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FACILITATION OF SYMPATHETIC NEUROTRANSMISSION IN THE RAT ANOCOCCYGEUS MUSCLE BY PROSTAGLANDINS D_2 AND $F_{2\alpha}$

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1 Investigations were made into the effects of prostaglandins D_2 (PGD_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) on the responses of the rat anococcygeus muscle to field stimulation of intrinsic motor (sympathetic) and inhibitory nerves, and to exogenous noradrenaline.

2 PGD_2 (2.8×10^{-6} mol/l) substantially increased the motor responses to field stimulation at all frequencies tested (2 to 32 Hz), and caused a smaller increase in the responses to noradrenaline.

3 $PGF_{2\alpha}$ (2.8×10^{-6} mol/l) strongly potentiated the motor responses to field stimulation (2 to 32 Hz) and also to noradrenaline. This prostaglandin had quantitatively similar effects on the responses to both types of stimulus.

4 PGD_2 was without effect on the inhibitory responses evoked by field stimulation in the presence of guanethidine. $PGF_{2\alpha}$ seemed to reduce the inhibitory responses to low frequencies of stimulation (0.5 to 1.5 Hz), but this effect was marginal.

5 The results suggest that PGD_2 facilitates sympathetic neurotransmission in this tissue by both pre- and post-junctional actions. The effect of $PGF_{2\alpha}$ seems likely to be mediated predominantly post-junctionally.

Introduction

Prostaglandins of the E series inhibit sympathetic neurotransmission in a variety of tissues, predominantly by a pre-junctional action (see Hedqvist, 1977). The effects of F prostaglandins on sympathetically innervated tissues have been investigated less widely, but in several such tissues prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) facilitates neurotransmission. Both pre- and post-junctional actions have been implicated in this effect. For example, in the dog saphenous vein and the rabbit kidney, $PGF_{2\alpha}$ increases the vasoconstrictor responses to both sympathetic nerve stimulation and exogenous noradrenaline, and appears not to facilitate, or may actually inhibit, noradrenaline release (Brody & Kadowitz, 1974; Frame, 1976; Hedqvist, 1976). The prostaglandin also increases the sensitivity of the canine pulmonary lobar artery and vein to noradrenaline (Brody & Kadowitz, 1974). On the other hand, the facilitation of sympathetically-evoked vasoconstrictor responses in the canine uterus, hindpaw and tibial artery by $PGF_{2\alpha}$ seems to result predominantly or entirely from a prejunctional action, i.e. an increase in noradrenaline release (Brody & Kadowitz, 1974). In contrast, this prostaglandin has been found to inhibit neuroeffector transmission in the field stimulated guinea-pig vas deferens, although it was far less active than prostaglandin E_2 (PGE_2) on this preparation (Baum & Shropshire, 1971; Hedqvist & von Euler, 1972).

The biological actions of D prostaglandins have been studied little in comparison with those of prostaglandins E and F. Some of the actions of prostaglandin D_2 (PGD_2) resemble those of $PGF_{2\alpha}$, whereas others are more akin to those of a prostaglandin E (Horton & Jones, 1974; Hamberg, Hedqvist, Strandberg, Svensson & Samuelsson, 1975; Nishizawa, Miller, Gorman & Bundy, 1975; Flower, Harvey & Kingston, 1976; Wasserman, DuCharme, Griffin, De Graaf & Robinson, 1977). However, there have been few studies concerned with the effects of PGD_2 on sympathetic neurotransmission. Hedqvist (1977) observed that PGD_2 inhibited the stimulus-evoked release of noradrenaline from the guinea-pig vas deferens, but with much less potency than PGE_2. Malik (1978) found only inconsistent effects of PGD_2 on the vasoconstrictor responses of the isolated splenic vasculature of the rat to sympathetic nerve stimulation or exogenous noradrenaline, but Jeffrey & Smith (1977) found that PGD_2, like $PGF_{2\alpha}$, potentiated the responses of the dog saphenous vein to noradrenaline.

