuously at 1-3 ml min$^{-1}$ with a physiological saline of the following composition (mM): NaCl 118.4, $NaHCO_3$ 25.0, $NaH_2PO_4$ 1.13, $CaCl_2$2.5, KCl 4.7, $MgCl_2$1.3 and glucose 11.0. The solution was gassed with a mixture of 95% $O_2$ and 5% $CO_2$ (to pH 7.4) and maintained at 36-37°C. The vas deferens was excited by electrical field stimulation (pulse width 0.5 or

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1 ms, 3–20 V) of the vas deferens nerves through a suction electrode into which the distal portion of the hypogastric nerve and the pelvic plexus were drawn. When stimulated in this manner e.j.ps are little changed in amplitude by bath application of 100 μM hexamethonium (i.e. demonstrating that the nerves are activated postganglionically. see Ferry, 1967; Brock & Cunnane, 1992b).

*Intracellular recording*

Intracellular recordings from smooth muscle cells on or near the surface of the vas deferens were made by use of borosilicate filament microelectrodes (Clark Electromedical Instruments, GC100F-15) coupled to an Axoprobe bridge amplifier (Axon Instruments). Microelectrodes were fabricated with a Flaming/Brown micropipette puller (model P-87) and had resistances of 80–160 MΩ when filled with 0.5 M KCl. Impalements were accepted only if the following criteria were satisfied: (1) the cell penetration was abrupt and the membrane potential increased to a value more negative than the initial potential, (2) the membrane potential was stable. Recordings were only made after the impalement had stabilized for at least 5 min. For each tissue the stimulus intensity was increased until contraction during a train of stimuli at 1 Hz dislodged the recording electrode. The stimulus intensity was then decreased to a level that did not cause contraction and thereafter was not modified. Typically the fully facilitated e.j.ps had amplitudes of about 20 mV. Recorded signals were amplified 100× and stored on magnetic tape for subsequent analysis.

*Extracellular recording*

The outer smooth muscle layer was exposed by carefully removing the overlying connective tissue. A bevelled glass electrode (tip diameter ~50 μm) was applied by slight suction (20–30 mmHg) to the surface of the vas deferens. The electrode was continually perfused (see Brock & Cunnane, 1995) at a rate of about 50 μl $min^{-1}$ with a HEPES buffered saline of the following composition (mM): NaCl 142.2, KCl 4.7, HEPES 5, $CaCl_2$2.5, $MgCl_2$ 1.3 and glucose 11.0 (pH adjusted to 7.4). Electrical activity was recorded through an a.c. amplifier (Neurolog NL104, gain 10,000 or 20,000×, low frequency cut-off 0.1 Hz) and the output filtered (Neurolog NL125, high frequency cut-off 3 kHz) and stored on magnetic tape.

*Data analysis*

A PC based data acquisition system (SCAN, see Dempster, 1993) was used to digitize (sampling frequencies of 0.3–5 kHz) and analyse electrophysiological signals previously recorded on tape. To assess the effects of drug treatments, e.j.ps, e.j.cs and averaged nerve terminal impulses evoked by trains of stimuli before and during drug treatment were compared. To allow for slight variations in the latency of individual nerve terminal impulses during the averaging process, the initial positive-going or negative-going component of each signal was aligned at its point of maximum rate of rise (see Brock & Cunnane, 1992b). The effect of $PGE_1$ or $PGE_2$ on s.e.j.p. amplitude frequency distributions was determined only in those experiments in which both control and test recordings were made during a single impalement. Statistical comparisons were made by paired or unpaired $t$ tests as appropriate. $P$ values $<0.05$ were considered significant.

*Drugs*

$PGE_1$ and $PGE_2$ were supplied by Sigma Chemical Company (Castle Hill, NSW, Australia). They were both prepared as 1 mM stock solutions in ethanol and serially diluted in the physiological saline or HEPES buffered saline to the required final concentration. The most concentrated solutions of the $PGE_1$ and $PGE_2$ (100 nM) used contained 0.01% (v/v) ethanol, which had no detectable effect on recorded electrical activity.

## Results

*Effects of $PGE_1$ and $PGE_2$ on resting membrane potential*

In control preparations the mean resting membrane potential was $-69.8 \pm 0.3$ mV ($n=69$ penetrations in 19 tissues, range $-66$ to $-74$ mV). Application of $PGE_1$ (1–100 nM) or $PGE_2$ (1–100 nM) had no significant (paired $t$ tests) effect on the resting membrane potential (control $= -71.5 \pm 0.5$ mV and 100 nM $PGE_1 = -70.8 \pm 1.2$ mV, $n=4$ tissues; control $= -68.3 \pm 0.9$ mV and 100 nM $PGE_2 = -68.5 \pm 1.8$ mV, $n=4$ tissues).

