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Modification by prostaglandin E_2 (PGE_2) of the response of guinea-pig isolated vasa deferentia and atria to adrenergic stimuli

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Summary

1. Prostaglandin E_2 (PGE_2) exerted positive cardiostimulant effects on isolated guinea-pig atria. The response was not altered by treatment of the animal with reserpine or by addition of propranolol to the organ bath. These results suggest that the cardiostimulatory actions of PGE_2 are not mediated through the release of catecholamines or stimulation of adrenoceptors.

2. On the electrically driven atria, PGE_2 consistently exerted a cardiostimulant action which was not appreciably altered by changes in calcium ion in the bathing medium. PGE_2 showed no effect on the transport of calcium by the fragments of heart sarcoplasmic reticulum.

3. PGE_2 reduced the responses to both noradrenaline and tyramine in the isolated atria. The shifted dose-response curve was not parallel to the original.

4. PGE_2 increased the contractor response of the isolated vas deferens to nerve stimulation or to direct electrical stimulation.

5. PGE_2 antagonized the increase caused by noradrenaline in contractor response of isolated vas deferens to direct electrical stimulation, whereas it affected the potentiation by noradrenaline differently when the vas deferens was contracting in response to nerve stimulation. In low concentration it inhibited and in large concentrations, it slightly enhanced the potentiation by catecholamine.

6. It is concluded that PGE_2 has actions on multiple sites. It has postjunctional as well as pre-junctional effects on adrenergic neurones.

Introduction

Several investigators have shown that prostaglandins of the E series modify the response of sympathetically innervated tissues to adrenergic stimuli (Berti & Usardi, 1964; Brundin, 1968; Hedqvist, 1970b). PGE_2 potentiated the contractor response of isolated guinea-pig vas deferens to hypogastric nerve stimulation (Naizmada, 1969; Ambache & Zar, 1970). On the other hand, infusion of PGE_2 into the isolated perfused rabbit heart in concentrations ranging from 8×10^{-8} to $5{\cdot}5 \times 10^{-7}$M markedly inhibited the increase in heart rate to nerve stimulation (Hedqvist, Stjärne & Wennmalm, 1970). Similarly, PGE_2 inhibited splenic contractor responses to nerve stimulation (Hedqvist, 1969, 1970a) in the cat by altering

noradrenaline release. The present work investigates the mechanism by which PGE_2 affects responses to adrenergic stimuli of guinea-pig vas deferens and atria.

Methods

Male guinea-pigs weighing 300–800 g were stunned and decapitated. Vasa deferentia with attached hypogastric nerves were dissected according to the procedure of Hukovic (1961), and placed in a 100 ml organ bath in a bathing solution which was maintained at $34 \pm 0{\cdot}5°$ C. A mixture of 95% oxygen and 5% carbon dioxide was bubbled through the bathing fluid via a sintered glass plate at the bottom of the bath. The vas deferens was attached to a myograph B (E & M Instrument Company, Houston, Texas) or to a Grass force-displacement transducer (resting tension of 0·5 g). The hypogastric nerve was placed within two platinum loop electrodes and, after resting for approximately 20 min, the preparation was stimulated at various frequencies for 4 s every 2 min at a submaximal voltage (2–8 V). The response of the tissue was recorded on a Grass polygraph or on a Physiograph. When PGE_2 was added, 15–20 min were allowed before repeating the electrical stimulation. In some experiments the calcium concentration of the bathing solution was changed. The force developed above resting tension was expressed in grammes.

The guinea-pig isolated stripped vas deferens preparation stimulated transmurally

The vasa deferentia were removed and their mesenteric integuments carefully stripped away. One end of the vas deferens was tied to a supporting hook and the other (upper) end was attached to a myograph B (resting tension of 0·5 g). The vas deferens was suspended between and parallel to two platinum electrodes. Square-wave pulses of 2 ms duration, were delivered at a frequency of 25 Hz and with a supra threshold voltage of 10–15 V for 4 s at 2 min intervals unless otherwise indicated.

