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A comparison of the effects of exogenous and endogenous prostaglandins on fast and slow contractions of field-stimulated guinea-pig vas deferens

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1 This study has compared the effects of exogenous and endogenous prostaglandins on the two phases of contraction of the guinea-pig vas deferens produced by electrical field stimulation. Prostaglandin E_2 (PGE_2), sulprostone and arachidonic acid dose-dependently and completely inhibited the first (fast) phase of contraction, with IC_{50}s of 2.6 nM, 0.65 nM and 2.2 μM, respectively.

2 Following desensitization of the receptor for adenosine triphosphate (ATP) with α, β-methylene ATP, PGE_2, sulprostone and arachidonic acid dose-dependently inhibited the second (slow) phase of contraction of the guinea-pig vas deferens produced by electrical field stimulation, but the inhibition was incomplete (up to only 30%). Indomethacin (2.8 μM) reduced the effect of arachidonic acid. On its own, indomethacin (0.3 to 6.0 μM) had no consistent effect although, on some tissues, a slight potentiation of the contractions was seen.

3 Cicaprost (a PGI_2 analogue) at low concentrations (0.5 to 30 nM) potentiated the first phase of contraction but even at high concentrations, had no consistent effect on the second phase of contraction of the guinea-pig vas deferens produced by electrical field stimulation.

4 PGE_2, sulprostone and cicaprost potentiated contractions of the guinea-pig vas deferens produced by exogenous ATP. PGE_2 and sulprostone also potentiated contractions produced by exogenous noradrenaline, whereas cicaprost had no consistent effect on the response to noradrenaline.

5 These findings indicate that prostaglandins of the E-series inhibit the second phase of contraction as well as the first phase of contraction of the guinea-pig vas deferens produced by electrical field stimulation. However, the extent of the inhibition is much less for the second phase than for the first phase. The reasons for this differential action of PGE are not clear.

6 Cicaprost potentiates the first phase but not the second phase of contraction. Since cicaprost potentiates the contractions produced by exogenous ATP, but not by exogenous noradrenaline, by an action presumably on post-junctional IP receptors, the potentiating action of cicaprost on the first phase of contraction produced by electrical field stimulation would appear to be satisfactorily explained through the action of cicaprost on these post-junctional IP receptors.

7 Exogenous arachidonic acid is apparently converted predominantly to PGE_2 by the vas deferens, since the action of arachidonic acid mimicked that of PGE_2 and was reduced by indomethacin. However, there was little evidence that sufficient PGE_2 is generated during a short period (15 s) of sympathetic nerve stimulation for it to have any significant inhibitory effect on the size of the contractions produced.

Keywords: Vas deferens; ATP; noradrenaline; prostaglandins; arachidonic acid; indomethacin

Introduction

Prostaglandins of the E-series (PGEs) have been shown in several tissues (including the vas deferens) to have an inhibitory effect on sympathetic neurotransmission, to reduce the amounts of noradrenaline released, and to be released in some tissues when the sympathetic nerves are stimulated. Thus it was proposed that PGEs modulate sympathetic neurotransmission by inhibiting noradrenaline release (see Hedqvist, 1977). However, since these initial studies were performed, it has been established that noradrenaline is not the only transmitter released from sympathetic nerve endings. In the guinea-pig vas deferens, the release of ATP is responsible for the first (fast) phase of contraction and the release of noradrenaline is responsible for the second (slow) phase of contraction produced by electrical field stimulation (see Burnstock, 1990). Thus, the proposal that PGEs reduce sympathetic neurotransmission by inhibiting the release of noradrenaline has had to be re-evaluated. Even in the early studies on the field-stimulated guinea-pig vas deferens, it was observed that PGEs were more effective in abolishing the initial fast contraction produced by a short chain of pulses than the more prolonged contraction produced by a long chain of pulses (Ambache & Aboo Zar, 1970; Hedqvist, 1974). In addition, endogenous PGE produced by field stimulation of the guinea-pig vas deferens (Swedin, 1971a; Hedqvist & von Euler, 1972) had a greater inhibitory effect on the first phase than on the second phase of contraction (Swedin, 1971a). More recent studies have shown that PGE_2 selectively inhibits the fast phase of contraction of the rat and guinea-pig vas deferens (Bedwani & Blanning, 1983; Venkova & Radomov, 1988; Hata *et al.*, 1991).