*Effects of $PGE_1$ and $PGE_2$ on e.j.ps evoked by short trains of stimuli*

Under control conditions e.j.ps evoked by the first 5–6 stimuli in a train of 10 at 1 Hz increased in amplitude (i.e. facilitated) and reached a plateau level (Figures 1a and c). Bath application of $PGE_1$ (1–10 nM, $n=4$) or $PGE_2$ (0.1–10 nM, $n=8$) inhibited in a concentration-dependent manner the amplitudes of all e.j.ps in a train (see Figures 1a,b,c and d). In the presence of $PGE_1$ or $PGE_2$, the period of facilitation was lengthened so that e.j.p. amplitude continued to increase throughout the train. Higher concentrations of $PGE_1$ or $PGE_2$ (i.e. 30 nM ($n=2$ tissues for $PGE_1$ and 6 tissues for $PGE_2$) and 100 nM ($n=4$ tissues for $PGE_1$ and 5 tissues for $PGE_2$)) practically abolished e.j.ps evoked by short trains of stimuli. The inhibitory effects of relatively low concentrations of $PGE_1$ and $PGE_2$ (up to 10 nM) on e.j.p. amplitude were readily reversed by wash but the effects of higher concentrations (30 and 100 nM) were only slowly and incompletely reversed during prolonged wash periods (>30 min).

*Effects of $PGE_1$ and $PGE_2$ on s.e.j.ps*

At concentrations up to 30 nM, $PGE_1$ and $PGE_2$ had no obvious effects on the amplitude frequency distributions of s.e.j.ps ($n=4$ for $PGE_1$ and 8 for $PGE_2$). Figures 2a and b show the effects 30 nM $PGE_2$ on e.j.p. amplitudes evoked by trains of 10 stimuli at 1 Hz and on the amplitude distributions of s.e.j.ps respectively. It was not possible to determine the effects of 100 nM $PGE_1$ or $PGE_2$ on s.e.j.p. amplitudes in single impalements, as the electrode was normally dislodged following application of this concentration. However, on impaling neighbouring cells, s.e.j.ps of normal amplitude could still be recorded. The failure to detect an inhibitory effect on s.e.j.p. amplitude indicates that $PGE_1$ and $PGE_2$ are without effect on the sensitivity of the postjunctional membrane to released ATP and therefore, the inhibitory action of these agents on e.j.p. amplitude is most probably at a presynaptic site inhibiting neurotransmitter release.

*Effects of $PGE_1$ and $PGE_2$ on e.j.ps evoked by long trains of stimuli*

When the period of stimulation was increased to 100 pulses at 1 Hz the e.j.ps evoked under control conditions normally changed little in amplitude after the initial period of facilitation (see Figure 3b). However, in a small number of tissues (2 of 11) the e.j.p. amplitudes declined after about 15 stimuli to reach a new plateau level after about 50 stimuli. This feature contributed to the shape of the control response shown in Figure 3a. Bath application of $PGE_1$ (1–100 nM, $n=4$) or $PGE_2$ (1–100 nM, $n=7$) inhibited the amplitudes of all e.j.ps in a train. Figures 3a and b show the effects of $PGE_1$ and $PGE_2$ (1 and 10 nM) respectively. In the presence of either agent, the amplitudes of e.j.ps evoked during the first 10–20 stimuli facilitated to reach a plateau level that was maintained for the remainder of the stimulation period. When the concentration of $PGE_1$ or $PGE_2$ was increased to 100 nM ($n=4$ for $PGE_1$ and 5 for $PGE_2$), e.j.ps evoked during trains of 100 stimuli at 1 and

