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Different effects of prostaglandins on adrenergic neurotransmission in atrial and ventricular preparations

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1 The effects of prostaglandin E_2 (PGE_2) and iloprost on the cardiac response to adrenergic nerve stimulation in guinea-pig atrial and ventricular preparations have been studied.

2 In guinea-pig isolated atria both PGE_2 (0.1–10 nM) and iloprost (0.1—3 μM) concentration-dependently reduced the cardiac response to adrenergic nerve stimulation.

3 The inhibition of cyclo-oxygenase by indomethacin and acetylsalicylic acid potentiated the response to nerve stimulation in the atrial preparations.

4 Arachidonic acid (1–10 μM) reduced the response to nerve stimulation in atria. This effect was prevented by indomethacin and acetylsalicylic acid.

5 In guinea-pig ventricles PGE_2 and iloprost were found to be effective at higher concentrations than in atrial preparations: arachidonic acid, indomethacin or acetylsalicylic acid did not modify the cardiac response to adrenergic nerve stimulation.

6 These results suggest a different modulator role for endogenous prostaglandins in atrial and ventricular tissue.

Introduction

It is widely accepted that prostaglandins of the E and I series are able to reduce the release of noradrenaline induced by sympathetic nerve stimulation in several tissues, including the rabbit and rat heart (Hedqvist *et al.*, 1970; Wennmalm, 1971; Fuder & Muscholl, 1974; Hedqvist, 1977; Khan & Malik, 1980; 1982).

Moreover, it has been demonstrated that stimulation of sympathetic nerves induces the output of a prostaglandin-like material from cardiac tissues (Junstad & Wennmalm, 1973; Khan & Malik, 1980), and that the inhibition of prostaglandin synthesis enhances the release of noradrenaline induced by adrenergic nerve stimulation (Samuelsson & Wennmalm, 1971; Chanh *et al.*, 1972; Fuder & Muscholl, 1974; Khan & Malik, 1982). These observations suggest that endogenous prostaglandins may have a physiological, modulator role on adrenergic neurotransmission in the heart.

In all previous studies the effects of prostaglandins have been tested on the whole isolated heart of the rabbit or rat. Hence, in the present study we decided to examine the effect of prostaglandins in two different sections of mammalian heart, namely atria and ventricular strips, in order to test whether the effects of prostaglandins may differ from section to section. Moreover, we have used two inhibitors of prostaglandin synthesis, indomethacin and acetylsalicylic acid, and arachidonic acid, to explore the possible modulator role played by endogenous prostaglandins in the atrial and ventricular tissues of guinea-pig heart.

Methods

Field stimulation of sympathetic nerve terminals during the functional refractory period was used in the experiments. Guinea-pig isolated preparations (atria or ventricular strips) were mounted in a glass chamber containing 15 ml of Tyrode solution bubbled with a gas mixture (95% O_2/5% CO_2) at a pH of 7.4 and at a temperature of 30°C. The preparations were driven at a constant rate (4 Hz for the atria, 2 Hz for the ventricular strips) by two pointed platinum electrodes. Field stimulation was applied through two platinum plates parallel to the preparation, during the absolute refractory period. A graded stimulation of sympathetic nerve terminals was induced by slightly different techniques in atrial and ventricular preparations. The method employed for the stimulation of ventricular preparations was almost identical to the one described by Blinks (1966): graded stimulus-inotropic response curves were obtained by applying one field pulse every 16 contractions (1/16) for 90 s, and then the frequency was doubled to one pulse every 8 contractions (1/8) and so on up to one pulse for every contraction (1/1), which produced the maximum increase in contractility. A second curve was determined after exposure to the selected drug, 40 min after the end of the first curve. For stimulation of atrial preparations a slight modification of the above-mentioned method was employed: trains of field pulses were applied at 3 min intervals, at a rate of one per contraction, and the number of field pulses per train was increased from 2 to 12, until the maximum positive inotropic response was reached (Ledda & Mantelli, 1984; Ledda *et al.*, 1984; 1985). The main reasons for this modification were the different responsiveness of atria and ventricles: in fact we previously observed that the total number of field pulses able to evoke a sympathetic response in atria was remarkably lower than that required for ventricular tissue, probably as a consequence of the greater innervation of atria. All the experiments in which the method of field stimulation was used were carried out in the presence of 1 μM atropine, in order to eliminate the parasympathetic component of the response (Blinks, 1966; Angus & Harvey, 1981). Dose-effect curves for noradrenaline were obtained by adding cumulative concentrations of the agonist. Statistical analysis was performed by Student's *t* test for comparisons between two groups of data. Analysis of variance and Tukey's test were used when a comparison between three or more groups was made. The following drugs were used: atropine sulphate (BDH), iloprost (kindly supplied by Schering), prostaglandin E_2 (PGE_2), acetylsalicylic acid, indomethacin, arachidonic acid (Sigma). All the drugs were dissolved in Tyrode solution except indomethacin and arachidonic acid, which were dissolved in ethanol and further diluted in Tyrode solution.