Sulprostone is a PGE_2 analogue with little activity on EP_2 receptors, but is more potent than PGE_2 on EP_3 receptors and less potent than PGE_2 on EP_1 receptors (Coleman *et al.*, 1987). Sulprostone was found to be more potent than PGE_2 at inhibiting the fast phase of contraction of the guinea-pig vas deferens (Christian & Poyser, 1994). This finding is consistent with the inhibitory action of PGEs on transmitter release from sympathetic nerve endings being mediated by the EP_3 receptor (Mantelli *et al.*, 1991; Lawrence *et al.*, 1992). The EP_3 receptor is linked to a G_i-protein which, directly or indirectly, appears to modify N-type calcium channels in sympathetic nerve endings so that the influx of calcium into the nerve induced by action potentials is reduced, thus reducing transmitter release (Mo *et al.*, 1985; Ikeda, 1992; Sugimoto *et al.*, 1992; 1993). However, although PGE_2 inhibits noradrenaline release from

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nerve endings in the guinea-pig vas deferens (Stjarne, 1972; Hedqvist, 1974; Ellis & Burnstock, 1990), the reported effects of PGE_2 on the second phase of the contraction are inconsistent with 'no effect', 'inhibition', and 'potentiation at higher concentrations' all of which have been stated (Ambache & Aboo Zar, 1970; Swedin, 1971b; Hedqvist, 1974; Venkova & Radomov, 1988; Hata *et al.*, 1991). One of the reasons for this is that noradrenaline apparently releases ATP from the smooth muscle, so this non-neuronal ATP may contribute to the response produced (von Kügelgen & Starke, 1991) especially as PGE_2 potentiates the post-junctional contractile effect of exogenous ATP on the guinea-pig vas deferens (Ellis & Burnstock, 1990). The main purpose of this study is to investigate the effects of exogenous PGE_2 and sulprostone, and of endogenous PGs on the second phase of contraction of the guinea-pig vas deferens in the presence of α,β-methylene ATP so that the action of noradrenaline is not complicated by the neuronal and non-neuronal release of ATP. The effects of PGs on the second phase have been compared with those produced on the first phase of contraction.

In contrast to PGE_2, cicaprost (an analogue of PGI_2) potentiates the fast phase of contraction of the guinea-pig vas deferens. At higher concentrations, cicaprost inhibits these contractions, but this is probably due to it cross reacting with the EP_3 receptor (Jones, 1993; Christian & Poyser, 1994). Since cicaprost does not potentiate the contractile effect of exogenous noradrenaline applied to the guinea-pig vas deferens, it was suggested that the IP receptor is situated pre-junctionally and that its activation leads to the enhancement of transmitter release (Jones, 1993). However, since the transmitter released during the initial fast phase of contraction is ATP and not noradrenaline, a post-junctional action of cicaprost has not been ruled out. Consequently, in the second part of this study we examined the effects of cicaprost on the contractions of the vas deferens produced by exogenous noradrenaline and ATP in comparison to the effects produced by exogenous PGE_2 and sulprostone, and the effects of cicaprost on the fast and slow components of the contraction of the guinea-pig vas deferens produced by electrical field stimulation.

Methods

Male guinea-pigs weighing between 300 and 450 g were killed by stunning and excising the neck. The vasa deferentia were dissected out, and any attached fat and connective tissue carefully removed. Each vas deferens was mounted in a 10 ml organ bath and connected to a Grass FT03 isometric transducer for the recording of tension by a recorder. The tissue was maintained in Krebs solution of the following composition (mM): NaCl 118, $NaHCO_3$ 25, glucose 12, KCl 4.7, $MgSO_4.7H_2O$ 0.6 and KH_2PO_4 1.2. The solution was kept at 37°C, and aerated with 95% O_2 and 5% CO_2. For all experiments, the effects of any one prostaglandin and of arachidonic acid were tested on 4 or 5 vasa deferentia.

