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PROSTAGLANDIN E AND $F_{2\alpha}$ RECEPTORS IN BOVINE CORPORA LUTEA: RELATIVE AFFINITIES OF PRODUCTS OF ARACHIDONIC ACID METABOLISM BY CYCLO-OXYGENASE

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SUMMARY

The relative affinities of products of arachidonic acid metabolism for binding to prostaglandin (PG)E and $F_{2\alpha}$ receptors in cells and plasma membranes of bovine corpora lutea were measured under conditions of reduced product breakdown. The PG endoperoxides exhibited moderately high affinity but this was attributable to products of their transformation. Following testing for the affinity of each of these products (i.e. thromboxane B_2, prostacyclin, 6-keto-$PGF_{1\alpha}$, PGD_2, PGE and $PGF_{2\alpha}$), it was concluded that only corresponding unlabeled PGs bind to PGE and $PGF_{2\alpha}$ receptors with high affinity.

INTRODUCTION

Prostaglandin (PG) endoperoxides are formed from arachidonic acid by the action of cyclo-oxygenase (1). These endoperoxides serve as common intermediates in the independent formation of PGE_2, $PGF_{2\alpha}$, PGD_2, thromboxanes (1) and prostacyclin (2). The PG endoperoxides, thromboxane (TX)A_2 and prostacyclin (PGI_2) are extremely potent biologically and their potencies are several fold higher than those of stable PGs (1,3). The TXA_2 and PGI_2, which are inherently unstable, spontaneously convert to stable end products, namely TXB_2 and 6-keto-$PGF_{1\alpha}$ respectively (1,2). These end products have also been shown to possess low intrinsic biological activity (4,5). These studies that provided the above information were conducted primarily on vascular tissues. The experiments designed to explore the above biosynthetic details with luteal tissue are lacking, but they are presumed to be the same as in other tissues. Great progress has been made in the last few years in identifying and

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characterizing PGE and $PGF_{2\alpha}$ receptors in cells (6,7) and plasma membranes (8-11) of bovine corpora lutea. In none of these studies, however, were experiments directed to examine the affinities of PG endoperoxides, TXB_2, PGI_2 or 6-keto-$PGF_{1\alpha}$ for binding to PGE and $PGF_{2\alpha}$ receptors. Such studies are obviously important in the evaluation of whether some of these products can serve as effective ligands to PGE and $F_{2\alpha}$ receptors.

MATERIALS AND METHODS

The sources of all the items used in the present study, details on the check of [^{3}H]PGs purity, purification if needed and isolation of cells and plasma membranes from bovine corpora lutea are the same as previously described (6,7,12-14). All the unlabeled compounds were dissolved and diluted in redistilled acetone and stored at -20^0 between uses.

A fixed number of cells (2 to 3 x 10^5) and plasma membrane protein (200 µg) were incubated for 10 min at 22^0 with [^{3}H]PGE_1 (8 nM), [^{3}H]$PGF_{2\alpha}$ (4 nM) and increasing amounts of various unlabeled compounds. The pH of incubation media was 7.0 except when experiments were conducted with PGI_2. Then the pH was 8.0. The selection of incubation temperature, time (< 10 min incubation could not be used because of very little binding of [^{3}H]PGs) and pH was primarily aimed at reducing the breakdown of PG endoperoxides and/or PGI_2. The competition experiments with corresponding unlabeled PGs were conducted simultaneously under the same conditions with respect to time, temperature and pH of incubation, for comprehensive assessment of results. All the experiments were conducted in quadruplicates and repeated three times. The rest of the details on binding methodology are the same as previously described (12).

The relative affinities for unlabeled compounds were calculated from molar concentrations required for 50% inhibition of [^{3}H]PGs binding and taking 100% for the corresponding PGs.

RESULTS

Table I shows that arachidonic acid has no measurable affinity for PGE receptors. Among the products of its metabolism by cyclo-oxygenase pathway, only PG endoperoxides exhibited moderate affinity as compared to unlabeled PGE_1. However, stable epoxymethano analogs of PG endoperoxide (PGH_2) had very little affinity unlike the parent unstable molecule. It should be noted that the % relative affinity values were about the same (one exception, i.e. PGG_2), regardless of the use of dispersed viable luteal cells or plasma membranes as a source of PGE receptors.

Arachidonic acid exhibited very little affinity to $PGF_{2\alpha}$ receptors (Table II). Among the products of its metabolism, PG endoperoxides, PGD_2 and possibly PGI_2 exhibited moderate affinity as compared to unlabeled $PGF_{2\alpha}$.

TABLE I

Percent relative affinities of various unlabeled compounds for binding to PGE receptors in cells and plasma membranes of bovine corpora lutea.

