Inotropic Effects of Prostaglandin E_2 on Isolated Cardiac Tissue (35463)

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This report describes the inotropic effects of prostaglandin E_2 (PGE_2) on isolated cardiac tissue. An attempt also has been made to clarify the mechanism of action of this agent in producing its effects on myocardial contraction.

These studies were prompted by reports that other prostaglandins, especially PGE_1, produced a positive inotropic effect both *in vitro* and *in vivo* in some species (1–7).

Materials and Methods. Electrically driven (120 beats/min) rabbit left atria were prepared for recording of isometric contractile force *in vitro,* using methods and techniques described previously (8–10). Isolated human atrial muscles, stimulated or spontaneously beating, were studied using procedures developed in our laboratories (11). Spontaneously beating rabbit right atria (9) were used for studying chronotropic effects. All experiments were done at 37.5°. The physiological salt solution was a modified Krebs–Henseleit buffer (10) which was gassed with a 95% O_2–5% CO_2 gas mixture. Following an equilibration period, the preparations were exposed to the agents under study. Changes in force of contraction produced by PGE_2 were expressed as a percentage change from the immediate pre-prostaglandin contraction value. The following drugs were used in the study: propranolol HCl, atropine sulfate, bretylium tosylate, phenoxybenzamine HCl, diphenhydramine HCl, reserpine, and fresh solutions, made daily, of prostaglandin E_2 (PGE_2), supplied by The Upjohn Co. as a white crystalline powder, using 95% ethanol as a solvent. Reserpine was given (5 mg/kg iv) to a series of rabbits 16–18 hr prior to their sacrifice in order to produce depletion of cardiac catecholamines (10). Appropriate controls were studied to evaluate the ethanol solvent effects in those volumes used in the PGE_2 experiments. Experiments were performed during the Fall and Winter months of 1969–70.

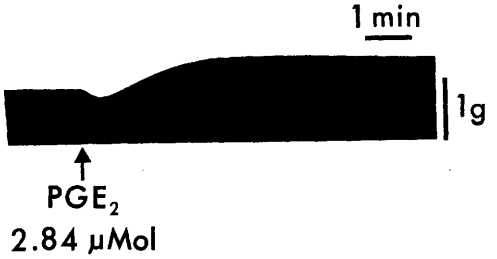

FIG. 1. Effect of PGE_2 (2.84 μM) on contractile force of a normal, electrically driven (120 beats/min) rabbit left atrial preparation.

Results. 1. Inotropic effects. Figure 1 illustrates the typical biphasic inotropic response to a 2.84 μM concentration of PGE_2 on an electrically driven rabbit left atrial preparation. Immediately following administration of the agent, there was a rapid but transient negative inotropic effect, followed by a gradual and sustained positive inotropic response.

Because of the reproducible responses achieved with this concentration of PGE_2, a series of experiments were performed to ascertain the mechanisms underlying the negative and chronotropic responses observed.

Table I summarizes the results of these experiments. When tested on fresh, previously untreated atria, PGE_2 produced a maximum decrease in contractile force of 18.5 ± 2.9% (mean ± SEM). The maximum positive inotropic effect was 38.7 ± 6.9%. Pretreating normal atria with atropine and propranolol, in concentrations known to block cholinergic and beta-adrenergic effects on the heart, failed to significantly modify the response to

TABLE I. Inotropic Effects of PGE_2 (2.84 μM) on Isolated Rabbit Left Atria.

Pretreatment	N	Response (max. % change)		p[c]	
		NIE[a]	PIE[b]	NIE	PIE
None	8	−18.5 ± 2.9	+38.7 ± 6.9	—	—
Atropine[d] + propranolol[e]	4	−12.0 ± 1.9	+37.9 ± 11.6	>.05	>.05
Bretylium[f]	6	−12.6 ± 1.7	+19.2 ± 2.0	>.05	<.02
Reserpine[g]	10	− 8.2 ± 1.3	+35.5 ± 14.0	<.01	>.05
Phenoxybenzamine[h]	4	−10.1 ± 2.1	+44.3 ± 15.9	<.05	>.05
Diphenhydramine[i]	7	− 1.7 ± 0.9	+15.7 ± 5.0	<.001	<.02

[a] NIE = negative inotropic effect.

[b] PIE = positive inotropic effect.

[c] p = p value for test groups vs no pretreatment group (Student's t test for uncorrelated means).

[d] 1.4 μM (5 min).

[e] 1.54 μM (5 min).

[f] 12.1 μM (15 min).

[g] 5 mg/kg, iv, 16–18 hr prior to experiment.

[h] 1.0 μM (60 min).

[i] 39.2 μM (15 min).

a subsequent challenge with PGE_2.