In one of the organ baths, the vas deferens was placed between two stainless steel electrodes and subjected to electrical field stimulation. A Grass S44 stimulator was used to give a supramaximal stimulus (80 to 100 V). Voltage-response curves were performed to ensure a supramaximal voltage was used. The vas deferens was stimulated with a train of 10 pulses (1 ms duration at 5 Hz for 2 s every 32 s) to produce the first (fast) phase of contractions. Subsequently, the second (slow) phase of contraction was obtained by stimulating the vas deferens with a train of 120 pulses (1 ms duration at 8 Hz for 15 s every 64 s), following incubation of the tissue with 9.9 μM, α,β-methylene ATP for 1 h. This pretreatment completely abolished the first phase of contraction for the remaining duration of the experiment. Prostaglandins were tested initially on the first phase and then on the second phase of contraction. PGE_2, sulprostone and cicaprost were added to the organ bath between stimulations and were washed out after an effect was observed. The tissue was then allowed to recover and the contractions return to normal before the next dose was added. Non-cumulative log concentration-response curves were obtained by doubling the dose of prostaglandin added each time. The effects of arachidonic acid on both phases of contraction were also investigated in the same manner as for the prostaglandins except that doses of arachidonic acid were added to the organ bath cumulatively. In addition, the effect of arachidonic acid on the second phase of contraction was examined in the presence of 2.8 μM indomethacin. The indomethacin was added to the organ bath 30 min before the cumulative log concentration-response curve for arachidonic acid was obtained. In an additional experiment to investigate whether the increased frequency and time of stimulation used in the second part of this study affected the actions of prostaglandins, the effect of PGE_2 on the first phase of contraction was examined when the tissue was stimulated with a the train of 120 pulses at 8 Hz in the absence of α,β-methylene ATP.

The other vas deferens from each animal was used to examine the effects of prostaglandins on the contractions produced by the addition of exogenous noradrenaline and ATP to the organ bath. A dose cycle of 5 min was used with contact times of 45 s and 30 s for noradrenaline and ATP, respectively. A fixed concentration of noradrenaline and ATP that produced a contraction approximately 45% (range 35 to 55%) of the maximum response was used for any one tissue. However, this concentration ranged from 12.5 to 30.0 μM for noradrenaline and 140 to 360 μM for ATP among the tissues. The effects of increasing concentrations of PGE_2, sulprostone and cicaprost on the contractions produced by noradrenaline and ATP were examined by adding the prostaglandin to the organ bath 60 s before adding the fixed dose of noradrenaline or ATP.

Sources of material

PGE_2, arachidonic acid and α,β-methylene ATP were purchased from Sigma Chemical Co Ltd, Poole, Dorset. Indomethacin was provided by Merck Sharp and Dohme Ltd. Hoddesdon. Sulprostone and cicaprost were kindly supplied by Schering AG, Berlin, Germany.

PGE_2, sulprostone, cicaprost, noradrenaline, ATP and α,β-methylene ATP were dissolved in 0.9% saline. Arachidonic acid and indomethacin were made up as strong solutions in ethanol, and a small aliquot of each was diluted at least 100 fold with 0.9% saline before use.

Statistical tests

The results were analyzed by the analysis of variance (ANOVA), and comparisons between two groups were made by Student's *t* test.

Results

All results are expressed as mean (±s.e.mean, $n=4$ or 5).

Effects of prostaglandins and arachidonic acid on contractions produced by electrical field stimulation

The first phase of contraction of the guinea-pig vas deferens produced by a train of 10 pulses at 5 Hz was inhibited by PGE_2 (0.28 to 28.0 nM) in a dose-dependent manner, and complete inhibition was achieved. The IC_{50} was 2.6 nM (Figure 1a). PGE_2 (0.28 to 28.0 nM) produced a similar, complete inhibition of the first phase of contraction when a train of 120 pulses at 8 Hz was used. The IC_{50} was 2.4 nM (Figure 2). The second phase of contraction produced by a train of 120 pulses, following desensitization of the ATP receptor with α,β-methylene ATP, was also dose-dependently inhibited by PGE_2 (0.28 to 28.0 nM) but only up to a maximum inhibition of 29.0±1.4%. The concentration which produced half this maximum inhibition was 3.2 nM (Figure 1a). PGE_2 had a significantly

