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EFFECTS OF PROSTAGLANDIN E_2 AND A PROSTAGLANDIN ENDOPEROXIDE ANALOGUE ON NEUROEFFECTOR TRANSMISSION IN THE RAT ANOCOCCYGEUS MUSCLE

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1 Investigations were made into the effects of prostaglandin E_2 (PGE_2) and a prostaglandin endoperoxide analogue (Upjohn compound U-46619) on the responses of the rat anococcygeus muscle to field stimulation of the intrinsic sympathetic nerves, and to exogenous noradrenaline. The effects of PGE_2 on responses to stimulation of intrinsic inhibitory nerves were also studied.

2 PGE_2 (5.6×10^{-8} or 2.8×10^{-6} mol/l) decreased motor (sympathetic) responses to field stimulation at all frequencies tested (2 to 24 Hz). The prostaglandin also reduced the inhibitory responses to field stimulation, seen when the tone of the preparation had been raised and its sympathetic innervation had been blocked by guanethidine. However, these inhibitory responses were also reduced by other spasmogens (carbachol and 5-hydroxytryptamine) which, like PGE_2, further increased the tone of guanethidine-treated preparations.

3 At a concentration of 5.6×10^{-8} mol/l, PGE_2 had no effect on responses to noradrenaline, whereas at a fifty-fold higher concentration the prostaglandin potentiated these.

4 Unlike PGE_2, U-46619 (5.6×10^{-8} mol/l) greatly potentiated motor responses to field stimulation, at frequencies from 0.75 to 24 Hz. This effect did not represent a specific facilitation of sympathetic neurotransmission, as responses to carbachol and 5-hydroxytryptamine, as well as to noradrenaline, were also potentiated.

5 The results are discussed in relation to the effects of prostaglandins and prostaglandin endoperoxides on neuroeffector transmission in other sympathetically innervated tissues. It is concluded that PGE_2 inhibits sympathetic neurotransmission in the rat anococcygeus muscle by a prejunctional action, whereas the predominant effect of U-46619 is direct excitation of the muscle. The effect of PGE_2 on inhibitory responses to field stimulation may represent an interference with inhibitory neuroeffector transmission in this tissue, or may simply be a consequence of the spasmogenic action of the prostaglandin.

Introduction

Prostaglandins of the E series have been shown to inhibit sympathetic neurotransmission in a variety of organs, including the cat spleen (Hedqvist & Brundin, 1969; Hedqvist, 1970a), rabbit heart (Hedqvist, Stjärne & Wennmalm, 1970), rabbit kidney (Frame, Hedqvist & Åström, 1974), guinea-pig vas deferens (Hedqvist & von Euler, 1972a, b) and human and rabbit oviduct (Brundin, 1968; Moawad, Hedqvist & Bygdeman, 1975). In most cases, this effect seems to be largely prejunctional, as it is associated with a reduction in the overflow of noradrenaline following stimulation of the sympathetic nerves to these organs, although in many tissues a postjunctional effect is also apparent, the prostaglandins modifying responses to exogenous noradrenaline. However, the nature of the postjunctional effect varies considerably in different tissues, and is also dependent on the dose and identity of the particular prostaglandin used. For example, in the cat spleen, low concentrations of prostaglandin E_2 (PGE_2) reduce the pressor responses to noradrenaline whilst higher concentrations potentiate them (Hedqvist, 1970a), whereas prostaglandin E_1 (PGE_1) appears only to reduce these responses (Hedqvist & Brudin, 1969; Hedqvist, 1970b). In the guinea-pig vas deferens, both PGE_1 and PGE_2 poten-

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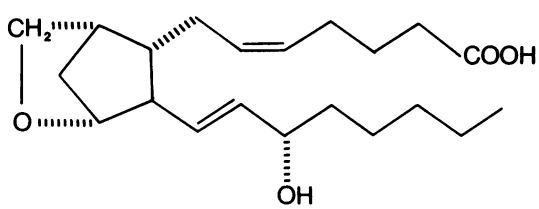
tiate contractile responses to noradrenaline, but the effect of these prostaglandins on neurotransmission is variable, changing from inhibition alone to inhibition followed by potentiation with increasing concentrations (Hedqvist & von Euler, 1972a). Thus, a dual action of prostaglandins on neurotransmission in the vas deferens seems likely, a prejunctional effect (inhibitory) predominating at low doses and a postjunctional effect (excitatory) predominating at higher doses.