The present experiments were carried out to investigate the effects of PGD_2 and $PGF_{2\alpha}$ on sympathetic neurotransmission in the rat anococcygeus muscle, a

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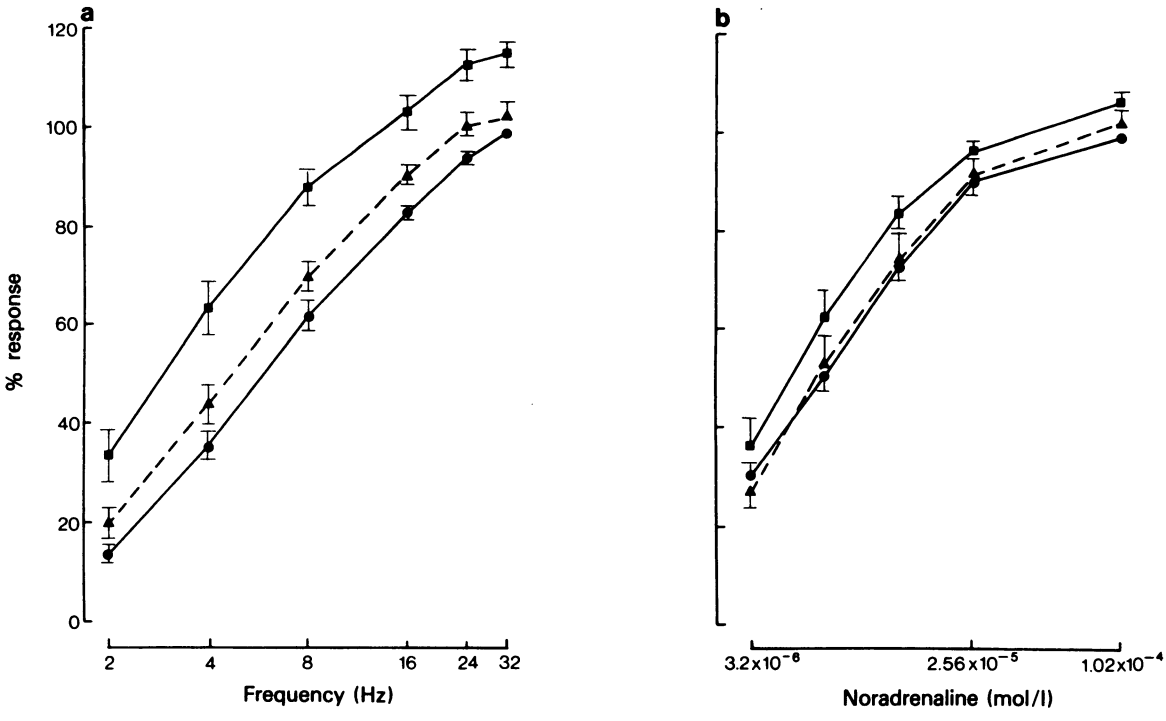

Figure 1 Rat anococcygeus muscle: (a) frequency-response curves for motor responses to field stimulation before (●), during (■) and after (▲) exposure to prostaglandin D_2 (PGD_2, 2.8×10^{-6} mol/l). Responses are expressed as percentages of the response to 32 Hz (which was maximal or near-maximal) before the addition of PGD_2. Mean results from 8 preparations: vertical bars represent s.e. mean. (b) Dose-response curves for noradrenaline. Responses are expressed as percentages of the response to 1.02×10^{-4} mol/l (which was maximal or near-maximal), before the addition of PGD_2. Other details as for (a).

preparation in which PGE_2 has been shown recently to inhibit transmission by a prejunctional action (Al-Timimi, Bedwani & Stanton, 1978).