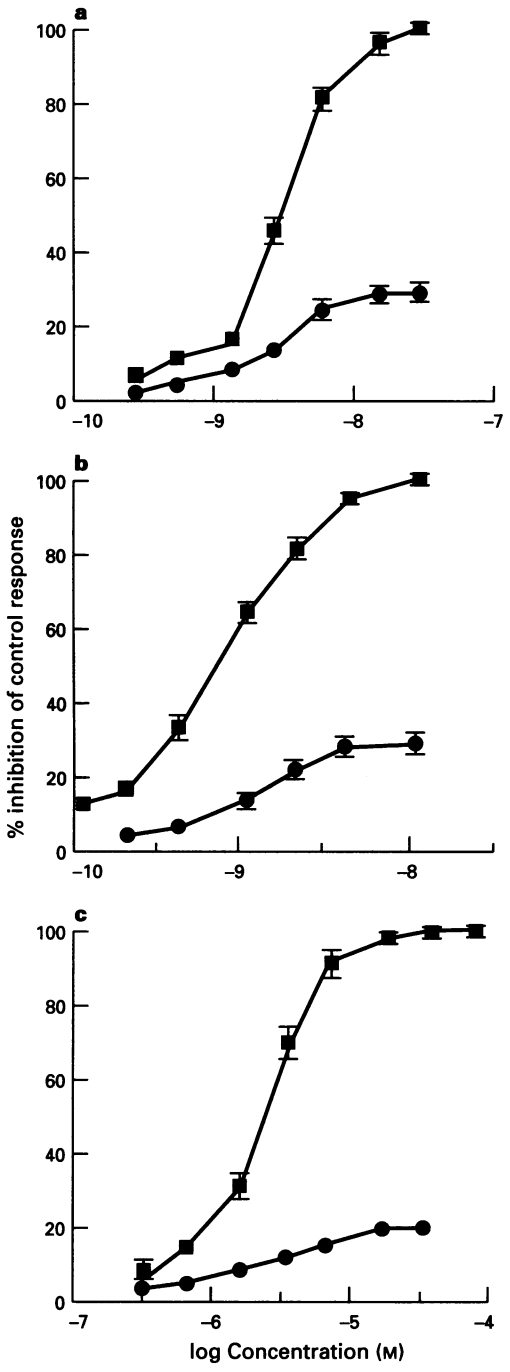

Figure 1 Mean (±s.e.mean, $n=4$)% inhibition of the first phase (■) and second phase (●) of contraction of the guinea-pig vas deferens produced by electrical field stimulation after treatment with (a) prostaglandin E_2, (b) sulprostone and (c) arachidonic acid. Where no standard error bars are shown, the standard error is smaller than the symbol used.

($P<0.05$) smaller inhibitory effect on the second phase of contraction at all concentrations used. Similarly, sulprostone (0.1 to 11.0 nM) dose-dependently inhibited the first and second phases of contraction; the first phase was inhibited completely whereas the second phase was inhibited up to a maximum of 29.0±1.6%. The IC_{50} for the first phase was 0.65 nM, whereas the concentration of sulprostone required to achieve half the maximum inhibition of the second phase was 1.2 nM (Figure 1b). Each concentration of sulprostone had a significantly ($P<0.05$) smaller inhibitory effect on the second phase compared to the first phase of contraction.

Arachidonic acid (0.33 to 33.0 μM) produced complete inhibition of the first phase and partial inhibition of the second

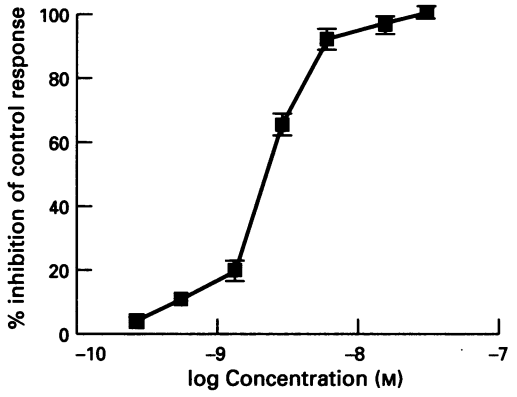

Figure 2 Mean (±s.e.mean, $n=4$)% inhibition by prostaglandin E_2 of the first phase of contraction of the guinea-pig vas deferens produced by electrical field stimulation at 8 Hz. Where no standard error bars are shown, the standard error is smaller than the symbol used.

phase of contraction (maximum inhibition = 20.4±0.8%) in a dose-dependent manner. The IC_{50} for the first phase was 2.2 μM, and the concentration which produced half the maximum inhibition of the second phase was 1.78 μM (Figure 1c). Arachidonic acid was 860 times and 556 times less potent than PGE_2 in inhibiting the first and second phases of contraction, respectively. The lengths of time taken for the highest concentration of PGE_2 to produce its effect were 1.7±0.3 min for the first phase and 3.7±0.3 min for the second phase of contraction. The corresponding times taken for the highest concentration of arachidonic acid were 4.4±0.3 min and 6.6±0.4 min, respectively, which were significantly ($P<0.05$) longer than those for PGE_2. Indomethacin (2.8 μM) significantly ($P<0.05$) reduced the effect of arachidonic acid on the second phase of the contraction and caused the log concentration-response curve to move 2.5 fold to the right (Figure 3). On its own, indomethacin (0.3 to 6.0 μM) slightly potentiated (up to 9%) either one or both phases of contraction of some tissues. However, on other tissues, indomethacin had no effect on the size of the contractions. Thus, indomethacin alone had no statistically significant effect.