Isolated atrial preparation

The atria were dissected from the hearts of freshly killed guinea-pigs (300 to 450 g body weight) and suspended in a bathing solution maintained at 34° C. A mixture of 95% oxygen and 5% carbon dioxide was bubbled through the bathing fluid via a sintered glass plate at the bottom of the bath. The atria were attached to an EM strain gauge, and the force of contraction was measured isometrically (resting tension of approximately 0·5 g). The rate of spontaneous beating was recorded with an EM Physiograph. The atria were allowed to equilibrate for at least 1 h after being placed in the bath.

Electrically driven, isolated atrial strips

The left atrium was tied to a plastic holder which contained punctate electrodes. The upper end was tied to a force displacement transducer (Grass FT-03), and the force of contraction was measured isometrically and recorded on a Grass polygraph. The atria were mounted in an organ bath of 100 ml capacity (unless otherwise indicated) and were electrically driven via platinum electrodes, placed parallel to, but not touching the tissue. The stimulus was delivered at various frequencies

with square-wave pulses of 5 ms duration at an intensity approximately 20% above the threshold level for each atrium. The intensity ranged generally between 5 and 7 V. The tissues were gently stretched until the tension failed to further augment the force of contraction; the resting tension was set at half that value. No further adjustments of the length of the strip were made thereafter. Sixty minutes were allowed for the atria to stabilize, and during this period the atria were washed with solution every 10 minutes. Frequency-force response curves were obtained in the presence, and in the absence, of PGE_2 at various calcium concentrations. The force of contraction was expressed in grammes. At a frequency of 1 Hz a force of 0·6 g was normal for control atria.

Bathing solution

The solution had the following composition: NaCl, 9·0 g; KCl, 0·42 g; CaCl, 0·24 g; $NaHCO_3$, 0·50 g; glucose 1·9 to 2·0 g in 1 litre of distilled water. The pH of the solution equilibrated with 5% CO_2 was approximately 7·4.

Log-concentration curve to sympathomimetic amines

Cumulative dose-response curves for various amines were obtained by a stepwise increase of the total concentration. Responses were calculated as increase in the heart rate above the initial rate of the atria in each experiment.

Two dose-response curves were determined on the same tissue with an interval of about 60 min between the end of the first and the beginning of the second. In experiments with PGE_2, it was added 20 min before the beginning of the second dose response curve. In the experiments with sympathomimetic amines, ethylenediaminetetra acetic acid (EDTA) was always present at a concentration of 10 μg/ml ($2{\cdot}7 \times 10^{-5}$ M) in the bath during the exposure to the amine to retard oxidation (Iversen, 1967). This concentration of EDTA had no effect on the tissue's force of contraction, the uptake, release, metabolism or effectiveness of noradrenaline.

For studying calcium transport in guinea-pig atria

Hearts were thoroughly washed with 0·25 M sucrose containing 1 mM EDTA, pH 7·0, and homogenized in 10 volumes of medium (10 mM sodium bicarbonate, 5 mM sodium azide and 15 mM Tris-HCl, pH 6·8) in a Waring blender for 45 seconds. The isolation of the fragments of the sarcoplasmic reticulum and the determinations of calcium binding (in the absence of oxalate), calcium uptake (in the presence of 5 mM potassium oxalate) and total ATPase in the presence of Mg^{++} and Ca^{++} were determined according to the procedures described earlier (Sulakhe & Dhalla, 1970; 1971). The protein concentration of this fraction was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). The calcium transport by this fraction was found to be unaffected by the presence of 5 mM sodium azide.

For calcium binding experiments, the reticular membranes (0·5 to 0·3 mg protein/ml) were incubated in medium containing 100 mM KCl, 20 mM Tris-HCl, pH 6·8, 0·1 mM ^{45}Ca Cl_2, 10 mM $MgCl_2$ in the absence or presence of PGE_2 (1 to 5 μg/ml) at 25° C. For calcium uptake and ATP hydrolysis experiments, the reticular membranes (0·02 to 0·04 mg protein/ml) were incubated in the above medium in the presence of 5 mM potassium oxalate at 37° C. The reaction was

started 2 min after incubation of these membranes with PGE_2 by the addition of ATP (4 mM final concentration) and stopped by Millipore filtration at the required time.

