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Interaction of prostaglandin E_1 and calcium in the guinea-pig myometrium

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Summary

1. Prostaglandin E_1 (PGE_1) increased the responses of guinea-pig myometrium in a low calcium medium to added Ca^{2+}, acetylcholine, vasopressin, Ba^{2+} and Sr^{2+}. The concentration of PGE_1 used (50 pg/ml) was clearly below the threshold for direct spasmogenesis. In the presence of PGE_1 the doses necessary for half-maximal contractions were decreased by factors of 2·6 for Ca^{2+}, 2·4 for acetylcholine, and 3·7 for vasopressin. The responses to Ba^{2+} or Sr^{2+}, though studied less extensively, were found to be affected in much the same manner.

2. The K^+ depolarized myometrium in a low Ca^{2+} medium contracts in response to added Ca^{2+}. These responses also were increased by low concentrations of PGE_1, but the effective concentration of PGE_1 was indistinguishable from that for direct spasmogenesis.

3. Possible mechanisms for the interaction of PGE_1 and Ca^{2+} in the myometrium are discussed. It is tentatively suggested that these findings may be relevant to the physiological control of human myometrium.

Introduction

In concentrations smaller than those needed directly to stimulate the guinea-pig myometrium, prostaglandin E_1 (PGE_1) increases the responses to other spasmogens such as vasopressin. This effect differs from other varieties of potentiation in that it is caused by a subthreshold dose of an agent which is itself a spasmogen, and that it persists for long after the agent has been removed from contact with the tissue ; it has been called ' enhancement ' (Clegg, Hall & Pickles, 1966). Enhancement may result from a direct facilitation of intracellular Ca^{2+} movements, possibly by the formation of a lipid-soluble PG-Ca complex (Pickles, Hall, Clegg & Sullivan, 1966).

van Dorp & Heertje (personal communication), elaborating on preliminary unpublished results by Advani & Pettit, have shown that PGE_1 and Ca^{2+} can form a complex with an association constant $[PG\text{–}Ca^+]/[PG^-]\cdot[Ca^{2+}]=24\pm8$. The biologically much less active $PGF_{1\beta}$ gave a constant indistinguishable from zero.

These results have prompted a further investigation of the possible physiological interaction of PGE_1 and Ca^{2+}. Whereas in the earlier experiments on ' enhance-

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ment' the agent directly causing the contraction responses was usually vasopressin or electrical stimulation, we have now used added calcium ion. Two kinds of suspension medium were used; a low-Ca^{2+} non-depolarizing solution, and one with high $[K^+]$ to give largely complete depolarization. In either of these the myometrium responded reproducibly to added Ca^{2+}, and to acetylcholine, vasopressin, Ba^{2+} or Sr^{2+} which were used for comparison. A preliminary account of some of these experiments has been published (Eagling, Lovell & Pickles, 1971).

Methods

Animals and materials

Whole uterine horns from young mature virgin guinea-pigs weighing 350–650 g and not in oestrus as shown by inspection of the vulva or by the Shorr-stained vaginal smear, were suspended at 37° C in a Krebs-Henseleit type of bicarbonate medium (Clegg, Hopkinson & Pickles, 1963) containing glucose 1 g/l., and aerated with 5% CO_2 in O_2. Preliminary experiments showed that $[Ca^{2+}]$ 0·1 mM and $[Mg^{2+}]$ 3 mM were suitable for non-depolarizing solutions. For depolarizing solutions, the NaCl and $NaHCO_3$ were replaced by K_2SO_4 and $KHCO_3$, the $[Ca^{2+}]$ and $[Mg^{2+}]$ both being 0·2 mM. These concentrations maintained the excitability without inducing spasm.

Except where otherwise stated, contractions were recorded isotonically with a load of 1·0 g.

The prostaglandins were kindly supplied by Prof. D. A. van Dorp and were known to be >99% pure on receipt. Since PGE_1 may alter during prolonged storage in solution even at −20° C, as may be shown by the development of additional spots on thin-layer chromatography, the stock was checked for homogeneity after the series was completed, and it appeared to be unaltered. In order to avoid the complicating effects of endogenous catecholamines on the sensitivity of the preparations, as suggested by Clegg (1963), phentolamine and propranolol were added routinely in concentrations of 10^{-6} g/ml in all but the earlier experiments with depolarizing solution. Preliminary experiments with added noradrenaline showed that these concentrations were adequate.