Cicaprost (0.5 to 30.0 nM) dose-dependently and significantly ($P<0.05$) potentiated the first phase of contraction (Figure 4). At higher concentrations (>30 nM), cicaprost began to inhibit these contractions. Cicaprost (0.5 to 540 nM)

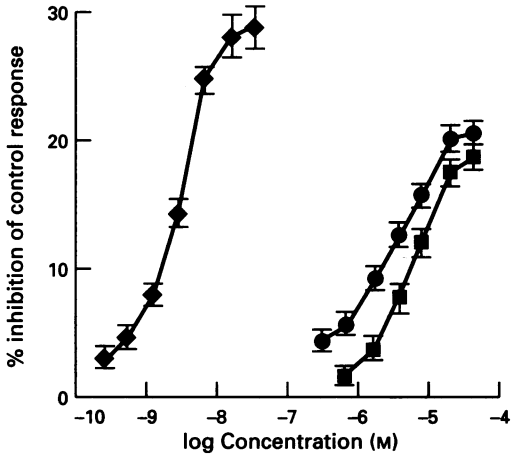

Figure 3 Mean (±s.e.mean, $n=4$)% inhibition of the second phase of contraction of the guinea-pig vas deferens produced by electrical field stimulation after treatment with prostaglandin E_2 (◆), arachidonic acid alone (●) and arachidonic acid in the presence of 2.8 μM indomethacin (■).

had no reproducible effect on the second phase of contraction. On some tissues there was slight inhibition (up to 8%), but other tissues showed no change.

Effects of prostaglandins on contractions produced by exogenous noradrenaline and ATP

PGE_2 (1.1 to 56.9 nM) and sulprostone (0.9 to 85.8 nM) significantly ($P<0.05$) potentiated contractions of the guinea-pig vas deferens produced by a submaximal concentration of noradrenaline. The % potentiation tended to increase with increasing concentrations of prostaglandin (Figure 5). Cicaprost (1.1 nM to 2.7 μM) had no consistent effect on the response produced by noradrenaline although, at the higher concentrations (200 nM to 2.8 μM), slight potentiation (up to 5%) was sometimes observed. PGE_2 (1.1 to 56.9 nM), sulprostone (0.9 to 85.8 nM) and cicaprost (1.1 to 109.0 nM) significantly ($P<0.05$) potentiated contractions of the guinea-pig vas deferens produced by a submaximal concentration of ATP, with the % potentiation tending to increase with increasing concentrations of prostaglandin (Figure 6).

Discussion

PGE_2 produced complete inhibition of the first phase of contraction of the guinea-pig vas deferens produced by electrical field stimulation, which is in agreement with previous studies (Ambache & Aboo Zar, 1970; Hedqvist, 1974; Venkova & Radomov, 1988; Hata *et al.*, 1991; Christian & Poyser, 1994). Sulprostone also completely inhibited this first phase of contraction, and was 4 times more potent than PGE_2. This is also in agreement with a previous study (Christian & Poyser, 1994) and, since sulprostone is more potent than PGE_2 on EP_3 receptors, is consistent with the EP_3 receptor being involved in this inhibitory response (Mantelli *et al.*, 1990; Lawrence *et al.*, 1992). PGE_2 potentiated contractions of the guinea-pig vas deferens produced by exogenous ATP, which confirms a previous report (Ellis & Burnstock, 1990). Sulprostone produced a similar potentiation and was slightly more potent than PGE_2. This finding suggests that the EP_3 receptor is also involved in

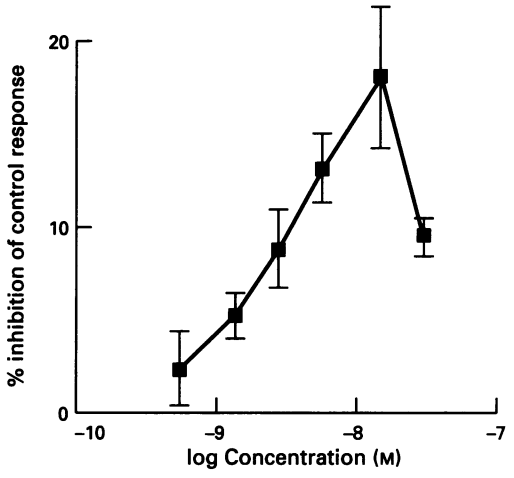

Figure 4 Mean (±s.e.mean, $n=4$)% potentiation by cicaprost of the first phase of contraction of the guinea-pig vas deferens produced by electrical field stimulation.