In this study, we have investigated the effects of PGE_2 on neurotransmission in another sympathetically innervated tissue, the rat anococcygeus muscle. This preparation consists almost entirely of smooth muscle, with an abundant sympathetic nerve supply which can be activated conveniently by field stimulation (Gillespie, 1972). PGE_2 was chosen because it is the most abundant naturally occurring prostaglandin of the E series, and its effects on neurotransmission have been widely studied in other preparations. Experiments were also carried out with the prostaglandin endoperoxide analogue (15S)-hydroxy-11α,9α-(epoxymethano)-prosta-5*Z*,13*E*-dienoic acid (Upjohn Company, compound U-46619; Bundy, 1975), to see whether this prostaglandin had similar effects to PGE_2 on sympathetic neurotransmission. The structure of this prostaglandin is shown below.



U-46619

Hitherto, there have been few reports concerning the effects of prostaglandin endoperoxides or their analogues on sympathetic neurotransmission, although Hedqvist (1976) reported that the naturally-occurring endoperoxides PGG_2 and PGH_2 inhibit the stimulation-induced release of noradrenaline from the guinea-pig vas deferens.

In addition to its sympathetic (motor) innervation, the rat anococcygeus muscle receives an inhibitory nerve supply. This can best be studied in preparations in which tone has been raised and responses to sympathetic nerve stimulation have been blocked by exposure to guanethidine. Under these conditions, field stimulation causes inhibitory responses (relaxations) which are abolished by low concentrations of tetrodotoxin (Gillespie, 1972). The neurotransmitter released by the inhibitory nerves has so far eluded identification (Gillespie & McKnight, 1976). However, as effects of prostaglandins on transmitter release or neuroeffector transmission are not confined to the sympathetic nervous system (see Bergström, Farnebo & Fuxe, 1973; Hedqvist, 1977), it was also of interest to see whether PGE_2 had any effect on inhibitory responses of the anococcygeus muscle to field stimulation.

Methods

Anococcygeus muscles were dissected from freshly killed adult male Sprague Dawley rats, as described by Gillespie (1972). Each muscle was suspended individually in an organ bath (volume 14 ml when field stimulation was employed, otherwise 6 ml) containing Krebs-Henseleit solution of the following composition (mmol/1): NaCl 118.8, KCl 4.15, $NaHCO_3$ 25.5, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.23 and glucose 11.1. The solution was maintained at 36 to 37°C and gassed with 95% O_2 and 5% CO_2. Tension was measured with a Devices 2STO2 strain gauge and recorded on a Devices M4 polygraph. The initial resting tension was set at approximately 1 gram.

Frequency-response curves for field stimulation of intrinsic motor and inhibitory nerves

The preparation was passed through a pair of silver ring electrodes connected to a Palmer square wave stimulator. Stimulation was carried out with a pulse width of 3 ms and a supramaximal voltage (usually 4 V). Motor responses were evoked by trains of stimuli applied for 15 s every 3 minutes. In experiments where responses to field stimulation of the inhibitory nerves were investigated, guanethidine was added to the Krebs-Henseleit solution in the reservoir supplying the organ bath (Gillespie, 1972) to give a final concentration of 6×10^{-5} mol/1, and the preparation was stimulated for 15 s every 2 minutes.

In each experiment, three sets of responses were obtained, as follows:

(1) before the addition of any prostaglandin (pre-prostaglandin control),

(2) after the addition of PGE_2 or U-46619, either to the organ bath directly or to the Krebs-Henseleit solution in the reservoir supplying the organ bath, and

(3) after wash-out of the prostaglandin from the organ bath (post-prostaglandin control). Each set comprised responses to increasing frequencies of stimulation, usually from 2 to 24 Hz (motor responses) or from 1 to 8 Hz (inhibitory responses). During both sets of control measurements, the dose of prostaglandin was substituted by an equivalent volume of the vehicle alone (0.9% w/v NaCl solution; saline).

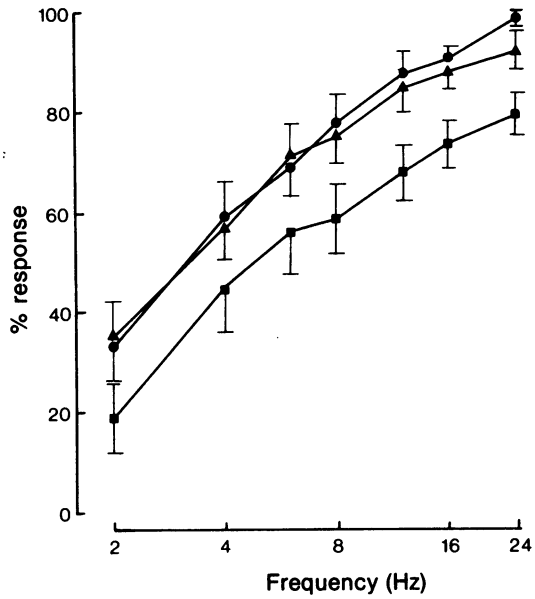


Figure 1 Frequency-response curves for motor responses of the rat anococcygeus muscle to field stimulation before (●), during (■) and after (▲) the presence of prostaglandin E_2 (PGE_2, 5.6×10^{-8} mol/litre). Responses are expressed as percentages of the maximal amplitude of contraction in response to field stimulation in the absence of exogenous PGE_2. Mean results from eight preparations; vertical bars represent s.e. mean.