Similarly, reserpinization *in vivo* with doses known to effectively deplete cardiac catecholamines (10) failed to prevent the typical positive inotropic response to PGE_2, although the negative inotropic response was significantly attenuated.

Since recent evidence suggests that rabbit atrial tissue may have alpha-adrenergic receptors that may subserve positive inotropic effects (12, 13), a group of muscles were treated with the alpha-adrenergic receptor antagonist phenoxybenzamine. As noted in Table I, this antagonist did not prevent the positive inotropic responses to PGE_2. However, there was a significant diminution of the magnitude of the negative inotropic effect.

Histamine release from isolated cardiac tissue is known to result in a positive inotropic response (14). Therefore, to test whether the positive inotropic effects of PGE_2 were mediated through a histaminergic mechanism, a group of muscles were pretreated with the antihistamine diphenhydramine, then challenged with PGE_2. The results of these experiments indicate a significant attenuation of both the normal positive and negative inotropic effects of PGE_2.

When tested on six human atrial appendages obtained from patients undergoing open-heart surgical procedures, PGE_2 failed to produce any measurable inotropic effects. This lack of a distinct inotropic effect was observed in both stimulated (60–90 beats/min) and spontaneously beating preparations.

In none of the studies with either rabbit or human muscles did the ethanol solvent alone (5 μl/50 ml bath) produce any measurable effect on contraction.

2. Chronotropic effects. PGE_2 (2.84 μM) produced a biphasic inotropic action on spontaneously beating rabbit right atria similar to that observed in left atria. However, measurement of the heart rate during the maximum negative and positive chronotropic phases of action revealed no changes in frequency of beating compared with the pre-PGE_2 heart rate.

3. Tachyphylaxis. In 11 experiments with both rabbit right and left atria, rechallenging the tissues with 2.84–14.2 μM PGE_2 following development of the maximum positive inotropic response to an initial concentration of 2.84 μM failed to produce either a characteristic negative or positive inotropic effect. That the lack of a positive inotropic effect was not due to the fact that the tissues were already in maximal contraction was indicated by the rapid positive inotropic response to isoproterenol (0.0032–0.081 μM).

Discussion. The salient feature of the action of PGE_2 on isolated rabbit atrial tissue is its biphasic inotropic action. The failure of atropine plus propranolol pretreatment to significantly inhibit either the negative or positive inotropic phases of PGE_2 action suggests that these responses are not mediated by a cholinergic or beta-adrenergic receptor mechanism.

The inability of reserpine pretreatment *in vivo* to prevent the normal positive inotropic effect of PGE_2 also suggests that the availability of endogenous catecholamines does not underlie the cardiotonic effect. Interestingly, however, the negative inotropic effect was significantly attenuated but not eliminated by prior reserpine treatment. No ready explanation for this attenuation is available, although one or several of the reported noncatecholamine actions of the alkaloid may be involved, including an action on Ca^{2+} exchange (10, 15).

An apparent contradiction to these findings is the fact that the sympatholytic agent bretylium significantly decreased (by 50%) the positive inotropic effect of PGE_2. Bretylium is thought to exert its sympatholytic or adrenergic blocking action by interfering with the release of norepinephrine at peripheral sympathetic nerve endings (16). However, bretylium also exerts an effect on the cardiac cell membrane analogous to local anesthetics (17), and this weak membrane stabilizing action may contribute to, or underlie its ability to modify the PGE_2 inotropism.

Similarly, pretreatment of rabbit left atria with the antihistamine diphenhydramine significantly attenuated, but did not completely block, the positive inotropic effect of PGE_2. Histamine release from cardiac muscle is known to occur with other agents and is accompanied by a positive inotropic action (14). The attenuation of PGE_2 effects by diphenhydramine might suggest a histaminergic action of PGE_2. However, a variety of pharmacologic effects are produced by antihistamines in addition to their primary antagonism of histamine action (18), and one of these may be operative here.

The complete failure of the alpha-adrenergic antagonist phenoxybenzamine to inhibit the positive inotropic effect of PGE_2 may be taken as presumptive evidence that the prostaglandin does not produce this effect via stimulation of cardiac alpha-adrenergic receptors. Agents such as phenylephrine are reported to produce their positive inotropic action on rabbit atria via cardiac alpha-receptors. This action can be blocked by alpha-receptor inhibitors (12, 13). The attenuation by 45% of the normal negative inotropic effect of PGE_2 produced by phenoxybenzamine is unexplained.

The lack of a distinct chronotropic effect on spontaneously beating rabbit atrial tissue emphasizes the separability of inotropic and chronotropic effects produced by PGE_2. This lack of effect on rate was observed even during the peak negative and positive inotropic effects on these preparations. Prostaglandin E_2 also has been reported to have variable or no effects on heart rate on perfused rat hearts (19). Langendorff-perfused rabbit hearts also were found to be unchanged in 9 out of 15 experiments using PGE_2 concentrations of 0.07–0.55 μM (20).

