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PROSTAGLANDINS E_1, E_2 AND I_2: EVIDENCE FOR THREE DISTINCT ACTIONS IN VASCULAR SMOOTH MUSCLE

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Summary: In the perfused mesenteric artery of the rat prostaglandins (PGs) E_1, E_2 and I_2 had distinct actions. PGE_2 potentiated pressor responses to noradrenaline, angiotensin II and potassium ions. PGE_1 potentiated responses to noradrenaline and angiotensin at low concentrations and inhibited them at high concentrations: no concentrations had any effect on potassium responses. PGI_2 inhibited responses to noradrenaline and angiotensin but had no effect on potassium responses. These three distinct actions suggest that the binding sites for the three PGs in this vascular muscle must be distinct.

Introduction

PGE_1 and PGE_2 have many similar actions. Also the recently discovered PGI_2 (prostacyclin) and PGE_1 have similar actions, particularly on adenylate cyclase (1). It is therefore often assumed that there must be considerable similarity between the receptors for the three PGs. We here present evidence that the three PGs have unequivocally distinct effects in rat mesenteric smooth muscle indicating three different receptors.

Methods and Materials

We used the perfused superior mesenteric vascular bed of the male rat as previously described in detail (2,3,4,5). Under ether anesthesia the artery was cannulated and its vascular bed dissected out and mounted in an organ bath. Using a peristaltic pump the preparation was perfused with Krebs-bicarbonate buffer at a flow rate of 3 ml/minute. Perfusion pressure was recorded via a side arm off the arterial cannula. Test injections into another side arm of pressor agents dissolved in 0.1 ml buffer caused brief vasoconstriction and hence elevation of pressure. We have previously shown that both the baseline pressure and the responses to a fixed dose of pressor agent remain stable for many hours (3,4). Actual doses of pressor agents used, each sufficient to give a 40-60% maximal pressor response, were 10 ng noradrenaline as the bitartrate (Sigma), 7 micromol of potassium chloride and 1 ng of angiotensin II (Sigma). The pressor effect of potassium injections is not dependent on release of noradrenaline from nerve endings since it persists even after complete abolition of adrenergic effects by phenoxybenzamine. Responses to potassium and vasopressin are rapidly abolished by use of a calcium-free buffer and presumably depend on stimulation of

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calcium entry from outside. Responses to noradrenaline and angiotensin remain at 60-80% of their original level on removal of extracellular calcium and presumably depend largely on calcium release from intracellular stores (3,4).

We tested the actions of PGE_2, PGE_1, and PGI_2 on pressor responses to noradrenaline (6 experiments each), angiotensin (4 experiments each) and potassium (6 experiments each). Once the preparation had stabilized, three test injections of the pressor agent to be used were given: the mean amplitude of these responses was taken as 100% and subsequent results were expressed as percentages of this. Progressively increasing concentrations of each PG were then added to the buffer, each being present for 10 minutes: at the end of that time the response to the same fixed dose of pressor agent being used was tested. PGE_1 and PGE_2 are stable and were supplied by Upjohn. PGI_2 as its sodium salt was synthesized as previously described (6) from $PGF_{2\alpha}$ methyl ester. This procedure provided a basic ethanolic solution of PGI_2 in a stable form (10^{-3}M PGI_2, 8 x 10^{-3}M sodium base, 10^{-3}M sodium iodide). This solution was directly diluted with the buffer being used as perfusate. The solvent without PGI_2 was similarly diluted and found to have no effects on baseline pressure or on responses to any of the pressor agents. Preliminary experiments showed that the PGI_2 effects were rapid, reached a plateau within five minutes and began to face at about 15 minutes, probably indicating degradation of the PGI_2. PGE_1 and PGE_2 effects also reached a plateau within 5 minutes and then the effect of each concentration remained unchanged for at least 30 minutes.

Results

None of the PGs had any effect on baseline pressure indicating an absence of direct vasodilating or constricting effects.

PGE_2 caused a progressive increase in response amplitude to injections of potassium and noradrenaline (fig. 1) and angiotensin (not shown). PGE_1 potentiated responses to noradrenaline at low concentrations but above 10^{-11}M this potentiation became progressively less and was converted to an inhibition above 10^{-7}M (fig. 2). PGE_1 effects on angiotensin responses were indistinguishable from those on noradrenaline, but PGE_1 failed to alter potassium responses.