Methods

Details of the methods used have been published previously (Al-Timimi *et al.*, 1978). Briefly, anococcygeus muscles from large male rats (body weight approximately 400 g) were suspended in an organ bath containing Krebs-Henseleit solution at 36 to 37°C. In each experiment, three sets of responses were obtained to field stimulation of intrinsic motor (sympathetic) or inhibitory nerves, or to exogenous noradrenaline, as follows: (1) before the addition of PGD_2 or $PGF_{2\alpha}$ (pre-prostaglandin control), (2) during the presence of PGD_2 or $PGF_{2\alpha}$ (added directly to the organ bath), and (3) after wash-out of the prostaglandin (post-prostaglandin control). Each set comprised responses to increasing frequencies of field stimulation, or to increasing doses of noradrenaline. The preparation was left unstimulated for 10 min between (1) and (2) (i.e. after the prostaglandin had been added), and between (2) and (3) (i.e. after the prostaglandin had been washed out). During both sets of control measurements, the dose of prostaglandin was substituted by an equivalent volume of the vehicle (0.9% w/v NaCl solution) alone.

Field stimulation was carried out with supramaximal voltage (usually 6 V) and a pulse width of 3 ms. Trains of stimuli were applied for 15 s every 3 min. Experiments with noradrenaline were conducted using a dose cycle of 8 to 12 min, with a contact time of 2 min. In each study, results from 5 to 8 experiments were pooled, enabling the construction of frequency- or dose-response curves for responses obtained before, during and after exposure to PGD_2 or $PGF_{2\alpha}$. Statistical analyses were carried out using the paired sample *t* test.

Drugs

PGD_2 and $PGF_{2\alpha}$ (tromethamine salt), donated by The Upjohn Company, were prepared as stock solutions of 1.4×10^{-4} mol/l in 0.9% w/v NaCl solution

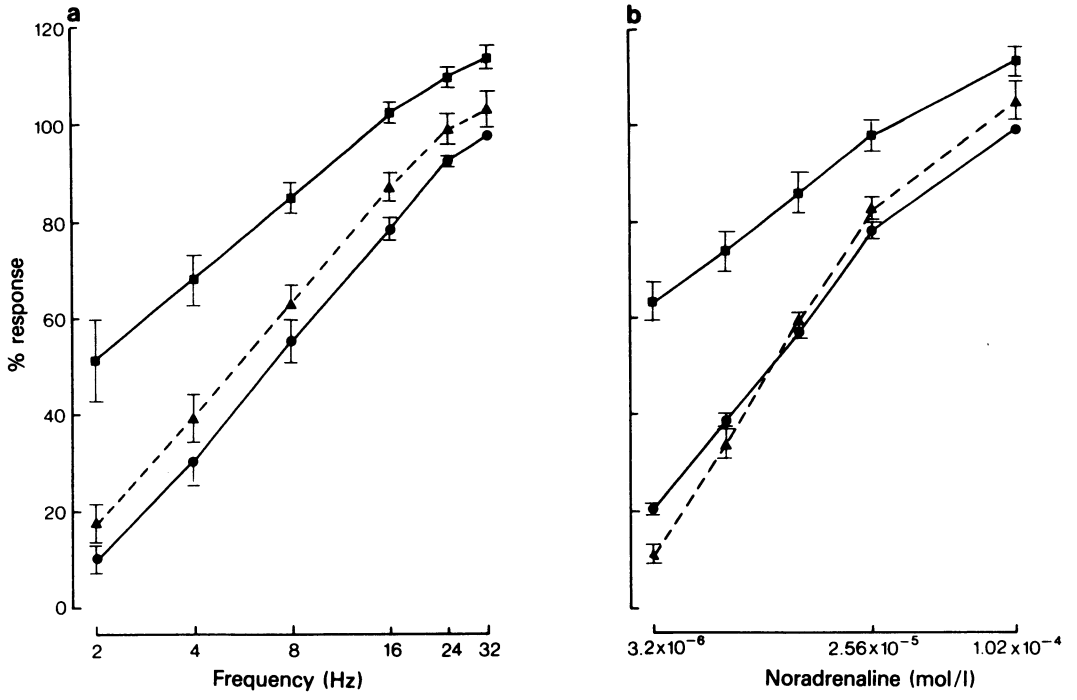

Figure 2 Rat anococcygeus muscle: effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on (a) motor responses to field stimulation (six experiments) and (b) responses to noradrenaline (five experiments). Other details as for Figure 1, but with $PGF_{2\alpha}$ (2.8×10^{-6} mol/l) in place of PGD_2.

