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Prostacyclin as an Endogenous Modulator of Adenosine Cyclic 3′,5′-Monophosphate Levels in Rat Myometrium and Endometrium

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SUMMARY

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Our previous study (Vesin *et al.*, 1978, *Mol. Pharmacol.* **14:** 24–37) showed that arachidonic acid, a precursor of prostaglandins, can elicit contractions of the estrogen-treated rat myometrium and stimulate cAMP accumulation in myometrium and endometrium. The present investigation characterizes the nature of the prostaglandin material(s) involved in the arachidonic acid responses. All prostaglandins tested were active contractile agents ($PGF_{2\alpha} > PG_2 > PGI_2 >$ 6-keto-$PGF_{1\alpha}$). Only PGE_2 and PGI_2, but not 6-keto-$PGF_{1\alpha}$ could induce, marked increases in intracellular cAMP in both tissues. PGI_2 was more active than PGE_2, although maximal responses were similar in magnitude for both compounds. Prior treatment of the uterine tissues with prostacyclin synthesis inhibitors *viz.* tranylcypromine and 15-hydroperoxy arachidonic acid (HP-AA), antagonized cAMP elevations caused by arachidonic acid, suggesting that PGI_2 might be involved. In contrast, tranylcypromine did not affect the arachidonic acid-induced contractions. Further experiments demonstrate that arachidonic acid treatment also stimulates the concomitant release from the myometrium and endometrium of a platelet antiaggregatory material that could be identified as PGI_2 by the following criteria: a) it shared the chemical lability of authentic PGI_2, b) its generation was abolished by prior treatment of the tissue with indomethacin, tranylcypromine or HP-AA and c) 6-keto-$PGF_{1\alpha}$ the end product of PGI_2 was the major prostaglandin generated from [^{14}C]arachidonic acid in myometrial and endometrial homogenates. No PGE_2 or PGD_2 could be detected. Basal values of PGI_2 released were similar for both uterine tissues (about 60 pmol/g tissue) and were increased with arachidonic acid to 130 pmol PGI_2/g tissue. Such local PGI_2 concentrations were sufficient to account for the twofold increment in tissue cAMP, which was similarly induced by corresponding concentrations of authentic PGI_2. Quantitative correlations, PGI_2 versus cAMP, could also be demonstrated under conditions of enhanced PGI_2 generation from endogenous arachidonic acid (*e.g.*, during isolation and stripping of the myometrium). These data indicate that locally generated PGI_2 could not be implicated in the contractile effect of arachidonic acid. They do offer strong suggestive evidence that endogenous PGI_2 largely contribute to the modulation of intracellular cAMP levels in the myometrium and endometrium.

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INTRODUCTION

It has been well documented that prostaglandins of the E and F (PGE and PGF)[1] series elicit contractions of the estrogen-dominated rat uterus (1–4). In the case of the PGF ($F_{1\alpha}$ and $F_{2\alpha}$)-stimulations, there was no alteration in the tissue cAMP content (4–6). On the other hand, PGE (E_1 and E_2) have been shown to be potent activators of the adenylate cyclase system in both the myometrium and endometrium (4–7) and such stimulations were accompanied by a parallel increase in intracellular cAMP binding (8) and protein kinase activation (9). Nevertheless, no direct causal relationship could be established between both PGE effects, *viz.*, stimulation of the cAMP system and uterine contractions (4, 8). More recent experiments (7) pointed to the fact that arachidonic acid, through its conversion to prostaglandin-like material, was also able to cause myometrial contractions and induce a significant increase in intracellular cAMP levels of both myometrium and endometrium. Although no definite answer could be provided for a role of cAMP in the regulation of uterine contractility (9), the latter findings clearly demonstrate that the endogenously synthetized prostaglandin(s) were equally effective in inducing contractions and cAMP accumulation.

The foregoing prompted us to investigate the nature of the prostaglandin material(s) synthetized from arachidonic acid and which contributed to the observed stimulatory effects. While it has been customary in the past to consider only PGE_2 and $PGF_{2\alpha}$ as the main and functionally significant products of the prostaglandin synthetase reaction, it became increasingly clear that other short-lived prostaglandin-like compounds including the endoperoxide themselves (10) as well as products of the thromboxane (11) and prostacyclin (12, 13) synthetase pathways may be more potent regulators of certain specific cellular functions. Particular attention was given to prostacyclin (PGI_2) in this study. This is because recent investigations have provided information regarding the synthesis of an active PGI_2-like substance by the myometrium of the pregnant rat (14) and because 6-keto-$PGF_{1\alpha}$, the end-product metabolite of PGI_2 has been previously recognized as a major prostaglandin compound in the pseudopregnant rat (15, 16). The experiments reported herein, describe a careful analysis of the effects of PGI_2 and 6-keto-$PGF_{1\alpha}$ both on uterine contractility and tissue cAMP levels. In addition this paper reports an evaluation of PGI_2 synthesis by the myometrium and endometrium challenged with arachidonic acid. Our findings demonstrate that neither PGI_2 nor PGE_2 can actually be considered as the local mediator of the arachidonic acid-induced contractions of the estrogen-pretreated rat myometrium. Evidence is however provided that PGI_2 synthetized *in situ* might be the major, if not the sole, prostaglandin compound participating in the modulation of rat uterus cAMP content.