Another prominent feature of the action of PGE_2 is the apparent rapid development of tolerance (tachyphylaxis) to its inotropic action. Thus, addition of normally effective concentrations of PGE_2 during the maximum positive inotropic phase produced by an initial challenging concentration (2.84 μM) failed to produce either a negative or positive inotropic effect. This tachyphylaxis persists even after washing the muscle chamber many times with a drug-free physiological salt solution. Rechallenging the tissue with PGE_2 immediately following this wash produced no response, or infrequently only a weak negative and/or positive inotropic effect.

Other investigators have reported a similar tachyphylactic response of rat myometrial tissue to the contractile effects of prostaglandins (21–23). It has been postulated that this tachyphylaxis is due to a desensitization phenomenon which is dependent on extracellular calcium and potassium concentration, since the tachyphylaxis was diminished when the concentrations of these two cations were increased (23).

The lack of a distinct inotropic effect of PGE_2 on human atrial muscle suggests an important species difference in the myocardi-

al contractile response to this agent. Others have reported differences in the inotropic responses to PGE_1 between various animal species (5). For example, Langendorff-perfused hearts from cats, rabbits, and rats show no inotropic response to PGE_1. Guinea pig and frog hearts respond with a positive inotropic effect, the latter species being the most sensitive and regular in its response. This positive inotropic effect of PGE_1 was not prevented by prior reserpine treatment or beta-adrenergic blockade (5), in general agreement with the findings of the present investigation with PGE_2. On the other hand, the failure to achieve any inotropic effect on human atrial muscle with PGE_2 (2.84 μM) may not represent a species difference, and may be the result of the unique clinical (disease) status of the patients from whom the tissues were obtained. As yet, no evidence of a direct inotropic effect of PGE_2 in man has been reported.

In general, attempts to characterize the nature of the negative and positive inotropic effects of PGE_2, using classical pharmacological antagonists and inhibitors, has not explained the mode of action of this agent. At the least, it appears that the negative and positive inotropic effects produced are not mediated by classical cholinergic, beta- or alpha-adrenergic, or catecholamine release mechanisms. The modification of PGE_2 effects by diphenhydramine suggests, but does not prove, that a histaminergic mechanism may be operative.

Because of the failure of classical antagonists to block the inotropic effects of agents such as PGE_1 (5), considerable attention has been given to the effect of the prostaglandins on cardiac membrane permeability to calcium (24). The available data point to a good relationship between the positive inotropic effects of PGE_1, and increases in calcium uptake by the heart, although total myocardial calcium content does not increase (24). It is postulated that the active prostaglandins produce their effect on contraction by increasing the availability of free calcium to the excitation-contraction coupling mechanism (4). This effect may be brought about through a direct increase in cellular membrane permeability to calcium, or by enhancing the uptake of calcium by the sarcoplasmic reticulum (25). Whether these reported and postulated effects and mechanisms are involved in the unique biphasic inotropic action of PGE_2 on isolated rabbit atria remains to be explored.

Summary. Prostaglandin E_2 (PGE_2) was studied for its effects on the contractile force of electrically driven or spontaneously beating rabbit left and right atrial preparations, as well as on human atrial appendages obtained from patients undergoing open-heart surgical procedures. A concentration of 2.84 μM produced a reproducible biphasic inotropic action on rabbit left atrial preparations characterized by a transient negative inotropic phase (—18.5%) followed by a sustained positive inotropic effect (+38.7%). A qualitatively similar inotropic response was observed using spontaneously beating rabbit right atria, although no significant chronotropic response was noted in these experiments. Human atrial appendage failed to respond to PGE_2 under the conditions used.

The negative and positive inotropic effects of PGE_2 on left atria are not significantly attenuated by classical cholinergic or beta-adrenergic blocking agents. Reserpine pretreatment *in vivo* also failed to modify the positive inotropic response, although the negative inotropic phase was significantly attenuated. Bretylium and diphenhydramine significantly attenuated but did not completely inhibit the positive inotropic phase. The antihistamine also produced a highly significant inhibition of the negative inotropic effect. Phenoxybenzamine failed to antagonize the positive inotropic response, although it reduced the magnitude of the negative inotropic effect.

An apparent tachyphylactic response to PGE_2 was observed in the rabbit atria experiments, as evidenced by failure to produce a normal inotropic effect upon rechallenging the tissues with PGE_2 following a prior response to an earlier dose.

The data suggest that a classical cholinergic, beta- or alpha-adrenergic, or catecholamine releasing mechanism is not involved in mediating the biphasic inotropic responses of rabbit left atria to a 2.84 μM concentration of PGE_2. A possible histaminergic mechanism

may be operative, but is not proved, because of known nonspecific actions of the histamine antagonist used.

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