Experimental design

For the experiments with non-depolarized preparations, the following pattern was adopted. One uterine horn was suspended in the modified Krebs solution (Ca, 0·1; Mg, 3·0 mM), whilst the other was suspended in a similar solution, to which PGE_1 had been added in a concentration of 0·05 μg/l. ($1{\cdot}4 \times 10^{-10}$M). For non-depolarized preparations, but not for depolarized ones, the PGE_1 concentration was clearly below the threshold for a direct spasmogenic action. These media flowed slowly through the organ bath so that the contents (2·5 or 5 ml) were replaced approx. every 2–3 minutes. After 10–20 min a large dose (100 μg/ml) of ACh was applied for 1 min to each horn, to record the 'maximum' responses. After a further 10–15 min, during which the preparations completely relaxed, a series of twenty-five applications was begun, identically to each horn. This series consisted of one of each of the three spasmogens at each of eight dose levels covering almost the full range of responses, with the addition that the first application was immediately

repeated. All applications were of 1 min duration and were followed by 5 min intervals irrespectively of the speed of relaxation, which was almost always complete. When the series of twenty-five applications to each horn had been completed, the reservoirs were interchanged and the whole procedure repeated. This formed one day's experiment.

Twenty-four such experiments were done. The order of application of the eight dose levels × 3 spasmogens was different each day, according to a predetermined plan which ensured that in the whole series any one dose level of any one spasmogen would occur once in each of the 24 possible positions and would be immediately preceded once by each of the 8 dose levels × 3 spasmogens (including itself). Repetition of the first application in each block or quarter-experiment was necessary to balance possible inflations of responses due to residual effects. Since the first trial in each block was not fully comparable it was excluded from the analysis.

Thus a total of forty-eight responses were available for each dose-level of each spasmogen in the presence of PGE_1, and the same number in its absence. Abbreviated forms of this experimental design were used for the experiments with Ba^{2+} and Sr^{2+}.

In the experiments with depolarized preparations, the sensitivity to Ca^{2+} or other spasmogens was first tested by 2 min to 5 min applications of various doses. When the dose producing a small but definite response had been established, this was then repeated at intervals of 10–20 minutes. The prostaglandin was added to the organ bath for 3 min before some applications of the spasmogen, and was washed out immediately afterwards.

Results

Non-depolarized preparations

The results are summarized in the figure of the preliminary account (Eagling *et al.*, 1971). The three spasmogens acetylcholine, vasopressin and Ca^{2+} all gave the usual sigmoid curves, with gradients and apparent maxima increasing in that order. In the presence of PGE_1, the curves were moved to the left by amounts corresponding to decreases in the dose necessary for a half-maximal contraction by factors of 2·4 (acetylcholine), 3·7 (vasopressin) and 2·6 (Ca^{2+}) (Table 1).

TABLE 1. *Effect of PGE_1 on responses of guinea-pig uterus*

	Ca^{2+}		Acetylcholine		Vasopressin	
	with PGE_1	no PGE_1	with PGE_1	no PGE_1	with PGE_1	no PGE_1
Estimated maxima	4,419	4,520	4,186	3,961	4,294	4,300
ED50	0·468 mM	1·214 mM	152·8 ng/ml	357·1 ng/ml	1·023 mU/ml	3·827 mU/ml
Log (ED50)	$\bar{1}$·6705	0·0844	2·1841	2·5527	0·0097	0·5829
S.E.	0·2354	0·2376	0·4320	0·4173	0·2845	0·2959
Slope	2·51	2·42	1·40	1·49	2·30	2·09

The maxima were estimated by extrapolation from eight points on each curve, each representing the total response to forty-eight applications of a dose and measured in mm on the kymograph tracings. ED50 is the dose necessary for a half-maximal contraction.

The estimated maximum responses for acetylcholine and vasopressin were 91% and 96% of that for the Ca^{2+} responses, but the differences were not statistically significant. There was no indication that the maximal responses in the presence of PGE_1 were materially different from the corresponding maxima in its absence (Table 1).