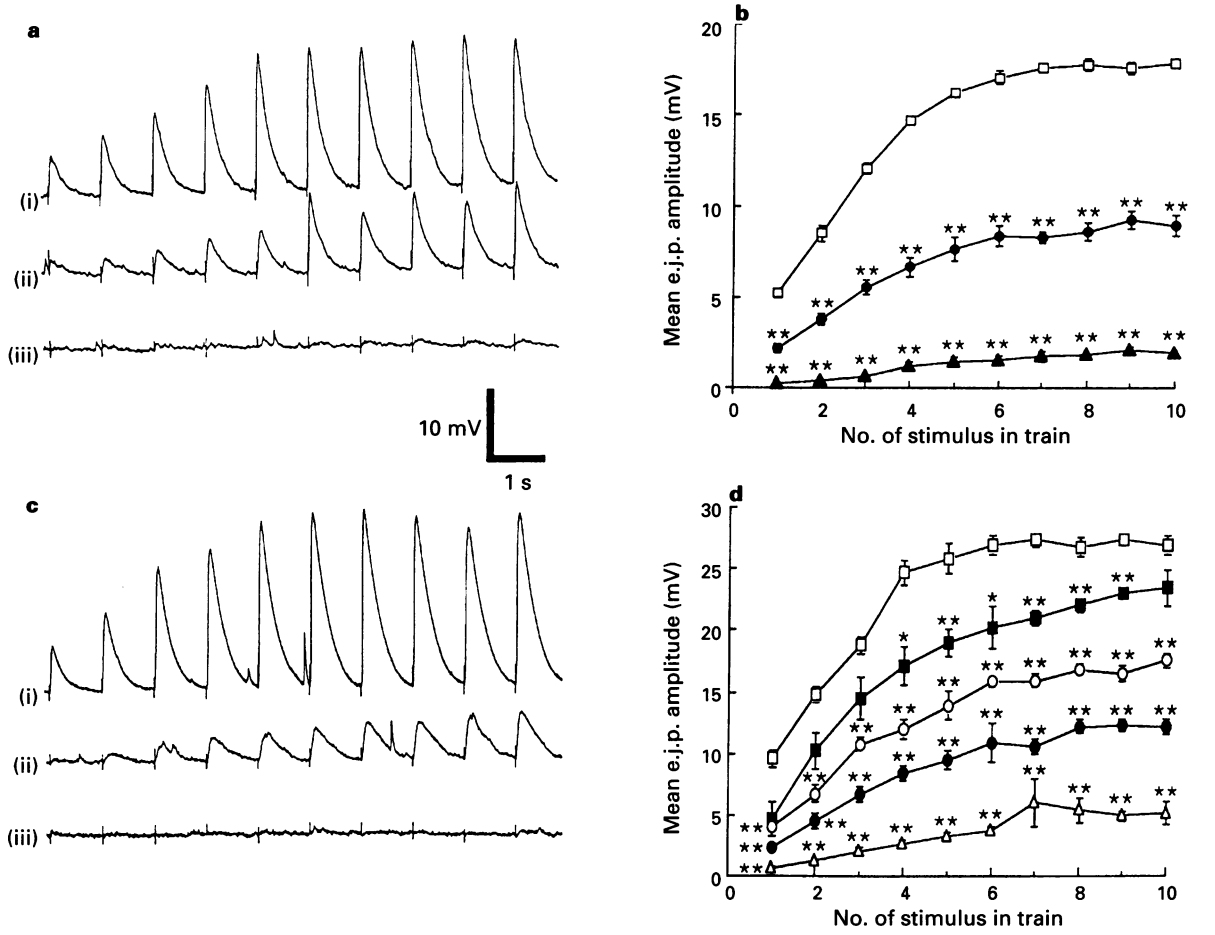


**Figure 1** Effects of $PGE_1$ (a and b) and $PGE_2$ (c and d) on excitatory junction potentials (e.j.ps) evoked by trains of 10 stimuli at 1 Hz. (a and c) Traces recorded before (i) and during the sequential addition of 1 nM (ii) and 10 nM (iii) $PGE_1$ or $PGE_2$. (b and d) Graphs showing the effects of a range of $PGE_1$ and $PGE_2$ concentrations (□ control; ■ 0.1 nM; ○ 0.3 nM; ● 1 nM; △ 3 nM and ▲ 10 nM) on the mean amplitudes of e.j.ps (± 1 s.e.mean) recorded in single impalements. The e.j.ps used to construct the graphs were recorded during 5 trains of stimuli in (b) and 4 trains of stimuli in (d) under each condition. Statistical comparisons between the control and PGE treated e.j.p. amplitudes were made by unpaired $t$ tests ($*P<0.05$, $**P<0.01$).