Monoamine oxidase (MAO) was assayed by measuring the conversion of ^{14}C-tryptamine to indoleacetic acid according to the method of Wurtman & Axelrod (1963), catechol-*o*-methyl transferase (COMT) was assayed by measuring the formation of ^{3}H-metadrenaline on incubation with (−) adrenaline and ^{3}H-methyl-s-adenosylmethionine (specific activity 8·9 Ci/mmol, Amersham/Searle. Illinois) as described by Axelrod (1959).

Drugs used

The following substances were used: prostaglandin E_2 (obtained from Upjohn Company, USA), (−) noradrenaline bitartrate monohydrate and tyramine hydrochloride.

Statistical evaluations (t-test) were performed according to Snedecor (1956). $P<0.05$ was regarded as significant.

Results

Response of the atrial pacemaker to PGE_2

PGE_2 (3.5×10^{-6}M) consistently exerted a cardiostimulatory positive inotropic and chronotropic response which was fairly well maintained but was terminated by three to four washings with bathing solution (Table 1). Following the positive response to PGE_2 the preparation was almost entirely refractory to subsequent addition of PGE_2 although noradrenaline still produced a chronotropic and inotropic response.

The cardiostimulant response to PGE_2 remained unaltered when the atria were depleted of catecholamines. Atria from guinea-pigs which had received intraperitoneal injections of reserpine (2·5 mg/kg) 24 h prior to the experiment responded to PGE_2 (Table 1), but the stimulatory response to tyramine was completely abolished. Similarly, the cardiostimulatory response to PGE_2 was unaffected by blockade of beta-adrenoceptors by propranolol (1.7×10^{-6}) (Table 1), although in these atria

TABLE 1. *The effect of PGE_2 (3.5×10^{-6}M) on spontaneously beating isolated guinea-pig atria*

Pretreatment	Rate of contraction Increase in beat/min Mean±S.E.		*P*
Control	43·7±5·7	(9)	
Reserpine	54·9±6·2	(9)	>0·05
Propranolol	52·9±4·3	(8)	>0·05
Pretreatment	**Force of contraction (% increase) Mean±S.E.**		
Control	43·3±18·2	(9)	
Reserpine	59·6±12·6	(9)	>0·05
Propranolol	61·2±14·3	(8)	>0·05

Reserpine was administered intraperitoneally 18 h prior to the experiment (2·5 mg/kg). Propranolol (1.7×10^{-6}M) was added to the bathing medium 20 min prior to the addition of PGE_2. $P<0.05$ indicates a significance of difference between control versus drug treatment.

addition of noradrenaline failed to elicit the usual stimulatory response. Thus, release of catecholamines or stimulation of adrenoreceptors by PGE_2 was not involved in its cardiostimulant effect.

Response to PGE_2 of isolated atria, driven electrically

PGE_2 increased the force of contraction of left atrial strips driven electrically at 1 Hz immediately after its addition to the bathing solution (Fig. 1). This enhancement reached its maximum within 15 to 20 minutes. In another series of experiments atria were driven at various rates. The force of contraction was a direct function of the rate of contraction in the lower range. At higher rates the force of contrac-

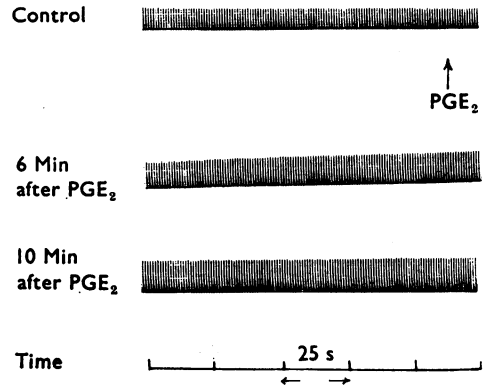

FIG. 1. The response to PGE_2 ($3{\cdot}5\times10^{-6}$M) of an isolated guinea-pig left atrial strip driven electrically at 1 Hz in bathing solution at 34° C. Record is from one typical experiment.