In each study, results from 6 to 9 experiments were pooled to enable the construction of frequency-response curves relating to responses obtained before, during and after the presence of PGE_2 or U-46619. Each muscle was used for one experiment only.

Dose-response curves for noradrenaline, carbachol and 5-hydroxytryptamine

The experimental design was analogous to that described above, i.e. sets of responses to increasing doses of the agonist under investigation were obtained before, during and after the presence of PGE_2 or U-46619. Experiments were normally conducted with a dose cycle of 6 to 8 min and a contact time of 2 min (noradrenaline), 3 min (carbachol) or 4 min (5-hydroxytryptamine). In some experiments with noradrenaline, however, a high concentration of PGE_2 (2.8×10^{-6} mol/l) was used, and in these the organ bath was not washed out after each dose of the agonist; instead cumulative dose-response curves were obtained using stepwise increments in noradrenaline concentration. This approach was adopted in order to conserve our supplies of PGE_2.

Drugs

PGE_2 and U-46619 were donated by the Upjohn Company. Both were prepared as stock solutions of 1.4×10^{-4} or 2.8×10^{-6} mol/l in saline. Other drugs used were: noradrenaline bitartrate (Sigma Chemical Co.), carbachol chloride (Sigma), 5-hydroxytryptamine bimaleinate (5-HT, Koch-Light Ltd.), phentolamine mesylate (Ciba) and guanethidine sulphate (Ciba). These were also dissolved in saline. The volume of drug solutions added to the organ bath did not exceed 6% of the bath volume.

Results

Effects of prostaglandin E_2 and U-46619 on motor responses to field stimulation

Field stimulation of the anococcygeus muscle produced frequency-dependent contractile responses which were blocked by phentolamine (5×10^{-7} mol/l). At this concentration, phentolamine had no effect on the contractile responses to submaximal concentrations of carbachol.

The effects of PGE_2 (5.6×10^{-8}mol/l) on the motor responses to field stimulation are shown in Figure 1. The prostaglandin reduced these responses, a significant effect being found at each frequency tested ($P < 0.05$ or < 0.01, paired sample t test comparing responses at each frequency before and during the presence of PGE_2). At this dose level, the prostaglandin generally had no effect on the resting tone of our preparations, although in some cases this was increased marginally.

Preliminary experiments had shown that the effect of PGE_2 was reversed rapidly upon removal of the drug from the organ bath. Accordingly, in all experiments with this compound, the third (post-prostaglandin control) set of measurements was started immediately after wash-out of the prostaglandin. It can be seen from Figure 1 that the responses obtained during the two sets of control measurements (pre- and post-prostaglandin) in this series of experiments were very similar.

A similar result was obtained in eight experiments in which PGE_2 was used at a fifty-fold higher concentration (2.8×10^{-6} mol/l). At this concentration, the prostaglandin consistently caused a small increase in the resting tone of the preparation. Motor responses to field stimulation were decreased at all frequencies tested (nine frequencies from 2 to 24 Hz, $P < 0.05$ or < 0.01, except at 6 Hz and 20 Hz where $0.1 > P > 0.05$, paired sample t test) and recovery

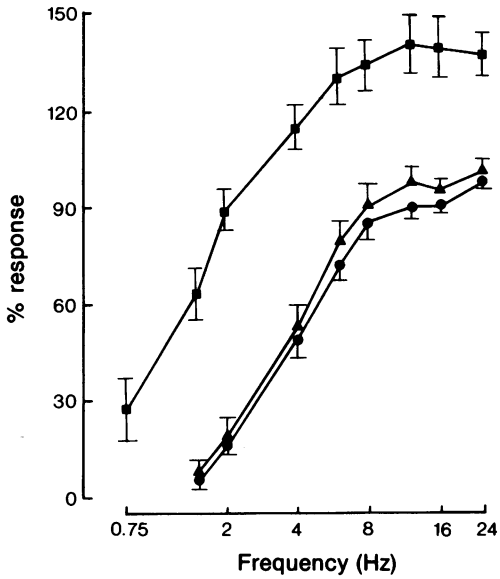


Figure 2 Legend as for Figure 1, but U-46619 (5.6×10^{-8} mol/l) was used in place of prostaglandin E_2. An interval of 40 min was left between the second (■) and third ( ) set of measurements. Mean results from six preparations.

again occurred after wash-out of the prostaglandin (results not shown).