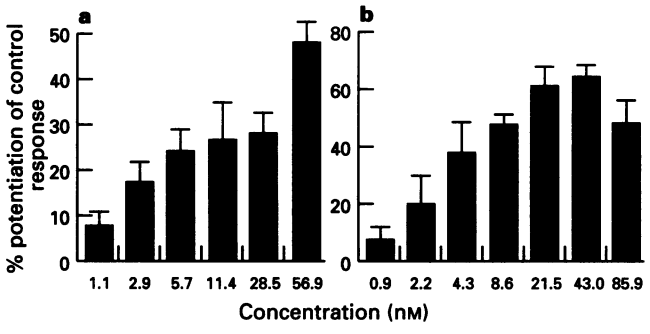

Figure 5 Mean (±s.e.mean, $n=4$)% potentiation by (a) prostaglandin E_2 and (b) sulprostone of the contractions of the guinea-pig vas deferens produced by noradrenaline.

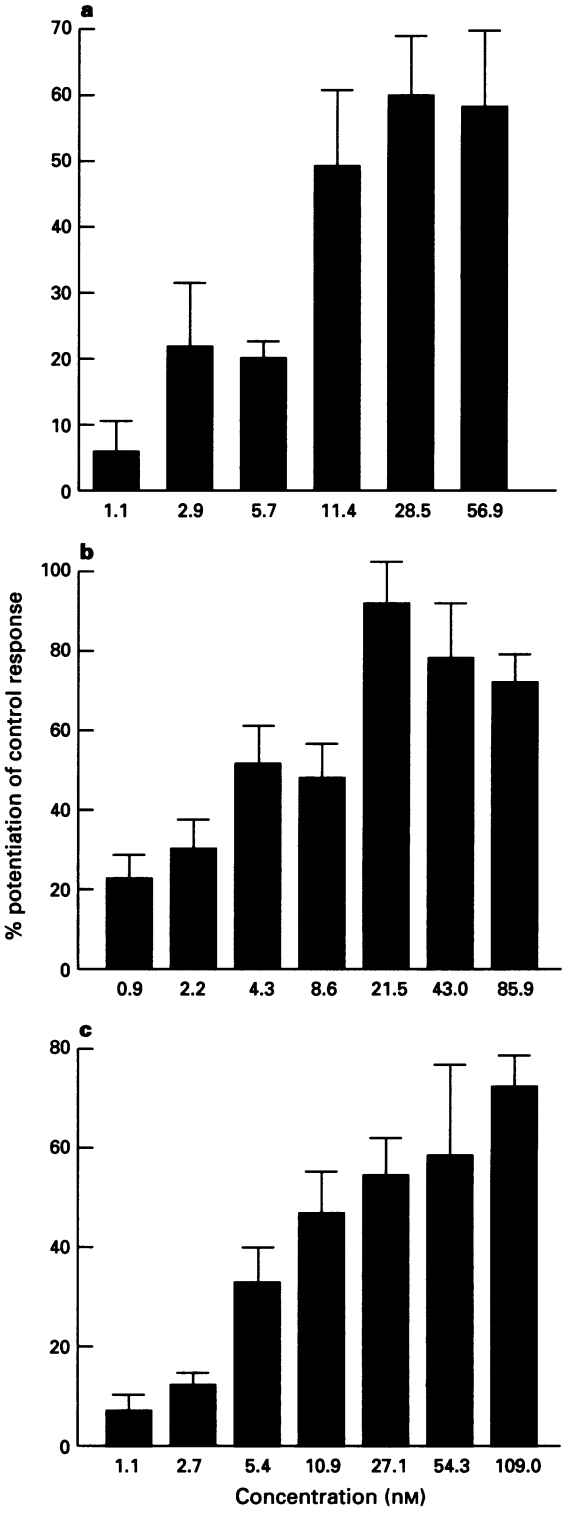

Figure 6 Mean (±s.e.mean, $n=5$)% potentiation by (a) prostaglandin E_2, (b) sulprostone and (c) cicaprost of the contractions of the guinea-pig vas deferens produced by ATP.