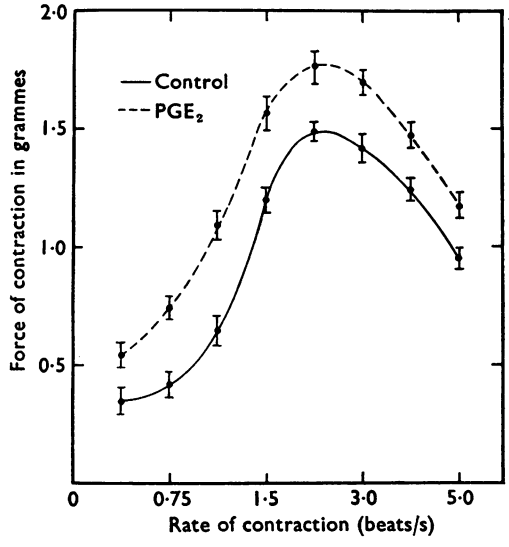

FIG. 2. The response to PGE_2 of an isolated guinea-pig atrial strip driven electrically at various frequencies. The atria were driven by a train of single pulses at various rates. The experiment was performed in the absence (——) and presence (- - - - -) of PGE_2 ($3{\cdot}5\times10^{-6}$M). Each point represents mean values ± S.E. for 8 observations.

tion began to decrease. The addition of PGE_2 ($3{\cdot}5 \times 10^{-6}$M) shifted the frequency-force response curve upward. The enhancement of the force of contraction after PGE_2 was significantly greater at all frequencies applied (Fig. 2).

In the third series of experiments the calcium concentration of the solution was either raised from 1·6 mM to 3·2 mM or decreased to 1·2 or 0·8 mM before the atrial strips were exposed to PGE_2. Lowering the calcium concentration depressed the resting tension. The percentage change in force at the lower calcium concentrations was greater, but the absolute force was less in response to PGE_2. When the calcium ion concentration was increased from 1·2 mM to 1·6 mM to 3·2 mM, the positive inotropic response to PGE_2 remained unaltered. The data are summarized in Table 2.

In another series of experiments, the effects of PGE_2 on the energy-linked calcium transport by fragments of cardiac sarcoplasmic reticulum was tested. PGE_2 (1 to 5 μg/ml) did not show any action on calcium binding, calcium uptake or on total ATPase activity of the cardiac sarcoplasmic reticulum (Table 3). In similar experiments it was shown that other derivatives of prostaglandins (E_1, F_1, F_2, A_2) had no action on the calcium transport by these membranes.

Effect of PGE_2 on the response of spontaneously beating atria to sympathomimetic amines

In the presence of PGE_2 10^{-7}M or more the dose response curve to noradrenaline was shifted to the right in a non-parallel fashion (Fig. 3), and the maximal response to noradrenaline was depressed. PGE_2 significantly reduced the response to

TABLE 2. *Effect of alterations in extracellular calcium on the response to PGE_2 $3{\cdot}5 \times 10^{-6}$M of the guinea-pig isolated left atrial strip*

Concentration of Ca^{++} mM	Force developed (g) Before PGE_2	After PGE_2
0·8	0·18±0·03 (8)	0·36±0·08 (8)
1·2	0·32±0·06 (7)	0·47±0·06 (7)
1·6	1·20±0·12 (11)	1·59±0·03 (11)
3·2	1·42±0·11 (11)	2·01±0·10 (8)

Isolated left atrial strips were driven electrically at 1·5 Hz. Results are means±S.E.M. and the number of experiments is given in parentheses.