Each value in this Table and Table 2 represents the mean of twelve observations in three independent experiments. The variation, expressed as % of mean, averaged about 5.0%.

Unlabeled Compound	Cells	Plasma Membranes
	Percent Relative Affinities	
PGE_1	100	100
Arachidonic acid	-	< 0.00[a]
PGG_2	51.98	16.77
PGH_2	50.00	56.80
PGH_2 Epoxymethano Analog I	0.55[b]	0.49[b]
PGH_2 Epoxymethano Analog II	0.10[b]	0.14[b]
TXB_2	0.43	0.90
PGI_2	3.32	9.69
6-keto-$PGF_{1\alpha}$	0.12	0.31
PGD_2	1.40	2.60

[a]This value, calculated from the data of equilibrium binding experiments, was taken from a previous publication from our laboratory (19).

[b]These values were calculated from the data of equilibrium binding experiments. Analog I=(15S)-hydroxy-9α,11α-(epoxymethano)prosta-5Z,13E-dienoic acid; Analog II=(15S)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid.

Stable epoxymethano analogs of PGH_2 exhibited very little affinity as compared to the unstable parent molecule. Although the absolute % relative affinity values calculated from the data on cells and plasma membranes varied, the general trend of relative affinities was the same in both cases.

DISCUSSION

Precautions were taken in these studies to reduce the product breakdown so that the relative affinity values obtained would be meaningful. In addition, where possible, analogs which are resistant to breakdown were also used.

PG endoperoxides exhibited moderately high affinity for PGE and $PGF_{2\alpha}$ receptors. However, this probably is not attributable to these compounds as

TABLE II

Percent relative affinities of various unlabeled compounds for binding to $PGF_{2\alpha}$ receptors in cells and plasma membranes of bovine corpora lutea.

Unlabeled Compound	Cells	Plasma Membranes
	Percent Relative Affinities	
$PGF_{2\alpha}$	100	100
Arachidonic Acid	-	0.02[a]
PGG_2	53.18	19.42
PGH_2	28.10	79.50
PGH_2 Epoxymethano Analog I	1.42	-
PGH_2 Epoxymethano Analog II	2.12	-
TXB_2	4.71	1.01
PGI_2	19.52	2.73
6-Keto-$PGF_{1\alpha}$	2.22	9.45
PGD_2	34.10	18.00

[a]This value, calculated from the data of equilibrium binding experiments, was taken from a previous publication from our laboratory (19).

such because two epoxymethano analogs of PGH_2, which are not subjected to rapid transformation, unlike their parent molecule, exhibited very little affinity to these receptors. The high affinity of PG endoperoxides must be due to products of their transformation, which are TXB_2, PGI_2, 6-Keto-$PGF_{1\alpha}$, PGE, $PGF_{2\alpha}$ and PGD_2. Each of these products and/or their stable metabolites were tested in an attempt to find out which of these can bind with relatively high affinity. The result was that only corresponding unlabeled PGs exhibited a high affinity to PGE and $F_{2\alpha}$ receptors. This finding suggests that formation of PGE and $PGF_{2\alpha}$ primarily accounts for the relatively high affinity observed for PG endoperoxides. The lower affinities of PG endoperoxides as compared to stable PGs is explained by the lack of quantitative conversion of PG endoperoxides to stable PGs. (15).

As a corollary to the main finding, it should be noticed that while PGI_2 and PGD_2 exhibited negligible affinity to PGE receptors, their affinity to

$PGF_{2\alpha}$ receptors was relatively high. It is not known at this time what, if any, functional significance can be attested to this finding.

The expectation that receptor binding affinity correlates with biological potency arises from previous studies, which demonstrated good parallelism between these two parameters (6-11,16-19). Since neither arachidonic acid nor any of the products of its metabolism other than corresponding unlabeled PGs exhibited high affinity, it is concluded that these other compounds could not serve as physiological endogenous or even exogenous ligands to PGE or $F_{2\alpha}$ receptors. This conclusion can only be overridden if these products can be demonstrated to be present *in vivo* in great excess to compensate for their low affinity. However, this appears very unlikely from what is known about the levels of these compounds in other tissues (20,21).

Thus the data presented in this paper indicate that PG endoperoxides, TXB_2, PGI_2, 6-keto-$PGF_{1\alpha}$ and PGD_2 have little direct interaction with PGE and $F_{2\alpha}$ receptors in luteal tissue. However, the possibility that some of these products bind to receptors which are distinct from those of PGE and $PGF_{2\alpha}$ and perform some function not yet known in luteal tissue remains to be explored.

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