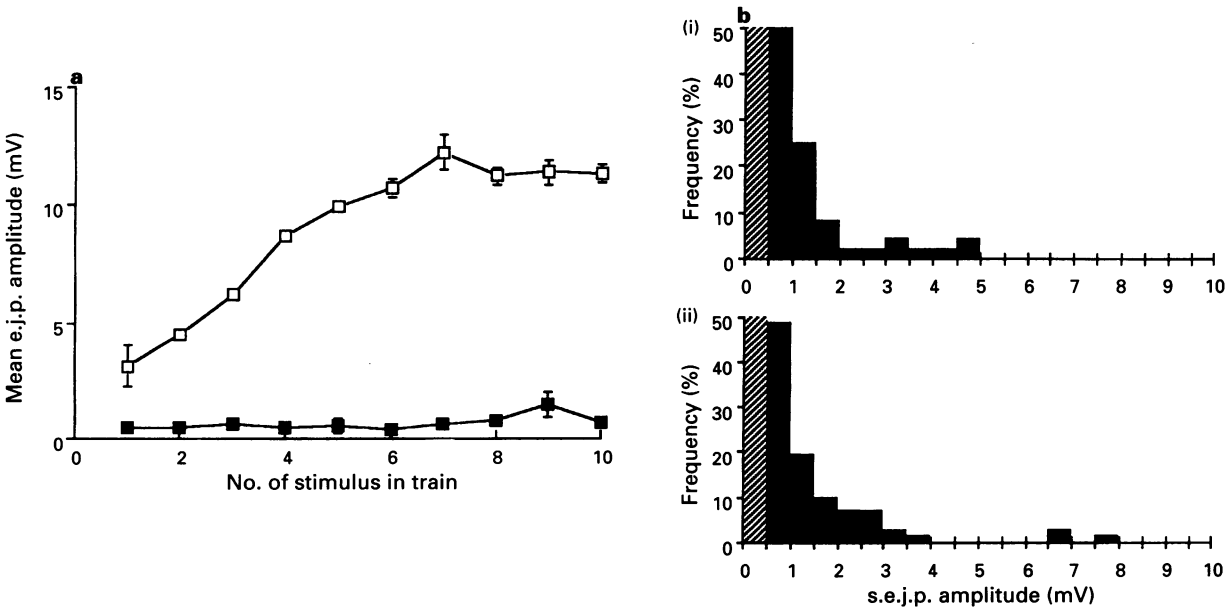


**Figure 2** Effects of $PGE_2$ on electrically evoked (a) and spontaneous (b) excitatory junction potentials (e.j.ps and s.e.j.ps) recorded in a single impalement. (a) Graph showing the mean e.j.p. amplitudes (± 1 s.e.mean) recorded during 4 trains of 10 stimuli at 1 Hz before (□) and during (■) the application of 30 nM $PGE_2$. (b) Amplitude frequency distributions of s.e.j.ps recorded during a 5 min period before (i) ($n=48$ s.e.j.ps) and during application of 30 nM $PGE_2$ (ii) (n = 72 s.e.j.ps).

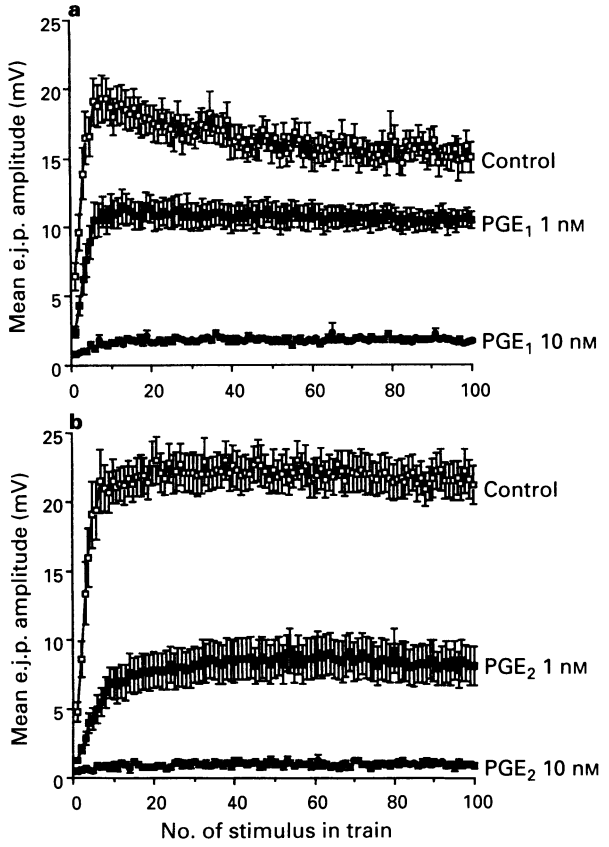


**Figure 3** Effects of 1 and 10 nM $PGE_1$ (a) or $PGE_2$ (b) on excitatory junction potentials recorded during trains of 100 stimuli at 1 Hz. Each point is the mean e.j.p. amplitude (±1 s.e.mean) for 8 impalements (2 from each of 4 tissues).

2 Hz were virtually abolished. These findings indicate that the inhibitory effects of $PGE_1$ and $PGE_2$ on e.j.ps cannot be overcome by lengthening the period of stimulation at these frequencies of stimulation.

### *Effects of $PGE_1$ and $PGE_2$ on e.j.cs*

The effects of $PGE_1$ and $PGE_2$ on sympathetic neuroeffector transmission were further investigated by use of focal extracellular recording techniques to measure e.j.cs and s.e.j.cs. In these experiments the prostaglandins were applied locally by internal perfusion of the suction recording electrode. Under control conditions, negative-going e.j.cs were evoked intermittently during trains of stimuli at 1 Hz (see Figure 4a), demonstrating that quantal secretion from individual varicosities occurs with a low probability (see Brock & Cunnane, 1993). A particular advantage of the extracellular recording technique is that on every occasion stimulus locked e.j.cs are recorded they are preceded by a non-intermittent fast transient signal that is the extracellular equivalent of the nerve terminal action potential (Figure 4a and b; see Brock & Cunnane, 1988). Thus, it is possible to determine directly whether prostaglandins inhibit transmitter release by modifying impulse propagation in sympathetic nerve terminals.