EXPERIMENTAL PROCEDURES

Chemicals. cAMP was obtained from P.L. Biochemical; β-oestradiol-3-benzoate and trans-2-phenyl-cyclopropylamine hydrochloride (tranylcypromine) from Sigma Chemical Co., St. Louis; 3-isobutyl-1-methyl-xanthine from Aldrich Chemical Company Inc. and Soyabean lipoxygenase (~250,000 units/mg) from Serva. [^{3}H]-cAMP (36 Ci/mmol) was a product of New England Nuclear Inc. and [1-^{14}C] arachidonic acid (56 mCi/mmol) was provided by Radiochemical Center, Amersham, England. Prostaglandins E_2, $F_{2\alpha}$, D_2, 6-keto-$PGF_{1\alpha}$ and prostacyclin were generous gifts from Dr. J. E. Pike of the Upjohn Company. Compound U44069, (15S)-hydroxy-9α,11α-(epoxymethano) prosta-5Z, 13E dienoic acid and compound U46619, (15S)-hydroxy-11α,9α-(epoxymethano) prosta-5Z, 13E-dienoic acid were obtained through the courtesy of Dr. G. L. Bundy (Upjohn).

[1] The abbreviations used are: PG, prostaglandin; PGI_2, prostacyclin; AA, arachidonic acid; HP-AA, 15-hydroperoxy arachidonic acid; cAMP, adenosine cyclic 3',5'-monophosphate; MIX, 3-isobutyl 1-methyl-xanthine; EDTA, ethylene diamine tetra-acetic acid; Tris, tris-(hydroxymethyl)-aminomethane; PRP, platelet rich plasma; RSV, ram seminal vesicles; tranylcypromine, trans-2-phenyl-cyclopropylamine hydrochloride; TLC, thin-layer chromatography; TCA, trichloroacetic acid.

Indomethacin was kindly supplied by Merck Sharp and Dohme.

Arachidonic acid was from Nu Chek Prep Inc. Solution of the ammonium salt was prepared by dissolving 9 mg/ml in NH_4OH for neutralization with agitation under a stream of N_2.

15-hydroperoxy arachidonic acid (HP-AA) was obtained by catalytic oxygenation of arachidonic acid using soyabean lipoxygenase (17). The reaction was carried out at room temperature in 2 ml of 0.1 M borate buffer, pH 9.0 with 50 μM arachidonic acid and 20 μl of the lipoxygenase solution (1000 U). Measurement of the absorbance at 238 nm indicated that the oxygenation was complete between 3 and 5 min. The reaction mixture was then extracted with 6 ml cold ether, evaporated to dryness under a stream of N_2 and the residue was redissolved in 1 mM NH_4OH. The oxidized product was further characterized by TLC on silica gel plates developed in ether/petroleum ether/acetic acid (100:100:2) and revealed by spraying with the ferrous thiocyanate reagent (18). The hydroperoxy arachidonic acid was also submitted to reduction by sodium borohydride (19) to the 15-hydroxy derivative before ether extraction and evaporation to dryness. Solutions of hydroperoxy- and hydroxy-arachidonic acid were usually obtained in 1 mM NH_4OH for use in the different incubation experiments.

Animals. Young virgin female rats (Wistar), 4–5 weeks old, were treated with 30 μg of estradiol for 2 days and used on the following day.

Tissue preparation. Rats were killed by decapitation, and their uteri were immediately removed and immersed in Krebs-Ringer bicarbonate buffer, pH 7.4 at 4°. The myometrium was separated by stripping away the endometrium as previously described (4).

Incubation experiments for assay of cAMP levels. Myometrial and endometrial preparations from about 20 rat uteri were cut into two segments, which were pooled and divided into equal parts. Incubations were carried out in Krebs-Ringer bicarbonate buffer at 37° (gas phase, 95% O_2–5% CO_2) under constant agitation. About 50 mg of myometrial or endometrial strips were added to 1.5 ml of buffer and allowed to equilibrate for 30 min at 37°. Incubations were further continued with or without the addition of 3-isobutyl-1-methylxanthine (MIX), final concentration 276 μM, and the various substances to be tested. Reactions were stopped at different times by immersing the tissue strips in 2 ml of cold 7% TCA followed by immediate homogenization of the samples and centrifugation for 30 min at 30,000 × *g*. cAMP was estimated in the trichloroacetic acid soluble extracts according to Gilman (20), as previously described (4). The centrifuged pellets were dissolved in 2 ml of 1 N NaOH for protein determination (21). cAMP levels were expressed as pmoles/mg of protein.