Results

Characteristics of the responses induced by field stimulation

In both atrial and ventricular preparations, trains of field pulses applied during the refractory period induced a graded

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positive inotropic effect. In preliminary experiments we verified the reliability of the two different methods of field stimulation, comparing the effects of a well-known prejunctional inhibitory agonist, i.e. clonidine, in atria and ventricles. The effectiveness of clonidine in reducing the positive inotropic effect of sympathetic stimulation in ventricular preparations was similar to that previously obtained in atrial preparations (Ledda & Mantelli, 1984). The IC_{50} value for clondine in ventricular tissue was 17.2 ± 1.8 nM (data not shown), a value very close to that previously detected in guinea-pig atrial preparations (Ledda & Mantelli, 1984).

Effect of PGE_2 on the sympathetic response to field stimulation

Exposure of guinea-pig atria to increasing concentrations of PGE_2 (0.1–10 nM) for 10 min before field stimulation produced a concentration-dependent and significant depression of the stimulus-response curve (Figure 1a). The concentration of PGE_2 able to induce a 50% inhibition (IC_{50}) of the response caused by an intermediate degree of sympathetic stimulation (i.e. the response induced by a train of 8 pulses) was 0.55 ± 0.11 nM. In guinea-pig ventricular preparations PGE_2 reduced the cardiac response to sympathetic nerve stimulation at higher concentrations than in guinea-pig atria (1–10 nM). The degree of inhibition of the cardiac response was lower in this tissue, and was significant at low and intermediate degrees of sympathetic stimulation but not at the maximal degree (see Figure 1b). The IC_{50} for PGE_2 (calculated for the response induced by intermediate stimulation, i.e. 1/2) was 4.06 ± 1.5 nM in ventricular tissue. In both tissues the inhibitory effect of PGE_2 was chiefly attributable to a prejunctional effect since, at the highest concentration tested (10 nM), PGE_2 did not modify cardiac contractility or the positive inotropic response to exogenous noradrenaline (see Table 1).

Effect of iloprost on the sympathetic response to field stimulation

A 10 min exposure of guinea-pig atria to iloprost, a synthetic stable analogue of prostacyclin, induced a concentration-dependent and significant rightward shift of the stimulus-response curve to sympathetic nerve stimulation (Figure 2a). Iloprost was active at concentrations ranging from 0.1 to 3 μM, and its IC_{50} (calculated as for PGE_2) was 0.74 ± 0.2 μM. In guinea-pig ventricular preparations iloprost was again active at ten fold higher concentrations; it was inactive at 0.1 μM (Figure 2b), and the IC_{50} = 1.5 ± 0.5 μM. It is interesting to note that the pattern of inhibition by iloprost of the sympathetic response in ventricular tissue was again different from