TABLE 3. *Influence of prostaglandin E_2 on calcium transport by fragments of sarcoplasmic reticulum of the guinea-pig heart*

	1 min	3 min	5 min
(A) Calcium binding (nmol Ca^{++}/mg protein)			
Control	25·2±3·1	37·1±2·4	—
PGE_2	29·8±2·9	35·0±2·6	—
(B) Calcium uptake (nmol Ca^{++}/mg protein)			
Control	474±42	1,322±62	2,034±124
PGE_2	459±17	1,238±45	1,932±93
(C) Mg^{++}—Ca^{++} stimulated ATP hydrolysis (nmol Pi released/mg protein)			
Control	2·44±0·23	6·56±0·48	9·11±0·76
PGE_2	2·13±0·26	5·67±0·48	8·57±0·83

The results are mean±S.E. of 5 to 6 different preparations. The data concerning the different concentrations of PGE_2 were grouped together since the values were overlapping.

tyramine in concentrations greater than 10^{-5}M, and also depressed the maximum response as shown in Fig. 4. Again the shift of the response curve was not parallel.

The effect of PGE_2 on contractions of the isolated vas deferens

PGE_2 in all concentrations potentiated the response of the isolated vas deferens to hypogastric nerve stimulation and to transmural stimulation (Table 4, Fig. 5).

Whereas PGE_2 potentiated the motor response of the smooth muscle to transmural electrical stimulation (Table 4), it inhibited the response to exogenous noradrenaline in the same preparation (Table 5).

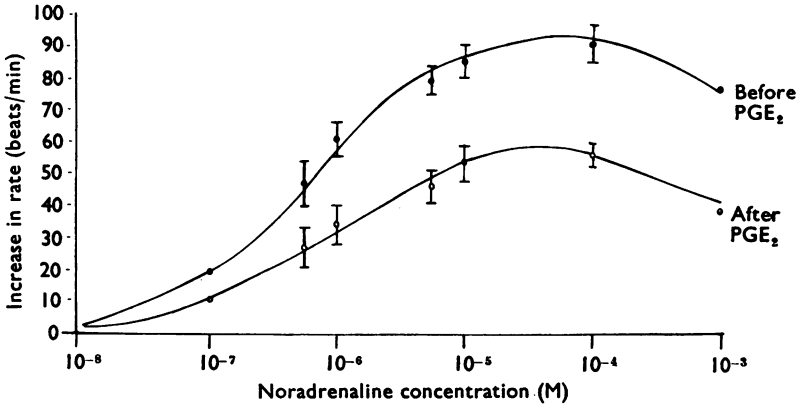

FIG. 3. Effect of noradrenaline on isolated, spontaneously beating guinea-pig atria: alone (upper curve), and in the presence of PGE_2 $3{\cdot}5\times10^{-6}$M (lower curve). Each point represents mean values ± S.E. for 9 observations.

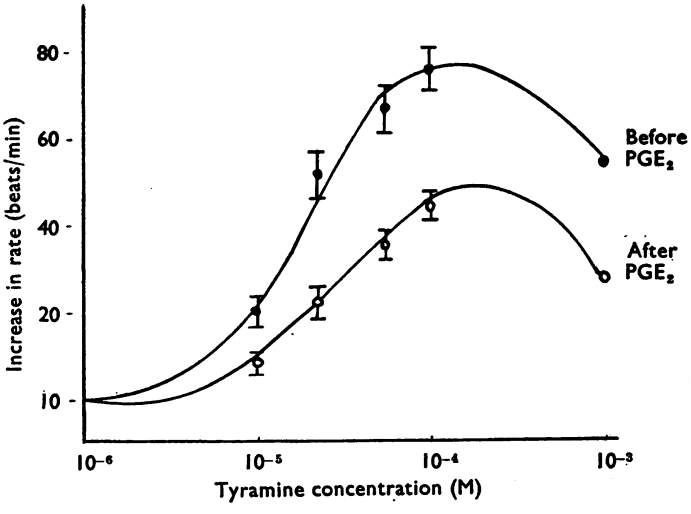

FIG. 4. Effect of tyramine on isolated, spontaneously beating guinea-pig atria, alone (upper curve), and in the presence of PGE_2 $3{\cdot}5\times10^{-6}$M (lower curve). Each point represents mean values ± S.E. for 9 observations.