the post-junctional potentiating effect of PGE_2 on the action of ATP. However, this suggestion requires further study using more selective EP receptor agonists, particularly as sulprostone is also active on the EP_1 receptor. The fact that PGE_2 inhibits the first phase of contraction of the guinea-pig vas deferens yet potentiates the action of exogenous ATP indicates that the inhibitory effect of PGE_2 is exerted pre-junctionally.

Following desensitization of the receptor for ATP by pre-treatment with α,β-methylene ATP, PGE_2 and sulprostone consistently and dose-dependently inhibited the second phase of contraction of the guinea-pig vas deferens induced by a train of 120 pulses at 8 Hz. Thus, the removal of any contribution to this response by ATP released from the neurones and/or smooth muscle (Venkova & Radomov, 1988; von Kulgelgen & Starke, 1991) showed that PGE_2 exerts a purely inhibitory effect on this second phase of contraction. Previous reports of PGE_2 having 'no effect' or producing a 'potentiation' of this response (Ambache & Aboo Zar, 1970; Swedin, 1971b; Hedqvist, 1974; Venkova & Radomov, 1988; Hata *et al.*, 1991) may have been due to the additional presence of endogenous ATP, particularly non-neuronal ATP which would have had its action potentiated by exogenous PGE_2. Sulprostone was 2.8 times more potent than PGE_2 in inhibiting the response, which is again consistent with EP_3 receptors being involved. However, PGE_2 and sulprostone were not able to inhibit the second phase of contraction completely, and a maximum of 29 to 30% inhibition was obtained. This difference is not attributable to the slightly higher frequency of stimulation (8 Hz) used to obtain the second phase of contraction, since PGE_2 was equally effective and equipotent at inhibiting the first phase of contraction when 5 or 8 Hz was used. PGE_2 and sulprostone potentiated the contraction of the guinea-pig vas deferens produced by exogenous noradrenaline. As with the potentiation of the response to exogenous ATP, sulprostone was slightly more potent than PGE_2 in potentiating the response to exogenous noradrenaline, which again suggests that the EP_3 receptor is involved in this post-junctional action of PGE_2 although further investigation is required. Nevertheless, the inhibitory action of PGE_2 on the second phase of contraction produced by electrical field stimulation is consistent with PGE_2 acting pre-junctionally to inhibit noradrenaline release (Stjärne, 1972; Hedqvist, 1974; Ellis & Burnstock, 1990).

The question is then raised as to how PGE_2, acting pre-junctionally via EP_3 receptors, exerts a greater inhibitory effect on the first phase than on the second phase of contraction induced by electrical field stimulation when ATP and noradrenaline are released as co-transmitters from sympathetic nerve endings in the guinea-pig vas deferens (Stjärne & Åstrund, 1985; Lew & White, 1987; Burnstock, 1990). From studies on the rabbit vas deferens, it has been proposed that the two neurotransmitters are released from different nerve types with the inference that PGE_2 inhibits transmitter release from one of these nerve types more effectively than from the other (Trachte, 1985). Another hypothesis proposes that ATP and noradrenaline are stored in separate populations of vesicles within the sympathetic nerves and that these pools are subject to differential presynaptic modulation (White & MacDonald, 1990). Somewhat surprisingly, PGE_2 has been reported to enhance ATP release from the guinea-pig vas deferens when stimulated at 2 Hz and to have no effect when stimulated at 20 Hz. In both instances, noradrenaline output was reduced (Ellis & Burnstock, 1990). However, the contribution of ATP released from non-neuronal tissue to the amount of ATP measured was not taken into account. In a similar study in which the release of non-neuronal ATP was prevented by the use of prazosin to block α_1-adrenoceptors and suramin to block P_{2x}-purinoceptors, ATP release from the guinea-pig vas deferens evoked by electrical field stimulation was reduced by 60% by 100 nM PGE_2. The release of noradrenaline was reduced by 78% (Driessen & Starke, 1994). It is clear that PGE_2 inhibits the release of both noradrenaline and ATP from sympathetic nerve endings. The molar ratio of noradrenaline to ATP in sympathetic nerve endings has been found to be between 6 and 8 (Lagercrantz, 1976; Fried *et al.*, 1978), which suggests that much more noradrenaline than ATP is released from sympathetic nerve endings. Consequently, although PGE_2 may inhibit noradrenaline and ATP release to similar degrees, the amounts of noradrenaline released may still be adequate to produce sub-maximal responses, whereas the amounts of ATP released may be below the threshold for producing any contractile response. In addition, the extent of the receptor reserve post-junctionally for the two transmitters may also be influential in the differential inhibitory effect of PGE_2 on the first and second phases of contraction.