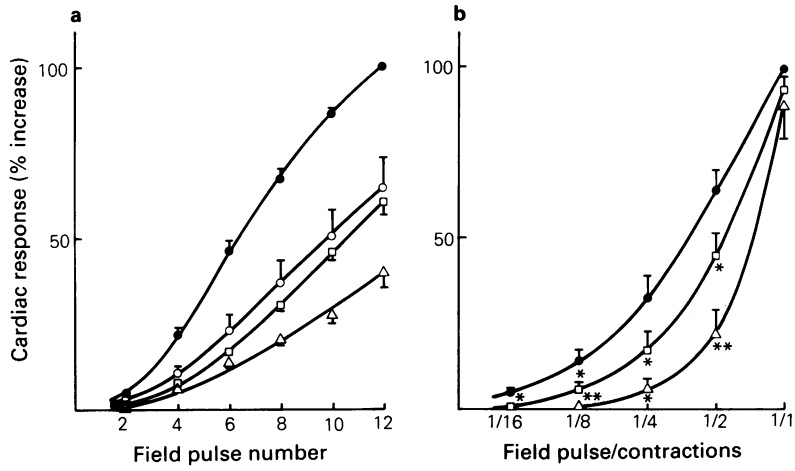

Figure 1 Effect of prostaglandin E_2 (PGE_2) on the stimulus-inotropic response curve obtained by increasing the number of field pulses in guinea-pig isolated atria stimulated at 4 Hz (a) and in ventricular strips stimulated at 2 Hz (b). (●) Control; (○) PGE_2 0.1 nM; (□) PGE 1 nM; (△) PGE_2 10 nM. Points represent means of 6 (a) and 5 (b) experiments; vertical lines show s.e.mean. In (a) all the points were statistically different from the control according to analysis of variance and Tukey's test ($P < 0.01$); symbols were omitted for clarity. In (b) $^*P < 0.05$; $^{**}P < 0.01$.

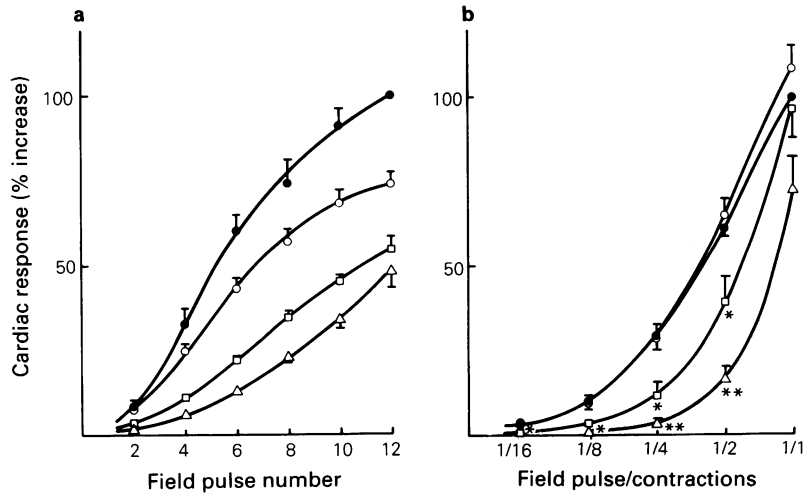

Figure 2 Effect of iloprost on the stimulus-inotropic response curve obtained by increasing the number of field pulses in guinea-pig isolated atria stimulated at 4 Hz (a) and in ventricular strips stimulated at 2 Hz (b). (●) Control; (○) iloprost 0.1 μM; (□) iloprost 1 μM; (△) iloprost 3 μM. Points represent means of 4 (a) and 6 (b) experiments; vertical lines show s.e.mean. In (a) all the points were statistically different from the control according to analysis of variance and Tukey's test ($P < 0.01$). In (b) $^*P < 0.05$; $^{**}P < 0.01$.