The effects of U-46619 (5.6×10^{-8} mol/l) on the motor responses to field stimulation were entirely different (Figure 2). This prostaglandin potentiated the responses of our preparations at all stimulation frequencies tested ($P < 0.01$ or <0.001; paired sample t test comparing responses before and during the presence of U-46619), including those which apparently gave a maximal response in the absence of the prostaglandin. In addition, U-46619 frequently decreased the rate at which the preparation relaxed when the stimulator was switched off. The prostaglandin also slightly increased the resting tone of the muscle.

Preliminary experiments had shown that the potentiating effect of U-46619 was fairly persistent, responses taking about 40 min to return to control values after wash-out of the prostaglandin. Accordingly, in all experiments with this compound, an interval of 40 min was allowed between the second (during U-46619) and third (post-U-46619) set of measurements. Under these conditions, the two sets of control responses (pre- and post-U-46619) in the present study were very similar (Figure 2).

Effect of prostaglandin E_2 on inhibitory responses to field stimulation

The addition of guanethidine to the Krebs-Henseleit solution bathing the anococcygeus was followed by a large increase in the tone of the muscle, and under these conditions field stimulation caused frequency-dependent relaxations (Gillespie, 1972).

At a concentration of 5.6×10^{-8} mol/l (Figure 3a), PGE_2 had no effect on the inhibitory responses to field stimulation at frequencies from 3 to 8 Hz, but appeared to reduce the responses to lower frequencies ($P < 0.05$ at 1, 1.5 and 2 Hz; paired sample t test comparing responses before and during the presence of PGE_2). After wash-out of the prostaglandin, the responses at 1 and 1.5 Hz were still substantially smaller than those during the pre-prostaglandin control period. When a fifty-fold higher concentration of PGE_2 was used (2.8×10^{-6} mol/l, Figure 3b) there was a marked reduction in the inhibitory responses to field stimulation, at all frequencies tested ($P < 0.05$ or <0.01; paired sample t test comparing responses as above). After wash-out of the prostaglandin, the responses returned almost to the pre-prostaglandin control levels, except at 1 Hz where they were still appreciably smaller.

The addition of PGE_2 to the organ bath in these experiments was followed by a further increase in the tone of the preparation, this effect being particularly noticeable at the higher dose level. It was important, therefore, to see whether other spasmogens similarly reduced the magnitude of the inhibitory responses of the anococcygeus evoked by field stimulation in the presence of guanethidine. Accordingly, experiments were carried out in which responses were obtained before, during and after the addition of carbachol (5×10^{-6} or 1.5×10^{-5} mol/l, 6 experiments) or 5-HT (5×10^{-6} or 1×10^{-5} mol/l, 4 experiments). It was found that, like PGE_2, both these drugs further increased the tone of our preparations, and also substantially reduced the magnitude of the inhibitory responses to field stimulation, at each frequency tested (2, 4 and 8 Hz; results not shown).

Effects of prostaglandin E_2 and U-46619 on responses to noradrenaline

Noradrenaline evoked graded contractile responses from the anococcygeus which were generally maximal at a dose of about 10^{-4} mol/l. Under our conditions, the maximum response obtainable with exogenous noradrenaline was greater than that obtainable by field stimulation.

The effect of PGE_2 (5.6×10^{-8} mol/l) on the responses to noradrenaline is shown in Figure 4a. The prostaglandin appeared to have no effect on these responses, the dose-response curves obtained before and