(saline). Other drugs used were: noradrenaline bitartrate (Sigma Chemical Co.), phentolamine mesylate (Ciba) and guanethidine sulphate (Ciba). These were also dissolved in saline.

Results

Effects of prostaglandin D_2 on motor responses to field stimulation and noradrenaline

Field stimulation of the anococcygeus muscle gave frequency-dependent contractions which were maximal or near-maximal at 32 Hz, and which were abolished by phentolamine (5×10^{-7} mol/l). The addition of PGD_2 (2.8×10^{-6} mol/l) to the organ bath generally had no effect on the resting tone of the muscle (in some experiments this was increased marginally), but substantially increased the response to each frequency of stimulation tested, i.e. from 2 to 32 Hz (Figure 1a; $P < 0.01$ or < 0.001 at each frequency, comparing responses before and during the presence of PGD_2). After wash-out of the prostaglandin, the responses returned almost to the pre-prostaglandin control levels, although they were still slightly enhanced ($P < 0.05$ or < 0.01 at each frequency), indicating some persistence of the effect of the prostaglandin.

Noradrenaline evoked contractile responses from the anococcygeus muscle, which were generally maximal at a dose of 1.02×10^{-4} mol/l. PGD_2 (2.8×10^{-6} mol/l) potentiated the responses of the preparation to noradrenaline (Figure 1b; $P < 0.01$ at each dose tested, except 3.2×10^{-6} mol/l, where $0.1 > P > 0.05$), but it is evident from a comparison of Figures 1a and 1b that this effect was smaller than the effect of the prostaglandin on the responses to field stimulation. The two sets of control responses (pre- and post-PGD_2) in this study with noradrenaline were very similar.

Effects of prostaglandin $F_{2\alpha}$ on motor responses to field stimulation and noradrenaline

The addition of $PGF_{2\alpha}$ (2.8×10^{-6} mol/l, as used for PGD_2) to the organ bath was frequently followed by a small but maintained increase in the resting tone of the preparation (by about 0.5 to 1 g; cf. a maximal response to noradrenaline which was typically in the region of 10 g). This prostaglandin greatly potentiated the responses to both field stimulation and added noradrenaline (Figure 2; $P < 0.001$ or < 0.01 at each