Figure 4a and b shows the effects of local application of $PGE_1$ (10 nM) on electrical activity recorded with a suction electrode during trains of 50 pulses at 1 Hz in a single attachment. Application of 10 nM $PGE_1$ markedly inhibited the occurrence of e.j.cs and this effect was reversed by wash (Figure 4a). Figure 4b shows averages of the traces shown in Figure 4a and demonstrates that $PGE_1$ is without any discernible effect on the size or configuration of the nerve terminal impulse. Similar results were obtained in 6 experiments with a range of $PGE_1$ concentrations (10–100 nM). The effects of $PGE_2$ (10–30 nM, $n=3$) on the electrical activity recorded with a focal recording electrode were similar to those of $PGE_1$.

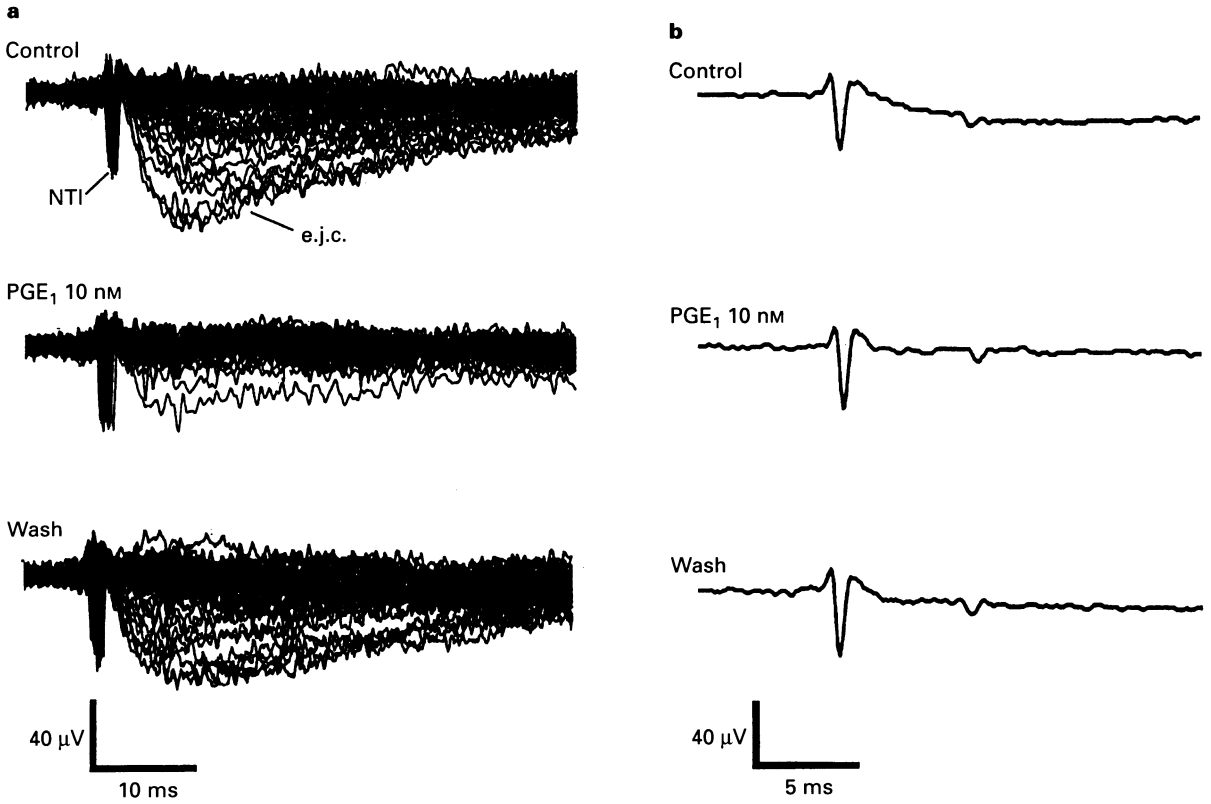


**Figure 4** Effects of locally applied $PGE_1$ on excitatory junction currents (e.j.cs) and the nerve terminal impulse (NTI) recorded in a single attachment. (a) Overlaid traces recorded during trains of 50 stimuli at 1 Hz before, during and after the addition of 10 nM $PGE_1$. (b) Averages of 10 traces recorded during the same trains as shown in (a).