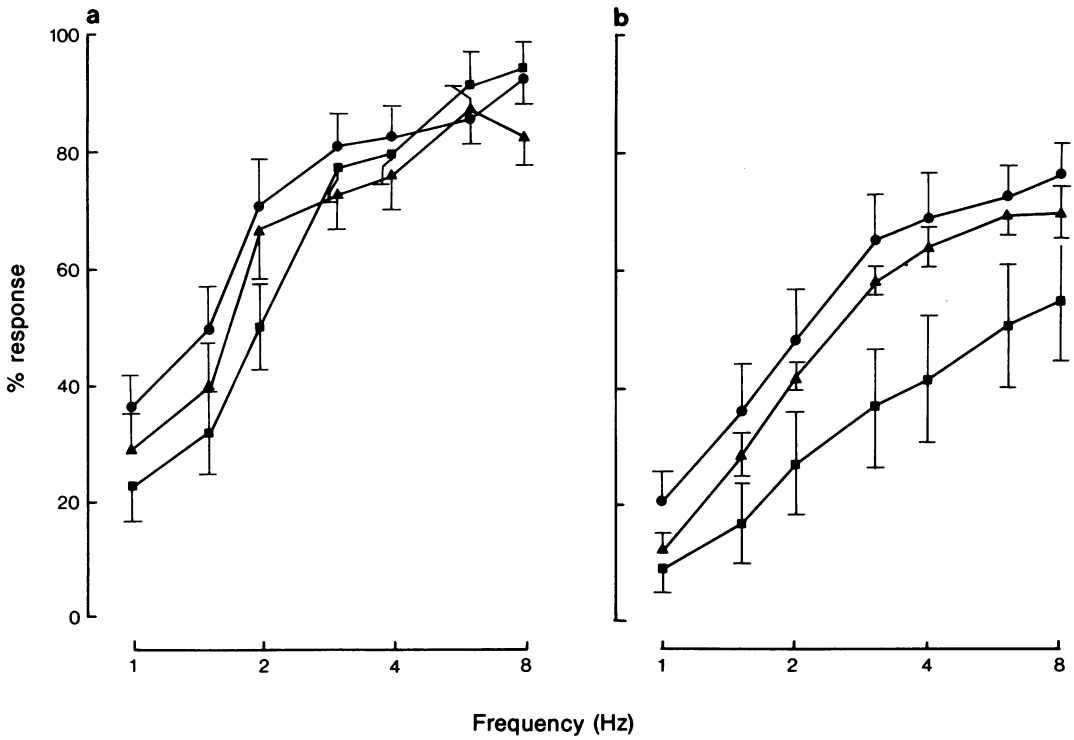


Figure 3 (a) Frequency-response curves for inhibitory responses of the rat anococcygeus muscle to field stimulation in the presence of guanethidine (6×10^{-6} mol/l), before (●), during (■) and after (▲) the presence of prostaglandin E_2, (PGE_2, 5.6×10^{-8} mol/litre). Responses are expressed as percentages of the maximal amplitude of relaxation in response to field stimulation in the absence of exogenous PGE_2. Mean results from nine preparations; vertical bars represent s.e. mean. (b) As for (a), except that PGE_2 was used at a concentration of 2.8×10^{-6} mol/litre. Mean results from nine preparations.

during the presence of PGE_2 being similar. However, with the lower concentrations of noradrenaline ($3.2 \times 10^{-6} - 1.28 \times 10^{-5}$ mol/l), responses obtained during the second (post-prostaglandin) control period were significantly lower than those recorded both in the first (pre-prostaglandin) control period, and during the presence of the prostaglandin ($P < 0.01$ or < 0.001, paired sample t test). This suggested that some deterioration in the responsiveness of our preparations occurred with repeated applications of noradrenaline.

Further experiments were carried out in which the prostaglandin was used at a fifty-fold higher concentration (2.8×10^{-6} mol/l, Figure 4b). As mentioned previously, these experiments involved the measurement of responses to cumulative doses of noradrenaline. It can be seen that the responses of our preparations were increased in the presence of the prostaglandin, this effect being significant ($P < 0.01$ or < 0.001, paired sample t test comparing responses before and during the presence of PGE_2) at each concentration of noradrenaline tested (up to 10^{-5} mol/litre). The two sets of control responses (pre- and post-prostaglandin) were very similar.

The effect of U-46619 on the responses of the anococcygeus to noradrenaline is shown in Figure 5. At a concentration of 5.6×10^{-8} mol/l, this prostaglandin potentiated the responses to submaximal doses of noradrenaline, a significant effect ($P < 0.05$ or < 0.01, paired sample t test comparing responses before and during the presence of U-46619) being found at each dose tested up to 2.56×10^{-5} mol/l, except the lowest (8×10^{-7} mol/l, $P > 0.1$). U-46619 had no significant effect ($P > 0.1$) on the responses to a maximal (or near-maximal) dose of noradrenaline (1.02×10^{-4} mol/litre).

The potentiation by U-46619 of the responses to noradrenaline was far less striking than its potentiation of the responses to motor nerve stimulation. However, as we observed in those experiments with