Table 1 Increase in contractile force induced by increasing concentrations of noradrenaline in guinea-pig atrial and ventricular preparations in the absence and presence of prostaglandin E_2 (PGE_2)

Noradrenaline concentration (μM)	*Control* (% *increase*)	PGE_2 (10 nM) (% *increase*)
Atrial preparations ($n = 6$)		
0.01	6.9 ± 1.2	6.1 ± 0.9
0.03	14.9 ± 2.2	14.4 ± 1.6
0.1	31.5 ± 3.8	32.2 ± 2.7
0.3	52.0 ± 5.1	51.0 ± 5.0
1	77.8 ± 5.4	76.8 ± 6.2
3	93.5 ± 2.6	92.5 ± 6.6
10	100.0 ± 0.0	100.4 ± 6.3
Ventricular preparations ($n = 4$)		
0.01	2.0 ± 0.7	2.0 ± 0.7
0.03	5.2 ± 1.4	4.3 ± 1.1
0.1	13.8 ± 2.6	11.9 ± 2.0
0.3	30.0 ± 5.2	26.3 ± 2.2
1	53.3 ± 5.0	52.7 ± 3.0
3	82.9 ± 6.6	87.9 ± 4.9
10	100.0 ± 0.0	109.4 ± 4.2

that observed in the atria, and was qualitatively similar to that observed with PGE_2 (compare Figures 1b and 2b). Iloprost (3 μM) was unable to affect the cardiac response to exogenous noradrenaline in ventricular preparations (data not shown); in atrial preparations, at the same concentration, it slightly but significantly reduced the positive inotropic effect of exogenous noradrenaline (Figure 3). However, its postjunctional inhibitory effect was very slight in comparison to the effect induced by the same concentration on the cardiac response to adrenergic nerve stimulation. Thus, the action of iloprost is mainly attributable to a prejunctional effect.

Effect of indomethacin and acetylsalicylic acid on the sympathetic response to field stimulation

A 40 min contact period of preparations with indomethacin and acetylsalicylic acid, at concentrations (3 μM and 500 μM respectively) able to block cyclo-oxygenase in isolated tissues (for a review see Flower, 1974), significantly potentiated the cardiac response to field stimulation in guinea-pig atrial tissue (Figure 4a). This effect was especially evident at a high intensity of nerve stimulation. On the other hand, neither indomethacin nor acetylsalicylic acid was able to modify the sympathetic response in guinea-pig ventricular preparations (Figure 4b).

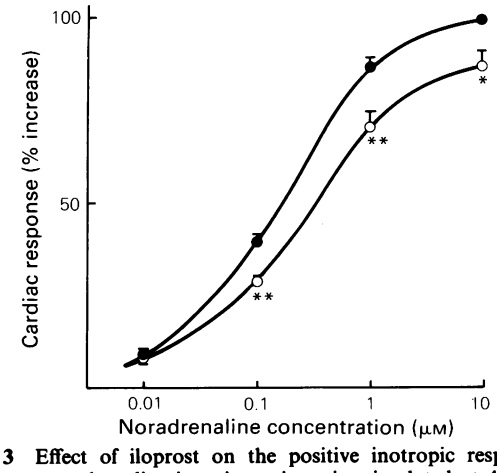

Figure 3 Effect of iloprost on the positive inotropic response to exogenous noradrenaline in guinea-pig atria stimulated at 4 Hz. The effect of the highest concentration of noradrenaline was taken as 100%. (●) Effect of noradrenaline alone and (○) in the presence of 3 μM iloprost. Points represent means of 14 experiments; vertical lines show s.e.mean. $*P < 0.05$; $**P < 0.01$.