Figure 5a illustrates the effects of 30 nM $PGE_2$ on electrical activity evoked by trains of 20 stimuli at 2 Hz. In this experiment, local application of $PGE_2$ inhibited the occurrence of negative-going e.j.cs without altering the configuration of the nerve terminal impulse but revealed a positive-going signal following the nerve impulse (Figure 5a). This positive-going signal is generated as a result of transmitter action at sites outside the recording electrode (Brock & Cunnane, 1988). Thus transmitter release at sites enclosed by the recording electrode is inhibited by $PGE_2$ applied locally but that occurring at sites outside the electrode is unaffected. In these experiments local application of $PGE_1$ and $PGE_2$ did not greatly change the amplitude frequency distribution of s.e.j.cs, further confirming that these agents are without effect on the sensitivity of the postjunctional membrane to ATP released from sympathetic nerve terminals. Figure 5b shows the effects of $PGE_2$ on the amplitude distribution of s.e.j.cs recorded in the same experiment as shown in Figure 5a.

### *Effects of stimulation frequency on the inhibitory effect of $PGE_1$ and $PGE_2$*

The effects of changing the frequency of stimulation on inhibitory effects of $PGE_1$ and $PGE_2$ were also investigated by the extracellular recording technique. The effects of 10 nM $PGE_1$ on the number of e.j.cs evoked during trains of 100 stimuli at 1, 2, and 4 Hz in a single experiment are shown graphically in Figure 6. As previously reported, under control conditions increasing the frequency of stimulation from 1 to 2 and 4 Hz increased the probability of e.j.c. occurrence (frequency-dependent facilitation; see Brock & Cunnane, 1988). Application of 10 nM $PGE_1$ ($n=2$) markedly decreased the number of e.j.cs evoked at all frequencies of stimulation, although the number of e.j.cs evoked still increased when the frequency of stimulation was raised. The effects of $PGE_1$ were reversed by wash. Similarly the inhibitory effects of 10 nM $PGE_2$ ($n=1$) on e.j.c occurrence were partially overcome by raising the frequency of nerve stimulation.

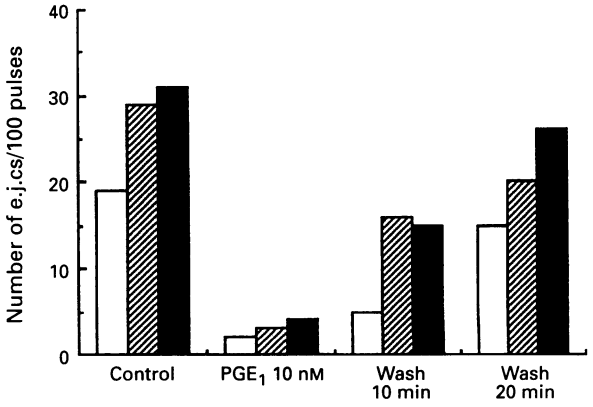


**Figure 6** Histogram showing the number of e.j.cs recorded during trains of 100 stimuli at 1 (open column), 2 (hatched column) and 4 Hz (solid column) before, during and after the application of $PGE_1$ 10 nM in a single attachment.