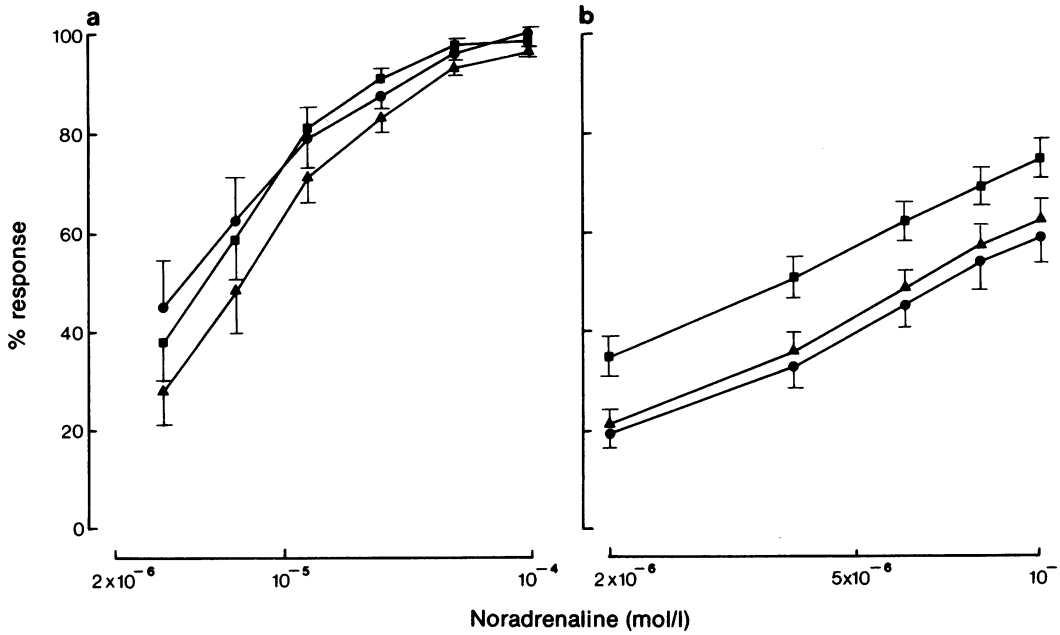


Figure 4 (a) Rat anococcygeus muscle: dose-response curves for noradrenaline before (●), during (■) and after (▲) the presence of prostaglandin E_2 (PGE_2, 5.6×10^{-8} mol/l). Responses are expressed as percentages of the maximal amplitude of contraction in response to noradrenaline, in the absence of exogenous PGE_2. Mean results from eight preparations; vertical bars represent s.e. mean. (b) Dose-response curves for cumulative doses of noradrenaline. Otherwise as for (a) above, except that PGE_2 was used at a concentration of 2.8×10^{-6} mol/1. Mean results from six preparations.

PGE_2 in which repeated doses of noradrenaline were applied, responses obtained with the lower concentrations of the catecholamine were significantly smaller during the second (post-prostaglandin) control period than during the first (pre-prostaglandin) control period ($P < 0.05$ or < 0.01 for concentrations of noradrenaline from 8×10^{-7} to 3.2×10^{-6} mol/l, Figure 5), substantiating our supposition that the sensitivity of our preparations deteriorated with repeated applications of noradrenaline.

Effects of U-46619 on responses to carbachol and 5-hydroxytryptamine

In view of the potentiating effect of U-46619 on the motor responses to field stimulation and to noradrenaline, it was of interest to see whether this compound also potentiated the responses of the anococcygeus to non-adrenoceptor agonists. Initially, the effects of U-46619 on responses to carbachol were investigated. The results are shown in Figure 6. U-46619 (5.8×10^{-8} mol/l) greatly increased the sensitivity of our preparations to carbachol, decreasing the threshold dose and also increasing the responses to both submaximal and maximal concentrations of this agonist. Three further experiments were carried out in which 5-HT was used as the agonist. U-46619 (5.6×10^{-8} mol/l) also decreased the threshold dose of 5-HT and strongly potentiated the responses to submaximal concentrations, although it appeared not to increase the maximal response obtainable with this agonist (results not shown). However, it was noted that the maximum contraction that could be evoked by 5-HT (or by noradrenaline) was greater than that obtainable by maximal doses of carbachol, in the absence of U-46619.

Discussion

The work presented here relates mainly to a study of the effects of prostaglandins on motor responses of the rat anococcygeus muscle to field stimulation. Gillespie (1972) has presented evidence that the motor responses evoked by such stimulation result from activation of intrinsic postganglionic sympathetic nerves. In particular, he found that field stimulation

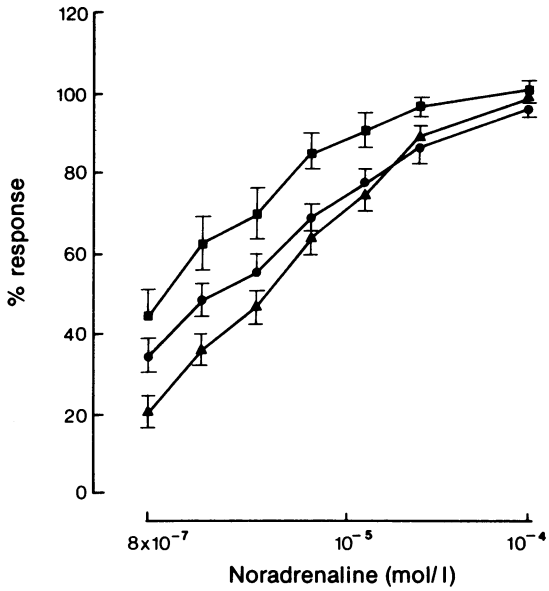


Figure 5 Legend as for Figure 4a, but with U-46619 (5.6×10^{-8} mol/l) in place of prostaglandin E_2. An interval of 40 min was left between the second (■) and third (▲) set of measurements. Mean results from nine preparations.