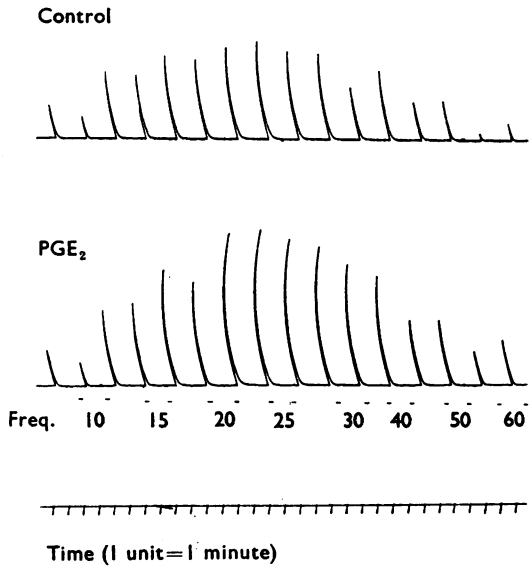

FIG. 5. The effect of PGE_2 on the response of the isolated guinea-pig vas deferens to electrical stimulation of the hypogastric nerve. The nerve was stimulated at various rates, at 6 V (2 ms duration) for 4 s every 2 minutes. The frequency response curve was determined before, and 20 min after, the addition of PGE_2 $3{\cdot}5\times10^{-6}$M. The record is typical of 12 preparations.

TABLE 4. *Effect of PGE_2 on the response of guinea-pig isolated vas deferens to hypogastric nerve and transmural stimulation*

Concentration of PGE_2	Force of contraction (% control) Nerve stimulation		Transmural stimulation	
$1{\cdot}4\times10^{-7}$M	115·3±23·5	(11)	115·2± 2·3	(6)
$7{\cdot}0\times10^{-7}$M	135·3± 9·2	(8)	155·1±10·5	(6)
$1{\cdot}4\times10^{-6}$M	162·3±15·3	(11)	145·3± 6·2	(6)
$3{\cdot}5\times10^{-6}$M	175·6±17·3	(12)	178·0±12·1	(10)
$7{\cdot}0\times10^{-6}$M	189·7±28·9	(12)	246·5±48·2	(8)

The hypogastric nerve was stimulated at 2 min intervals at a rate of 15 Hz (duration 2 ms) for 4 seconds. Results are means±S.E.M. and the number of animals is given in parentheses. In the absence of PGE_2, nerve stimulation and transmural stimulation produced about 52% of the maximum response.

TABLE 5. *Effect of PGE_2 on the response of guinea-pig isolated vas deferens to hypogastric nerve and transmural stimulation in the presence of noradrenaline* 2×10^{-6}M

Concentration of PGE_2	Response to noradrenaline force of contraction (% control) Nerve stimulation		Transmural stimulation	
None	147·3± 6·31	(25)	181·5±13·0	(15)
$1{\cdot}4\times10^{-7}$M	114·9± 8·5	(11)*	—	
$7{\cdot}0\times10^{-7}$M	168·3±39·9	(8)	136·5±17·51	(6)*
$1{\cdot}4\times10^{-6}$M	186·4±31·8	(11)	128·4±12·53	(10)*
$3{\cdot}5\times10^{-6}$M	145·6±12·9	(12)	134·6±10·71	(16)*
$7{\cdot}0\times10^{-6}$M	179·1±44·6	(10)	144·1±12·91	(18)*

The isolated hypogastric nerve of the vas deferens was stimulated at 15 Hz and 6 V (1·5 ms duration) for 4 s every 2 minutes. Results are means±S.E.M. and the number of experiments is given in parentheses. The response of the vas deferens to noradrenaline is expressed as a percentage of the response immediately prior to the addition of noradrenaline (with or without PGE_2). * Significantly lower ($P<0{\cdot}001$) when compared with control (first value in each vertical column).