PGI_2 failed to change responses to potassium but caused a progressive inhibition of responses to noradrenaline (fig. 3). In six preparations each, before adding the increasing concentrations of PGI_2, either 10 ng/ml PGE_2 or 10 μg/ml indomethacin was added to the buffer and left there while the PGI_2 dose response curve was carried out. The PGE_2 increased responses to about 150% of those obtained with buffer alone. These increased responses were taken as the 100% starting values for the PGI_2 curve. The presence of

Table I. *Inhibitory potencies of Leu-enkephalin and fluorescent peptides on the contractions of guinea-pig ileum and on the binding of ^{3}H-Leu-enkephalin by membranes from mouse striatum.*

Compound	Guinea-pig ileum		^{3}H-Leu-enkephalin binding
	IC_{50} (nM)	Relative Potency	Ki (nM)
Leu-E	67 ± 12	100	5 ± 1
Met-E-$(CH_2)_2$ dansyl	50 ± 11	86	10 ± 3
D-Ala_2-Met-E-$(CH_2)_2$ dansyl	27 ± 4	147	5 ± 2
Met-E-$(CH_2)_5$ dansyl	288 ± 15	13	21 ± 5
N-dansyl-Met-E	1,306 ± 328	4	50 ± 25

for β-adrenergic receptors (17). The fine analysis of energy transfer within such molecules in solution can provide interesting data regarding their preferred conformation (18) and could be compared to those provided by other approaches (19,20, 21). In addition, the energy transfer between the Tyr and the dansyl groups of the molecule provides an indirect index of the integrity of the molecule : since the biological inactivation consists primarily in the hydrolysis of the Tyr-Gly bond (22), Met-E-$(CH_2)_2$ dansyl can be used as a substrate for the peptidases involved in this cleavage and, monitoring the *in vitro* changes in fluorescence, provides a convenient mean to evaluate the activity of these enzymes and the actions of inhibitors.

Finally, and perhaps more interestingly, the analysis of energy transfer between tryptophane residues of the receptor molecule and the dansyl group of the probe (8,23) or between Tyr and dansyl chromophores within the latter, could provide interesting data regarding the conformational changes possibly occuring during attachment of the peptide to its recognition sites. Such an approach can be extended to the different endorphins which contain only one Tyr residue at the N-terminal part of the peptide.

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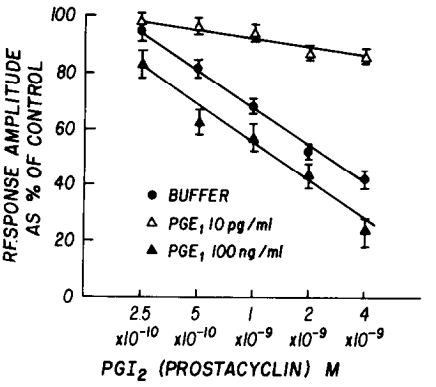

Fig. 4. Changes in response to fixed doses of noradrenaline produced by PGI_2 in plain buffer, and buffer containing 10 pg/ml (2.8×10^{-11}M) PGE_1 or 100 ng/ml (2.8×10^{-7}M) PGE_1. Each point represents the mean ± SEM for 6 experiments.

PGE_2 failed to alter the PGI_2 effects (fig. 3). Similarly indomethacin which reduced responses to noradrenaline to about 40% of their amplitude in buffer alone (3) failed to modify the PGI_2 curve (fig. 3).

In contrast to these results, PGE_1 did change the PGI_2 effects. 10 pg/ml (2.8×10^{-11}M) PGE_1, the concentration which produces a maximum potentiating effect, markedly attenuated the PGI_2 effect when the experiments were performed as described for the PGE_2/PGI_2 interaction. In contrast, 100 ng/ml (2.8×10^{-7}M) PGE_1 moved the PGI_2 curve slightly to the left (fig. 4).

Discussion

The three PGs have clearly distinct actions on pressor responses in this preparation indicating that three different receptor sites are involved. PGE_2 had equipotent effects on responses dependent on extracellular and intracellular calcium whereas PGE_1 and PGI_2 modified only responses to noradrenaline and angiotensin which seem to be largely dependent on intracellular calcium release. This does not necessarily mean that these PGs act intracellularly because intracellular calcium release could be changed by plasma membrane receptors.

The PGI_2 effects were unaltered by either exogenous PGE_2 or by reduction of endogenous PG synthesis by indomethacin suggesting that they were not

dependent on antagonism of action of an endogenous PG. They were attenuated by a very low concentration of PGE_1 indicating that PGE_1 and PGI_2 may oppose one another's effect on intracellular calcium release. PGE_1 altered the slope of the dose response curve indicating that the interaction between the two PGs was either due to an allosteric mechanism or to a physiological antagonism rather than to competition for the same receptor site. In contrast, the high PGE_1 concentration which itself was weakly inhibitory moved the PGI_2 curve slightly to the left but apparently in parallel. This might possibly be due to activation of PGI_2 receptors by high concentrations of PGE_1 or to stimulation of PGI_2 synthesis or to some other unknown mechanism.

We conclude that the different effects of the three PGs are probably related to their ability to activate specific receptors selectively.

Acknowledgments

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