Cicaprost at low concentrations potentiated the first phase of contraction of the guinea-pig vas deferens produced by field stimulation, thus confirming previous reports (Lawrence *et al.*, 1992; Christian & Poyser, 1994). Cicaprost also potentiated the contractions produced by exogenous ATP. Cicaprost even at high concentrations had no consistent effect on the second phase of contraction. In addition, cicaprost did not have any consistent effect on contractions produced by exogenous noradrenaline, which is in agreement with a previous study (Jones, 1993). As cicaprost has a different profile of action from PGE_2, it is likely that this PGI_2 analogue is exerting its effects via the IP receptor. It has been proposed (Jones, 1993) that, since cicaprost does not potentiate the action of exogenous noradrenaline yet potentiates the first phase of contraction of the guinea-pig vas deferens produced by field stimulation, this IP receptor is situated pre-junctionally and exerts its effect by enhancing transmitter release. If this were so, stimulation of the IP receptor would appear to increase selectively the release of ATP. The present study has shown that cicaprost potentiates the contractile effect of exogenous ATP presumably by a post-junctional action. Therefore, it appears more likely that the IP receptor is situated post-junctionally. This would satisfactorily account for the potentiation of the first phase of contraction by cicaprost without the need for a pre-junctional IP receptor. This point can only be resolved by examining the effect of cicaprost on ATP release.

Arachidonic acid completely inhibited the first phase of contraction and partially inhibited the second phase of contraction. The findings that the time taken for arachidonic acid to produce its effect were 1.8 to 2.6 fold longer than for PGE_2, and indomethacin reduces the action of arachidonic acid on the first phase (Christian & Poyser, 1994) and second phase of contraction indicate that the arachidonic acid is converted to PGE_2 by the tissue in order to produce its effect. The fact that no potentiation of the first phase of contraction is seen with arachidonic acid indicates that the amounts of PGI_2 produced by the guinea-pig vas deferens from exogenous arachidonic acid are relatively small compared to the amounts of PGE_2 produced. It is known that the concentrations of PGE_2 in the rabbit and rat vas deferens are significantly higher than the concentrations of PGI_2 (Swan & Poyser, 1983).

Although prostaglandins affect transmitter release and the post-junctional events in the vas deferens, the question is raised as to whether these actions of prostaglandins are of any physiological significance. It has been reported that repeated periods of prolonged electrical field stimulation of the guinea-pig vas deferens result in the release of PGE_2-like material which is able to inhibit both phases of contraction, with the second phase being inhibited to a lesser extent (Swedin, 1971a; Hedqvist & von Euler, 1972). Inhibitors of prostaglandin synthesis, such as eicosatetraynoic acid (ETYA) and indomethacin, sometimes produce slight increases in the contractions of the guinea-pig vas deferens produced by electrical field stimulation following the acute addition of the inhibitor to the organ bath (Swedin, 1971a; Hedqvist & von Euler, 1972; Fujita *et al.*, 1992; Christian & Poyser, 1994). These findings indicate that the quantities of PGE_2 released from the guinea-pig vas deferens during a short period of electrical field stimulation are too small to have any appreciable inhibitory effect on the contractions produced. However, during repeated periods of prolonged electrical field stimulation of the guinea-pig vas deferens, the contractions of the tissue become gra-

dually smaller. This inhibitory effect is prevented by ETYA (Swedin, 1971a). Consequently *in vivo*, a short period of nerve stimulation to the guinea-pig vas deferens may produce insufficient PGE_2 for it to have any inhibitory effect on sympathetic nerve transmission in the tissue, whereas a prolonged period of stimulation may allow sufficient PGE_2 to accumulate in the tissue for it to have an inhibitory effect on transmission provided by the sympathetic nerves. The results of the present study indicate that the second phase of the contraction would be inhibited to a much lesser extent than the first phase of contraction by this endogenous PGE_2.

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