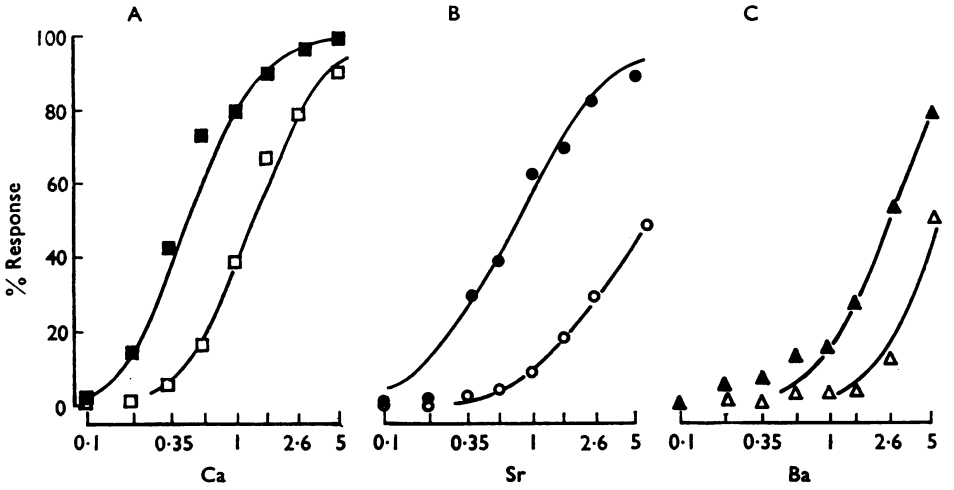

FIG. 1. Effect of PGE_1 on responses of guinea-pig uterus. Each point represents the mean of two contractions. Abscissae: concentrations of added salts (mM); ordinate: isotonic shortening. The lines are derived from straight lines fitted by eye to plots on log×probability paper, on the assumption that the maximal contractions are the same throughout and that the effect of the PGE_1 is a parallel shift to the left. The effects of the PGE_1 on the responses to Sr^{2+} (B) and to Ba^{2+} (C) are similar to that on Ca^{2+} (A). (A) ■, With PGE_1; □, without PGE_1. (B) ●, With PGE_1; ○, without PGE_1. (C) ▲, With PGE_1; △, without PGE_1.

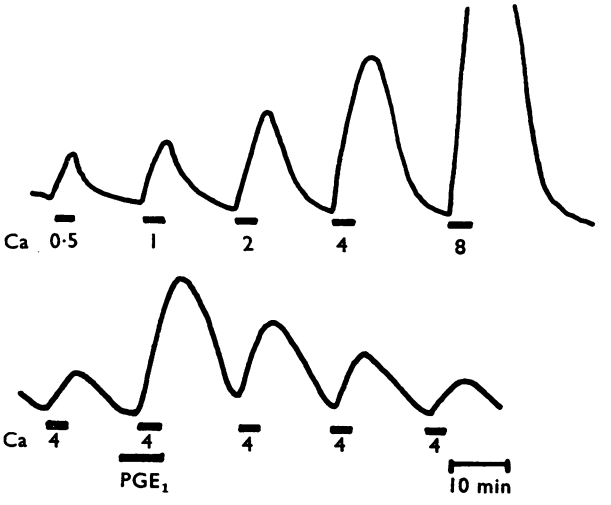

FIG. 2. Isotonic responses of a depolarized guinea-pig uterine horn in K_2SO_4-Krebs containing Ca^{2+} (0·2 mM) and Mg^{2+} (0·2 mM). Additional $CaCl_2$ was added for periods of 3 min in the concentrations shown (mM), one of these applications being preceded by PGE_1 0·1 ng/ml for 3 minutes. The record is not continuous, and during the part not shown the preparation became steadily less sensitive to added calcium.

The effects of PGE_1 on the responses to Ba^{2+} and to Sr^{2+} were generally similar to those on the Ca^{2+} responses though somewhat larger doses of the ions were needed (Fig. 1). When tests with the three spasmogens were continued throughout the period following a change from a solution containing PGE_1 to one without, the potentiation declined slowly as in the earlier 'enhancement' experiments (Clegg *et al.*, 1966). The use of a muscle-lever (Brown, 1971) that allowed a ready change from isotonic to auxotonic operation during the course of the experiment showed that the main effects described did not depend on the form of recording.

Depolarized preparations

PGE_1, in doses near or a little above the threshold for direct spasmogenic action, markedly increased the responses to raised $[Ca_2{}^+]$. Figure 2 shows one such experiment. The PGE_1 effect often persisted for approximately 40 min after the PGE_1 had been washed out of the organ bath.

Some of these experiments suggested that the corresponding effect of PGE_1 on acetylcholine responses was much less, but factors such as the slowness of relaxation made it impossible to make adequate comparisons. A few experiments showed that the dose of $PGF_{1\beta}$ needed to potentiate the Ca^{2+} responses was greater by a factor of the order of 100 than that of PGE_1.