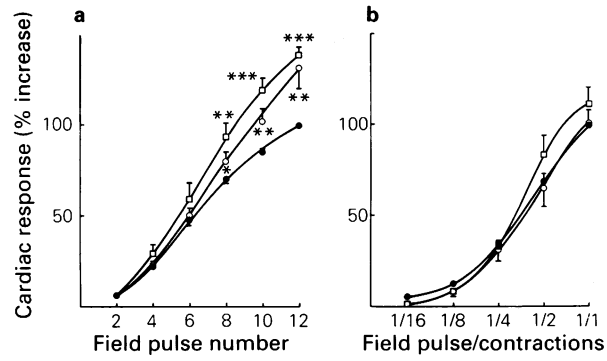

Figure 4 (a) Effect of indomethacin and acetylsalicylic acid on the stimulus-inotropic response curve obtained by increasing the number of field pulses in guinea-pig isolated atria stimulated at 4 Hz. (●) Control ($n = 12$); (○) indomethacin 3 μM ($n = 7$); (□) acetylsalicylic acid 500 μM ($n = 5$). $*P < 0.05$; $**P < 0.01$; $***P < 0.005$. (b) Effect of indomethacin and acetylsalicylic acid in guinea-pig ventricular strips stimulated at 2 Hz. (●) Control ($n = 12$); (○) indomethacin 3 μM ($n = 6$); (□) acetylsalicylic acid 500 μM ($n = 6$).

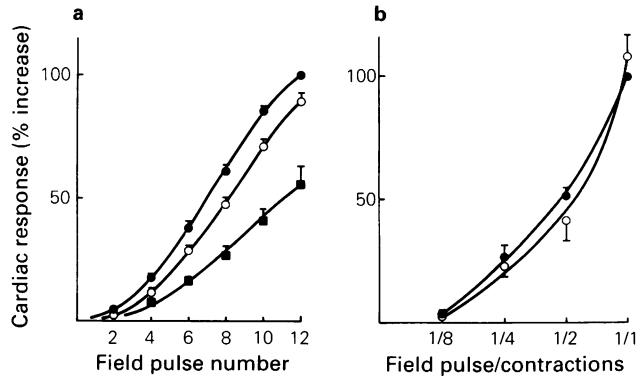

Figure 5 (a) Effect of arachidonic acid on the stimulus-inotropic response curve obtained by increasing the number of field pulses in guinea-pig isolated atria stimulated at 4 Hz ($n = 6$). (●) Control; (○) arachidonic acid 1 μM; (■) arachidonic acid 10 μM. All the points were statistically different from the control according to analysis of variance and Tukey's test. $P < 0.05$ for arachidonic acid 1 μM and $P < 0.01$ for arachidonic acid 10 μM. (b) Effect of arachidonic acid in guinea-pig ventricular strips stimulated at 2 Hz ($n = 4$). (●) Control; (○) arachidonic acid 10 μM.

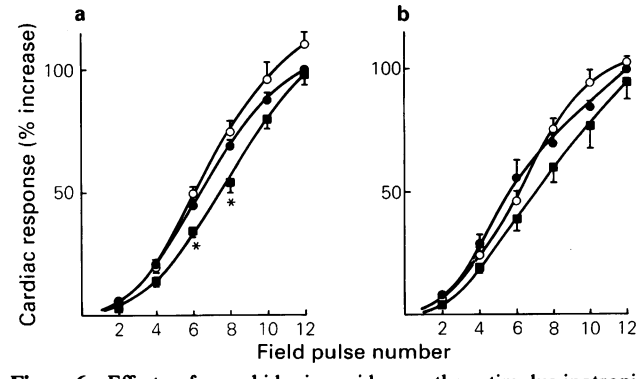

Figure 6 Effect of arachidonic acid on the stimulus-inotropic response curve obtained by increasing the number of field pulses in guinea-pig isolated atria stimulated at 4 Hz, in the presence of indomethacin 3 μM (a, $n = 4$) and acetylsalicylic acid 500 μM (b, $n = 4$). (●) Control; (○) arachidonic acid 1 μM; (■) arachidonic acid 10 μM. $*P < 0.05$.