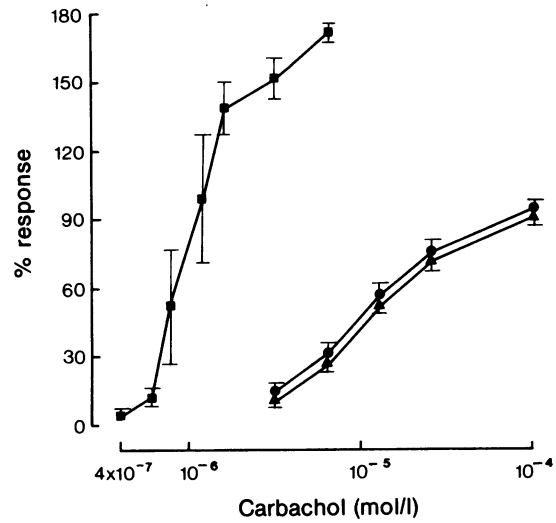


Figure 6 Rat anococcygeus muscle: dose-response curves for carbachol before (●), during (■) and after (▲) the presence of U-46619 (5.6×10^{-8} mol/l). Responses are expressed as percentages of the maximal amplitude of contraction in response to carbachol, in the absence of U-46619. An interval of 40 min was left between the second (■) and third (▲) set of measurements. Mean results from six preparations; vertical bars represent s.e. mean.

gave identical responses to stimulation of extrinsic nerves to this tissue, the effects of both being blocked by phentolamine. Responses to field stimulation were unaffected by hexamethonium. Furthermore, Creed, Gillespie & Muir (1975) found it impossible to stimulate directly the muscle fibres of this preparation by field stimulation in the presence of phentolamine, even with pulse widths as long as 10 milliseconds. In view of the foregoing, and our observation that the motor responses to field stimulation were blocked by a low concentration of phentolamine, it seems a valid assumption that the motor responses we obtained to field stimulation were indeed due to the activation of intrinsic sympathetic nerves.

The effect of PGE_2 on the motor responses of the rat anococcygeus to field stimulation suggests that the prostaglandin inhibits sympathetic neurotransmission in this tissue, as in several other tissues. In our experiments, the prostaglandin seemed to act predominantly prejunctionally, as the lower dose used (5.6×10^{-8} mol/l) had no apparent effect on responses of the tissue to noradrenaline, and the higher dose (2.8×10^{-6} mol/l) actually increased these. Thus, at the higher concentration, PGE_2 seemed to have opposing pre- and post-junctional effects on sympathetic neurotransmission, decreasing motor responses to field stimulation but potentiating responses to added noradrenaline. Similar opposing pre- and post-junctional effects of E prostaglandins on neurotransmission have been reported in other sympathetically innervated tissues, notably the guinea-pig seminal vesicle (Hedqvist, 1972) and vas deferens (Ambache & Zar, 1970; Baum & Shropshire, 1971; Hedqvist & von Euler, 1972a). E type prostaglandins have also been reported to increase the sensitivity of vascular smooth muscle to catecholamines, whilst inhibiting vasoconstrictor responses to sympathetic nerve stimulation (see Hedqvist, 1977).

Field stimulation of the anococcygeus will evoke activity in both the motor and the inhibitory intramural nerve fibres, although in the absence of initial tone only the motor response is apparent (Creed *et al.*, 1975). It was possible, therefore, that the effect of PGE_2 in reducing the motor responses to field stimulation could have resulted from a potentiation by the prostaglandin of the effects of the inhibitory innervation. However, this possibility was excluded by our finding that PGE_2 did not increase the relaxations of the muscle evoked by field stimulation in the presence of guanethidine. On the contrary, the lower concentration of PGE_2 (5.6×10^{-8} mol/l) appeared to decrease the inhibitory responses of the

muscle at low frequencies of stimulation, and with the higher concentration (2.8×10^{-6} mol/l) a reduction in these responses was apparent at all frequencies tested.

From the above results, it seemed that PGE_2 might interfere with transmission not only from the intramural sympathetic nerves but also from the inhibitory nerves to the smooth muscle of the anococcygeus. The prostaglandin could have reduced either the release of transmitter from these inhibitory nerves, or the sensitivity of the muscle to the inhibitory transmitter. However, since PGE_2 caused a further increase in tone of the guanethidine-treated muscle, and other agonists (carbachol and 5-HT) similarly reduced its inhibitory responses to field stimulation, it is possible that the prostaglandin was acting in a non-specific manner, opposing relaxations of the muscle simply by its own spasmogenic action. Thus, our results are not necessarily indicative of a direct effect of PGE_2 on inhibitory neuroeffector transmission in the rat anococcygeus.