Discussion

It was already known that PGE_1 potentiated (enhanced) the effects of other spasmogens such as vasopressin. If this were the result of a direct physico-chemical association of the prostaglandin with intracellular calcium, facilitating its release or transport, and if in addition PGE_1 could facilitate the influx of extracellular Ca^{2+} as indicated by the experiments on depolarized myometrium, then the potentiation of added Ca^{2+} might prove to be greater than that of other spasmogens. The experiments on the non-depolarized preparations did not show this. The dose-response curve for Ca^{2+} was shifted a little more than that for acetylcholine but a little less than that for vasopressin. A few experiments showed that similar effects were also given with Ba^{2+} or Sr^{2+} as the potentiated spasmogen.

The fact that the responses to other spasmogens are enhanced in essentially the same way by PGE_1 as those to Ca^{2+} suggests either that the PGE_1 acts at a point common to the actions of the various spasmogens, or that it modifies the effect of each of the spasmogens separately but similarly at an earlier stage. The first of these possibilities is the simpler. The second possibility might accord with the proposal of Smythies (1971) that prostaglandins (and cation) could be involved in receptor formation. In the present experiments the addition of PGE_1 might be supposed to increase the affinity of incomplete receptors for the three different spasmogens to differing extents, thus accounting for the minor differences seen between the potentiating effects of PGE_1 in the three cases. This hypothesis would not explain the enhancement by PGE_1 of the responses to electrical field stimulation (Clegg *et al.*, 1966).

In depolarized preparations also, PGE_1 clearly increased the responses to added Ca^{2+}, but here the threshold dose of PGE_1 for this effect was not distinguishable from that for an apparently direct spasmogenic response. The results of Edman & Schild (1962) are compatible with the supposition that the response of depolarized

myometrium to added Ca^{2+} is caused by a passive influx through the cell membrane. This might further be supposed to involve a carrier for which Mg^{2+} and Ca^{2+} compete. PGE_1 is known to localize in the uterus, amongst other sites, after intravenous injection (Samuelsson, 1964). In the present experiments PGE_1 absorbed into the cell membrane might act as an additional carrier by virtue of its ability to form a loose complex with Ca^{2+}. The lack of a clear difference between the threshold doses of PGE_1 for spasmogenesis and for potentiation of added Ca^{2+} in depolarized preparations is primarily due to lowering of the threshold for spasmogenesis. In these circumstances the carrier may have less importance.

Khairallah, Page & Türker (1967) suggested that low concentrations of a PGE might cause localized areas of depolarization in smooth muscle, thereby increasing its responsiveness to other spasmogens. This could not be true of myometrium that is already fully depolarized. Sucrose-gap recordings in the earlier 'enhancement' experiments (Clegg *et al.*, 1966), gave no evidence of any decrease in the resting potential of polarized myometrium in the presence of PGE_1, and it seems unlikely that the suggestion of Khairallah *et al.* is correct in this instance.

The possibility that cyclic AMP may be an intermediate in the 'enhancement' action of PGE_1 is being explored in experiments with dibutyryl cyclic AMP (Pickles, 1972).

Whatever the mechanism may be by which the myometrium is stimulated, a limiting factor is the influx of (extracellular) Ca^{2+} through a membrane. The initial hypothesis that PGE_1 facilitates this influx by the formation of a readily dissociable complex has not been disproved. The finding that $PGF_{1\beta}$ does not readily either form a complex with Ca^{2+} (van Dorp & Heertje ; personal communication) or cause enhancement, is compatible with the hypothesis.

It may be noted that the concentrations of Ca^{2+} giving marked responses include the physiological range in plasma. The concentration of PGE_1 used is even less than that quoted for PGE_2 in human plasma by Samuelsson, Granström, Gréen & Hamberg (1971). Roth-Brandel, Bygdeman & Wiqvist (1970) have shown that intravenous injection of PGE_1 stimulates the human non-pregnant myometrium, instead of inhibiting contractions as previously found from experiments *in vitro*. These facts together raise the question of whether the contractility of the non-pregnant as well as of the pregnant human myometrium may be modulated not only by $PGF_{2\alpha}$ liberated from the endometrium (Pickles, Hall, Best & Smith, 1965), but also by circulating PGE_1 in association with calcium.

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