Effect of arachidonic acid on the sympathetic response to field stimulation

Exposure of guinea-pig atria for 30 min to arachidonic acid (1–10 μM) produced a concentration-dependent and significant depression of the stimulus-response curve (Figure 5a). On the

other hand, in the ventricles 10 μM arachidonic acid was completely ineffective (Figure 5b). Preincubation with indomethacin 3 μM or acetylsalicylic acid 500 μM almost completely abolished the inhibitory effect of arachidonic acid in atrial preparations (see Figure 6).

Discussion

The results of the present study demonstrate that both PGE_2 and iloprost, a stable analogue of prostacyclin (PGI_2), concentration-dependently reduce the cardiac response to sympathetic nerve stimulation in guinea-pig atrial and ventricular preparations. These results are in agreement with previous observations demonstrating an inhibitory effect of prostaglandins of the E and I series on noradrenaline release induced by sympathetic nerve stimulation in rabbit and rat heart (Wennmalm, 1971; Hedqvist, 1977; Khan & Malik, 1980; 1982).

PGE_2 appeared to be much more active than iloprost in our study, thus suggesting that PGE_2 may be a more important modulator of sympathetic transmitter release in the heart than PGI_2, as already proposed by Wennmalm (1978). In fact PGE_2 reduced the cardiac response to field stimulation at a 1000 fold lower concentration than iloprost in both atrial and ventricular strips. The inhibitory effect of PGE_2 was completely attributable to a prejunctional action; in fact at the highest concentration tested PGE_2 did not modify the cardiac response to exogenous noradrenaline in either preparation. In the case of iloprost, the possibility of a postjunctional component of the inhibitory effect in ventricular preparations alone was ruled out. However, in the atrial tissue a small part of the inhibitory effect was attributable to a postsynaptic component, since the highest concentration of iloprost tested slightly reduced the positive inotropic response to exogenous noradrenaline.

An interesting observation arising from this study was the quantitative difference between the prostaglandin effects in the two different cardiac tissues, i.e. atrial and ventricular preparations. The IC_{50} for the inhibitory effect of PGE_2 on the cardiac response to adrenergic stimulation was 0.55 nM in atrial versus 4.06 nM in ventricular tissues; the IC_{50} for iloprost was also lower in atrial (0.7 μM) than in ventricular (1.5 μM) preparations. Moreover, the patterns of cardiac response to sympathetic stimulation by the two prostaglandins were also quite different in the two tissues: the whole stimulus-response curve was shifted to the right by prostaglandins in the atrial section, whilst the prostaglandin-induced inhibitory effect was evident only at low or intermediate degrees of stimulation, in the ventricular tissue. The possibility that these differences were due to the different methods of field stimulation used for atria and ventricles is unlikely, as the response to clonidine, a prejunctional inhibitory agonist, was similar in the two tissues. Also, we have previously observed that dynorphin, an opioid agonist able to inhibit the adrenergic response to field stimulation, was active in ventricles at lower concentrations than in atria (Ledda *et al.*, 1985; Mantelli *et al.*, 1987). Thus, it seems reasonable to conclude that the inhibitory effect of prostaglandins observed in the present study was stronger in atrial than in ventricular preparations. Moreover, inhibition of prostaglandin synthesis, induced by indomethacin and acetylsalicylic acid, enhanced the cardiac response to sympathetic nerve stimulation only in atrial preparations. Enhancement of noradrenaline overflow elicited by nerve stimulation with indomethacin has previously been observed in rabbit (Chanh *et al.*, 1972; Starke & Montel, 1973; Fuder & Muscholl, 1974) and rat heart (Khan & Malik, 1982). Thus our results are in agreement with previous observations and suggest that endogenous prostaglandins may have a modulator role in adrenergic neurotransmission only in the atrial section of the mammalian heart. This last hypothesis is supported by the finding that arachidonic acid reduced the cardiac response to sympathetic nerve stimulation only in atrial preparations, and that indomethacin and acetylsalicylic acid prevented this effect.

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