PGE_2 inhibited the response of the transmurally stimulated vas deferens to noradrenaline at all concentrations: it had a different effect on its response to nerve stimulation in the presence of exogenous noradrenaline. In low concentrations ($1{\cdot}4 \times 10^{-7}$M) PGE_2 inhibited the response ($P<0{\cdot}01$) to noradrenaline, while in higher concentrations it slightly enhanced ($P>0{\cdot}05$) it (Table 5).

Effect of PGE_2 on the cardiac monoamine oxidase and catechol-o-methyl transferase activities

Reduced sensitivity to noradrenaline could occur if PGE_2 increased the metabolic degradation of noradrenaline. The results in Table 6 show that the activity of enzymes responsible for degradation of noradrenaline were not changed after addition of PGE_2 in the homogenates of heart.

TABLE 6. *Effect of PGE_2 on monoamine oxidase (MAO) and catechol-o-methyl transferase (COMT) activity in the rat heart*

Treatment	MAO activity	COMT activity
Control	2,148±126	0·0059±0·0001
PGE_2 ($2{\cdot}8 \times 10^{-6}$M)	2,179±105	0·0056±0·0003
PGE_2 ($2{\cdot}8 \times 10^{-7}$M)	2,333± 55	—

The activity of MAO and COMT are expressed as nmol of product formed per g of tissue during 1 h incubation at 37° C. Product refers to ^{14}C-indole acetic acid or ^{14}C-normetadrenaline. The results are the mean±S.E.M. of 6 experiments.

Discussion

PGE_2 exerted positive inotropic and chronotropic effects on isolated, spontaneously beating atria and increased the force of contraction of electrically driven strips of guinea-pig left atrium. The enhancement of the force of contraction was significantly greater at all frequencies applied. PGE_2 also potentiated the response of the isolated vas deferens to hypogastric nerve and transmural stimulation. These results suggest that PGE_2 exerts a stimulatory effect on the smooth and cardiac muscle.

Since the cardiostimulant response to PGE_2 remained unaltered when the β-adrenoceptors were blocked by propranolol and when catecholamine stores were depleted by prior treatment of animal with reserpine, this suggests that these stimulatory effects of PGE_2 are not mediated through the release of catecholamine or through the stimulation of adrenoceptors. They may, therefore, be due to a direct effect on the muscle.

The cardiostimulant effects of PGE_2 were found to be independent of the increase in calcium ion concentration in the bathing medium. However, when the calcium ion concentration was decreased from 1·6 mM to 0·8 mM the positive inotropic response to PGE_2 was enhanced twofold. This is consistent with the findings of Mantegazza (1965) who showed that in guinea-pig hearts the chronotropic and inotropic responses to PGE_1 were increased when calcium concentration in the perfusion medium was lowered.

The finding that PGE_2 reduced the sensitivity of the tissues to noradrenaline could be explained either by an increase in the capacity of the tissue to take up noradrenaline or by an enhancement of its enzymatic degradation, since either

effect would result in a diminution of the response to this amine. However, both explanations seem unlikely, since firstly PGE_2 does not alter the removal of tritium labelled exogenous noradrenaline infused into the spleen (Hedqvist, 1970b) and secondly the activities of monoamine oxidase and catechol-*o*-methyl transferase, enzymes responsible for degradation of noradrenaline, were not altered by PGE_2.

Furthermore, the reduced responsiveness of the tissue to noradrenaline in the presence of PGE_2 could not be explained by the decreased availability of calcium since the present findings suggest that the ability of the sarcoplasmic reticulum to accumulate calcium or to hydrolyse ATP was not affected by PGE_2.

It is concluded that PGE_2 has actions on multiple sites. In smooth and cardiac muscle it seems to act in at least two places, on the muscle cells to induce contractions and probably, as shown in the present experiments, on the adrenoceptors to reduce the response to noradrenaline. Furthermore, Hedqvist (1970b) has shown that it inhibits the release of noradrenaline from the nerve terminals and thus may reduce the contractions by this mechanism as well.

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