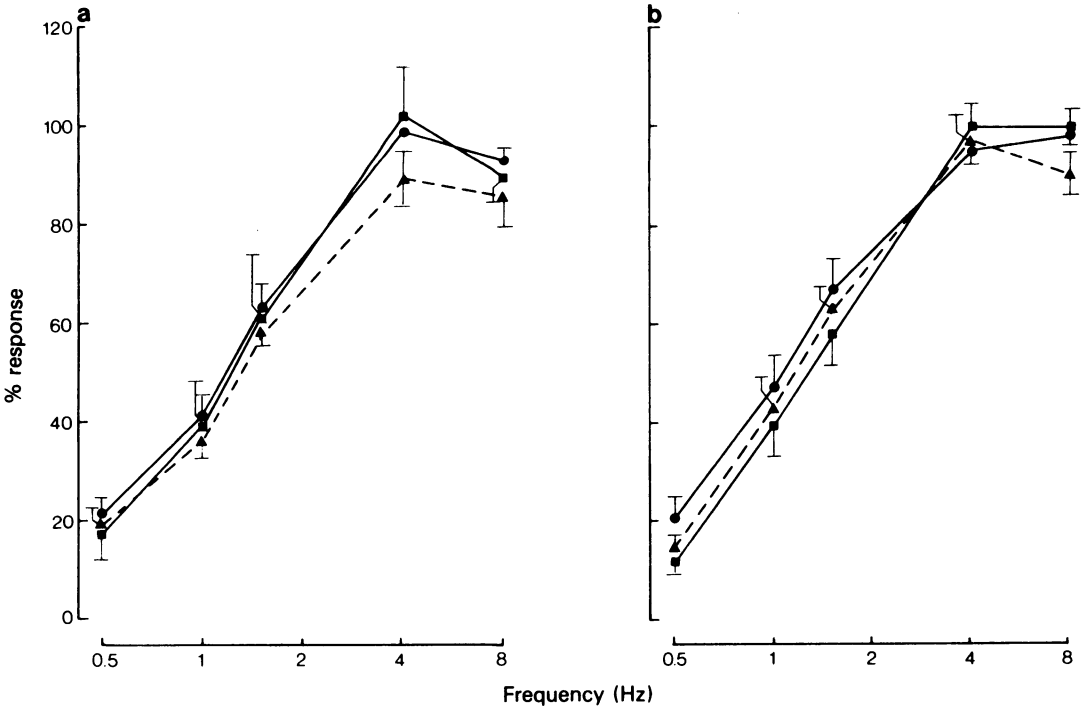

Figure 3 Rat anococcygeus muscle: (a) frequency-response curves for inhibitory responses to field stimulation in the presence of guanethidine (6×10^{-5} mol/l), before (●), during (■) and after (▲) exposure to prostaglandin D_2 (PGD_2) (2.8×10^{-6} mol/l). Responses are expressed as percentages of the maximal amplitude of relaxation in response to field stimulation, before the addition of PGD_2. Mean results from 6 preparations; vertical bars represent s.e. mean. (b) As for (a), but with $PGF_{2\alpha}$ (2.8×10^{-6} mol/l) in place of PGD_2. Mean results from eight preparations.

frequency or dose tested, except at 1.02×10^{-4} mol/l noradrenaline where $P < 0.05$, comparing responses before and during the presence of the prostaglandin). After wash-out of the prostaglandin, the responses returned to levels similar to those in the pre-prostaglandin control periods, although in the field stimulation study the post-prostaglandin control responses were still slightly enhanced ($P < 0.05$ at each frequency, except at 2 and 32 Hz where $0.1 > P > 0.05$, comparing responses before and after $PGF_{2\alpha}$).

It is apparent from a comparison between Figures 2a and 2b that the effects of $PGF_{2\alpha}$ on the responses to field stimulation and to exogenous noradrenaline were similar in magnitude. In this respect, $PGF_{2\alpha}$ differed from PGD_2, which increased the responses to field stimulation considerably more than those to noradrenaline.

A comparison of Figures 2a and 1a shows that $PGF_{2\alpha}$ potentiated the responses to the lower frequencies of field stimulation rather more than PGD_2, whereas at maximal and near-maximal frequencies the effects of the two prostaglandins were quantitatively similar.

Effects of prostaglandins D_2 and $F_{2\alpha}$ on inhibitory responses to field stimulation

Field stimulation of the rat anococcygeus muscle is likely to activate intramural inhibitory as well as motor nerve fibres (Creed, Gillespie & Muir, 1975). Thus, the potentiating effects of PGD_2 and $PGF_{2\alpha}$ on the motor responses to field stimulation could possibly have been due to an inhibition of transmission from the inhibitory nerve fibres. It was important, therefore, to examine the effects of these prostaglandins on the responses to the inhibitory innervation. These responses were revealed as relaxations by field stimulation in the presence of guanethidine (6×10^{-5} mol/l), which raises the tone of this preparation and abolishes the effects of stimulating the sympathetic innervation (Gillespie, 1972).

The effects of PGD_2 (2.8×10^{-6} mol/l) on these inhibitory responses are shown in Figure 3a. It can be

seen that the responses obtained in the presence of the prostaglandin were similar to those in the pre-prostaglandin control period (no significant difference at any frequency tested, $P > 0.1$), and also to those in the post-prostaglandin control period (although less so at the two highest frequencies where the post-prostaglandin control responses showed evidence of deterioration). Thus, PGD_2 appeared to have no effect on the responses of the anococcygeus muscle to inhibitory nerve stimulation.

The effects of $PGF_{2\alpha}$ (2.8×10^{-6} mol/l) on the inhibitory responses to field stimulation are shown in Figure 3b. This prostaglandin also had little effect on these responses, although it seemed to reduce slightly the relaxations evoked by low frequencies of stimulation ($P < 0.01$ at 0.5 Hz, $0.1 > P > 0.05$ at 1 and 1.5 Hz, comparing responses before and during the presence of the prostaglandin). After wash-out of the prostaglandin, the responses to these low frequencies returned towards those seen during the pre-prostaglandin control period, although they were still somewhat smaller.