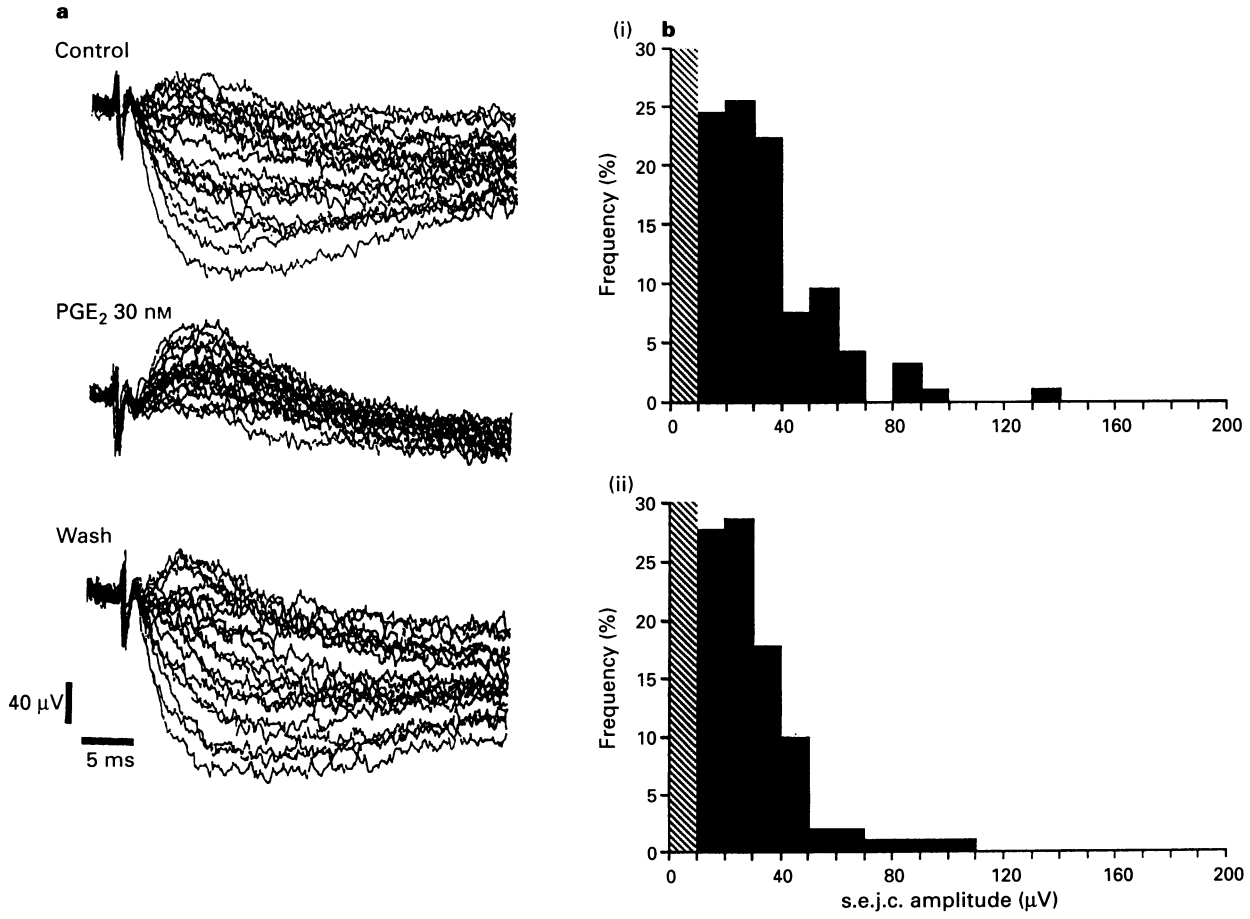


**Figure 5** Effects of locally applied $PGE_2$ on evoked and spontaneous excitatory junction currents (e.j.cs and s.e.j.cs) recorded in a single attachment. (a) Overlaid traces recorded during trains of 20 stimuli at 2 Hz before, during and after the addition of 30 nM $PGE_2$. (b) Amplitude frequency distributions of s.e.j.cs recorded during a 5 min period before (i) ($n=98$ s.e.j.cs) and during application of 30 nM $PGE_2$ (ii) ($n=104$ s.e.j.cs).

## Discussion

This study demonstrates that both $PGE_1$ and $PGE_2$ inhibit e.j.ps in the guinea-pig vas deferens without changing the amplitudes of s.e.j.ps. These findings support those previously reported by Sjöstrand (1972) and by Ito & Tajima (1979). Sjöstrand (1972) used the sucrose gap technique and demonstrated that $PGE_1$ inhibited e.j.ps in the guinea-pig vas deferens but in addition caused a depolarization of the muscle cell membrane, an effect not observed in the present study. However, the threshold for inhibition of e.j.ps in his experiment was approximately 30 nM while higher concentrations were required to produce depolarization (up to 300 nM). In the present experiments 1–100 nM $PGE_1$ and $PGE_2$ were without effect on membrane potential. Ito & Tajima (1979) reported that $PGE_1$ and $PGE_2$ (0.1–100 nM) inhibited e.j.ps in the guinea-pig vas deferens without changing the membrane potential, the passive or active electrical properties of the smooth muscle or the amplitude of s.e.j.ps. Similar inhibitory effects on the e.j.p. have also been reported for $PGE_2$ in the mouse vas deferens (Taylor & Einhorn, 1972).

The e.j.p. in the guinea-pig vas deferens is believed to be mediated by ATP released from the sympathetic nerve terminals by action potentials (see Brock & Cunnane, 1992a). Thus the present findings indicate that $PGE_1$ and $PGE_2$ inhibit purinergic transmission by reducing the release of ATP from sympathetic nerve terminals. However, these results do not agree with those of Ellis & Burnstock (1990) who reported that $PGE_2$ inhibited the increase in overflow of [$^3$H]-NA induced by trains of stimuli at 2 Hz but potentiated that of endogenous ATP. Ellis & Burnstock (1990) assumed that the stimulation-induced increase in ATP overflow was due solely to neuronally released ATP and they concluded that $PGE_2$ potentiated the release of ATP. However, in the guinea-pig vas deferens it has recently been demonstrated that a large proportion of the stimulation induced increase in ATP overflow is due to transmitter induced activation of the smooth muscle (e.g. Vizi *et al.*, 1992; Driessen *et al.*, 1993). Importantly, Ellis & Burnstock (1990) noted that $PGE_2$ markedly increased the amplitude of contractions evoked by trains of stimuli at 2 Hz. Thus it would seem likely that the $PGE_2$ induced increase in ATP overflow is due to this enhanced responsiveness of the smooth muscle to nerve stimulation. In support of this conclusion, Driessen & Starke (1994) have demonstrated that when the postjunctional effects of NA and ATP were prevented, the remaining stimulation induced overflow of endogenous ATP was inhibited by $PGE_2$.