The effects of the cyclic endoperoxide precursors of prostaglandins E, F and D on sympathetic neurotransmission have not been studied widely, although these compounds have been shown to have extremely potent actions in certain other biological systems (Hamberg, Svensson, Wakabayashi & Samuelsson, 1974). Hedqvist (1976) found that the prostaglandin endoperoxides, PGG_2 and PGH_2, inhibited the stimulation-induced release of noradrenaline from the guinea-pig vas deferens, although these compounds were less than half as potent as PGE_2. However, a difficulty encountered in experiments with the cyclic endoperoxides is that they are unstable in aqueous media, undergoing spontaneous conversion ($T_{\frac{1}{2}}$ approximately 5 min) mainly to prostaglandins E; thus some of the effects seen with PGG_2 and PGH_2 might be accounted for at least in part by the formation of PGE_2. In view of the problems involved in the preparation and use of the natural prostaglandin endoperoxides, we have used the stable endoperoxide analogue U-46619 in our experiments. This analogue, and the closely related substance (15S)-hydroxy-9α,11α-(epoxymethano) prosta-5*Z*,13*E*-dienoic acid (Upjohn Company compound U-44069) were originally synthesized by Bundy (1975) as compounds which were similar structurally to PGG_2 and PGH_2, but which were more stable. Like the natural endoperoxides they are potent bronchoconstrictors, platelet aggregators and spasmogens of aortic smooth muscle (Beckmann & Leovey, 1976; Wasserman, 1976; Chijimatsu, Nguyen & Said, 1977). At present it is too early to say how closely these analogues resemble the naturally occurring prostaglandins or prostaglandin endoperoxides in their biological activities.

We found that the effect of U-46619 on motor responses of the anococcygeus to field stimulation differed markedly from that of PGE_2, as the endoperoxide analogue strongly potentiated these responses. This effect did not reflect a specific facilitation of neurotransmission, since responses of the tissue to noradrenaline and also to carbachol and 5-HT were potentiated as well. It appeared that the compound had a direct effect on the anococcygeus muscle, causing a small increase in tone when given on its own, and greatly potentiating responses to other agonists. The latter effect was rather long-lasting as recovery was only found to be complete about 40 min after wash-out of the U-46619. The effect of the analogue on the smooth muscle of the anococcygeus may be similar to, but stronger and more persistent than, that of PGE_2. Thus, the latter compound, when used at a fifty-fold higher concentration than U-46619, also caused a small contraction of the muscle and potentiated its responses to noradrenaline. Moreover, the possibility that U-46619 may affect stimulation-induced noradrenaline release in a manner analogous to that of PGE_2 and the natural prostaglandin endoperoxides (Hedqvist, 1976) cannot be excluded from our experiments, as its direct effect on the muscle may have been large enough to mask any such prejunctional effect. Alternatively, U-46619 may have biological actions more in common with those of $PGF_{2\alpha}$, which has been reported to facilitate sympathetic neurotransmission in some tissues, an effect in which both pre- and post-junctional actions have been implicated (see Brody & Kadowitz, 1974; Hedqvist, 1977). Experiments involving measurements of noradrenaline overflow are required to determine what effect, if any, U-46619 has on transmitter release from sympathetic nerve endings.

An interesting finding was that U-46619 clearly increased the maximum responses of the anococcygeus to field stimulation and to carbachol, although it appeared not to increase the maximum response obtainable with added noradrenaline. The fact that the maximum responses that we could evoke with noradrenaline in our preparations were greater than those which we were able to obtain by field stimulation or with carbachol may be relevant to this observation.

There is substantial evidence that the actions of prostaglandins on sympathetic neurotransmission in various tissues reflect a physiological modulatory role of these compounds. For example, the release of prostaglandins, or material identified as prostaglandin-like on the basis of its chromatographic and biological behaviour, has been demonstrated from spleen (Gilmore, Vane & Wyllie, 1968; Davies, Horton & Withrington, 1968; Bedwani & Millar, 1975), heart (Samuelsson & Wennmalm, 1971) and vas deferens (Hedqvist & von Euler, 1972b) in response to sympathetic nerve stimulation. In addition, the prosta-

glandin synthetase inhibitor, indomethacin, has been found to augment the effects of nerve stimulation and of noradrenaline on the capsular and vascular smooth muscle of the cat spleen (Ferreira, Moncada & Vane, 1973), and to increase the outflow of noradrenaline from the field stimulated guinea-pig vas deferens (Stjärne, 1973). A similar effect on noradrenaline release has been observed in these organs, and in the rabbit heart, with a different inhibitor of prostaglandin synthesis, 5,8,11,14-eicosatetraynoic acid (Hedqvist, Stjärne & Wennmalm, 1971; Samuelsson & Wennmalm, 1971; Stjärne, 1973). Experiments on the rat anococcygeus with inhibitors of prostaglandin synthesis, and measurements of prostaglandin release, would be of interest to see whether prostaglandins are likely to have a physiological role as modulators of sympathetic neurotransmission in this tissue also.

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