Discussion

The contractions evoked by field stimulation of the rat anococcygeus muscle have been shown to result from the activation of intrinsic sympathetic nerve fibres, and not from a direct excitation of smooth muscle fibres (Creed *et al.*, 1975). Thus, the results presented here indicate that PGD_2 and $PGF_{2\alpha}$ facilitate sympathetic neurotransmission in this tissue. The sites of this facilitation cannot be stated with certainty, because no measurements of noradrenaline release were made. However, PGD_2 appeared to potentiate the responses to field stimulation considerably more than those to exogenous noradrenaline, so it seems likely that this prostaglandin was acting both pre- and post-junctionally, i.e. increasing the release of noradrenaline from the intramural sympathetic nerve terminals and also increasing the responsiveness of the muscle fibres. If this interpretation is correct, it appears that the effect of PGD_2 on the rat anococcygeus muscle differs from that on the guinea-pig vas deferens, where Hedqvist (1977) found it to reduce transmitter release. However, because PGD_2 was so much less potent than PGE_2 on his preparation, Hedqvist could not exclude the possibility that this effect was due to contamination of his sample of PGD_2 with traces of PGE_2. The potentiation of the responses of the anococcygeus muscle to noradrenaline by PGD_2 is in accordance with the effect of this prostaglandin on the dog saphenous vein (Jeffrey & Smith, 1977).

The possibility that PGD_2 may have increased the responses of the rat anococcygeus muscle to field stimulation by interfering with transmission from the inhibitory nerve fibres present in this preparation can be excluded, since we found it to have no effect on the inhibitory responses revealed by field stimulation in the presence of guanethidine.

$PGF_{2\alpha}$ also potentiated the motor responses of the anococcygeus muscle to field stimulation but unlike PGD_2, it had just as large an effect on the responses to exogenous noradrenaline. It seems likely, therefore, the $PGF_{2\alpha}$ was acting predominantly or entirely postjunctionally (i.e. on the cell membrane, the excitation–contraction coupling process or the contractile machinery of the smooth muscle fibres). However, it is also conceivable that the prostaglandin was acting by inhibiting the neuronal or extraneuronal uptake of noradrenaline. The effects of $PGF_{2\alpha}$ on sympathetic neurotransmission and the responses to noradrenaline in this preparation are analogous to those in the dog saphenous vein (Brody & Kadowitz, 1974) and the rabbit kidney (Frame, 1976; Hedqvist, 1976).

There was a tendency for the inhibitory responses of the anococcygeus muscle to low frequencies of field stimulation (0.5 to 1.5 Hz) to be reduced slightly in the presence of $PGF_{2\alpha}$. Thus, it is possible that this prostaglandin might have had a weak inhibitory effect on transmission from the inhibitory nerve fibres. However, it is difficult to come to any firm conclusion about this because the effect was small and the responses did not return completely to the pre-prostaglandin control levels after wash-out of the prostaglandin (Figure 3b). In any case, any effect that $PGF_{2\alpha}$ might have had on low-frequency transmission from these inhibitory nerve fibres is unlikely to have contributed to the large potentiation of the motor responses to field stimulation seen in the presence of the prostaglandin, because this occurred at higher frequencies of stimulation (i.e. 2 to 32 Hz).

It is apparent from the work presented here that the effect of PGD_2 on sympathetic neurotransmission in the rat anococcygeus muscle differs markedly from that of its isomer, PGE_2 (Al-Timimi *et al.*, 1978). PGD_2 resembles $PGF_{2\alpha}$ to the extent that, at the dose level tested, both prostaglandins facilitate sympathetic neurotransmission in this preparation but our results suggest that there are differences between the sites of action of the two prostaglandins. Further experiments, including measurements of noradrenaline overflow, are required before any definite conclusions can be drawn as to the relative effects PGD_2 and $PGF_{2\alpha}$ on neurotransmitter release, effector sensitivity and neurotransmitter re-uptake in this tissue.

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