When studied by the extracellular recording approach, $PGE_1$ and $PGE_2$ reduced the frequency of occurrence of e.j.cs without altering the size or the configuration of the nerve terminal impulse. These findings indicate that $PGE_1$ and $PGE_2$ inhibit transmitter release by interfering with depolarization-secretion coupling. Previous studies also support the view that prostaglandins of the E series inhibit transmitter release by an action on stimulus-secretion coupling and more specifically on the availability of $Ca^{2+}$ for the release mechanism (see Hedqvist, 1977; Malik & Sehic, 1990). The evidence can be briefly stated. First, procedures expected to increase the entry of $Ca^{2+}$ during action potential propagation (e.g. increasing the external $Ca^{2+}$ concentration; application of $K^+$ channel blockers) decrease the inhibitory effects of both $PGE_1$ and $PGE_2$ on NA release (Stjärne, 1973a, b; Hedqvist, 1976). Second, the effects of $PGE_1$ and $PGE_2$ on NA release can be partially overcome by increasing the stimulation frequency, a procedure which would be expected to increase the axoplasmic $Ca^{2+}$ levels (Junstad & Wenmalm, 1973). These observations may suggest that $PGE_1$ and $PGE_2$ inhibit $Ca^{2+}$ entry through voltage-sensitive $Ca^{2+}$ channels. Consistent with this suggestion, $PGE_2$ inhibits the depolarization-induced $Ca^{2+}$ current in rat cultured superior cervical ganglion neurones, an effect that is due to inhibition of N-type $Ca^{2+}$ channel opening (Ikeda, 1992). Alternatively, these findings might be explained if these agents modulate some step between $Ca^{2+}$ entry and the release of transmitter; e.g. they may (1) alter the sensitivity of the release mechanism to $Ca^{2+}$ or (2) alter the sequestration of axoplasmic $Ca^{2+}$.

Measurements of the stimulation-induced increase in [$^3$H]-NA and endogenous ATP overflow and their modulation by various agents have questioned whether these co-transmitters are co-released (e.g. see Driessen *et al.*, 1993). The findings of the present study demonstrate that ATP release, like that of NA, is inhibited by $PGE_1$ and $PGE_2$. However, it is interesting to note that while 100 nM $PGE_2$ in the present study almost completely inhibited e.j.ps evoked by long trains of stimuli at 2 Hz, the stimulation induced increase in [$^3$H]-NA overflow in the study of Ellis & Burnstock (1990) was only decreased by about 40%. Since the extracellular studies clearly demonstrate that inhibition of the e.j.p. is due to a decrease in quantal secretion, it would appear that 100 nM $PGE_2$ almost completely) inhibits ATP release. There are two possible explanations for this apparently differential effect of $PGE_2$ on NA and ATP release: (1) $PGE_2$ may more potently inhibit the release of ATP (i.e. the release of NA and ATP can be modulated separately) or (2) different functional pools of NA exist in the nerve terminal which may be differentially labelled by exogenously applied [$^3$H]-NA (Scömig *et al.*, 1990) and therefore, the measured overflow of [$^3$H]-NA may not accurately reflect the quantal release of endogenous NA. In support of the first explanation, contractions of the guinea-pig vas deferens evoked by short trains of stimuli, which are now known to be primarily purinergic (see Driessen *et al.*, 1994), are powerfully inhibited by $PGE_2$ (Ambache & Zar, 1971) but those evoked by longer trains of stimuli, which are primarily noradrenergic (see Driessen *et al.*, 1994), are potentiated (Ambache & Zar, 1971; Ellis & Burnstock, 1990). This finding is most readily explained if $PGE_2$ inhibits the release of ATP more markedly than that of NA. The finding that $PGE_2$ potentiates the response to exogenously applied NA would explain why, although the release of NA is decreased, the contraction of the vas deferens is increased.

To conclude, the findings of the present study demonstrate that $PGE_1$ and $PGE_2$ inhibit purinergic transmission by inhibiting the quantal release of ATP. Further, the results demonstrate that biochemical measurements of ATP overflow can give misleading results. Thus there is a need to identify accurately the component of the stimulation induced overflow of ATP that can be considered to be junctionally active.

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