Methods for recording uterine responses. The contractile activity of isolated uterine strips was measured with an isometric transducing device as described previously (4). The segments were loaded at a basal tension of 0.2–0.3 g and bathed at 37° in 15 ml Krebs-Ringer bicarbonate buffer (95% O_2–5% CO_2). The tissue was equilibrated in the organ bath for 30–40 min, with several changes of the bathing fluid. When a steady-state pattern of basal contraction evolved and the baseline did not change, the pharmacological agents to be tested were added at the indicated concentrations.

Platelet aggregation experiments. Blood was collected from unanaesthetized rabbits by cardiac puncture and collected into plastic tubes containing a 3.8% w/v sodium citrate solution (9 ml blood + 1 ml Na citrate). Platelet-rich plasma (PRP) was prepared by centrifuging blood (200 × g, 15 min) at room temperature. Aggregation experiments were carried out with 0.3 ml PRP, diluted with 0.9% NaCl to a final volume of 0.5 ml, using a Chrono-log aggregometer and following changes in light transmission. Aggregation was usually induced with 130 μM arachidonic acid and results were reported as percent transmission. To assay for the ability of the uterine tissues to release antiaggregatory activity, myometrial and endometrial strips (about 200 mg) were added to 1.5 ml Krebs-Ringer buffer, allowed to equilibrate for 30 min at

37° and further incubated with or without 3 mM arachidonic acid under conditions described for the cAMP experiments. At the end of incubation the tissue strips were immediately transferred into 0.5 ml (50 mM Tris–0.9% NaCl, pH 8.0 for 2 min at 37°). Following a rapid centrifugation (5 min at 3000 × *g*) aliquots of the supernatant fluids (25–200 μl) were added to PRP, 1 min before challenging with 130 μM arachidonic acid and their antiaggregatory activity was quantitated against the antiaggregatory effect of authentic PGI_2.

Biotransformation of [^{14}C]arachidonic acid by myometrial and endometrial homogenates. Preparations were obtained from both myometrium and endometrium by homogenizing 400 mg of each tissue in 2.0 ml 50 mM Tris-HCl, 1 mM EDTA pH 8.0, using an Ultra-Turrax homogenizer (twice 15s). Incubations were carried out at 37° in the presence of 20 μM [^{14}C]arachidonic acid (1×10^6 cpm) for different periods of time (3–20 min) under an atmosphere of 95% O_2, 5% CO_2. Reactions were stopped by the addition of 5 volumes cold ethanol and after standing for 15 min at 0°, the reaction mixtures were filtered as described by Pace-Asciak *et al.* (22). Standard prostaglandins (PGE_2, $F_{2\alpha}$, A_2, 6-keto-$F_{1\alpha}$) were added and after evaporation to dryness under a stream of N_2, the residues were acidified to pH 4.0 and extracted three times with ether (total volume: 6 ml). The ether extracts were pooled and evaporated to dryness. The residue was finally redissolved in chloroform/methanol (2:1) and aliquots were quantitatively spotted on silica gel plastic plates (F 1500, Schleicher et Schüll) for TLC analysis. The chromatograms were developed in two different solvent systems, namely system A: the organic phase of a mixture of ethylacetate/acetic acid/iso-octane/H_2O (110:20:50:100) and system B: ethyl acetate/acetic acid (99:1) as has been proposed by Sun *et al.* (23) for the separation of 6-keto-$PGF_{1\alpha}$ from PGE_2 and $PGF_{2\alpha}$. Double development was routinely carried out in order to gain adequate resolution of these compounds. The positions of standard prostaglandins and excess arachidonic acid on the plates were visualized with iodine vapor. Radioactive zones were located using Kodak X-ray films (Kodirex). In some experiments, the intensity of the different areas on the developed films was monitored with a densitometer. In all cases, the corresponding radioactive areas on the TLC plates were cut out and the radioactivity was estimated by liquid scintillation counting. Remaining areas on the plate were divided into 0.5 cm bands and treated similarly. Recovery was evaluated by comparison of the sum of the total radioactivity recovered from the plates with that of originally added [^{14}C]arachidonic acid. From the knowledge of the specific activity of arachidonic acid at the onset of incubation, it was possible to estimate the amount of prostaglandin material generated by each tissue preparation. The ability of the uterus homogenates to convert arachidonic acid to different prostaglandin products was compared with that of the ram seminal vesicle (RSV) microsomes prepared as described by Wlodawer and Samuelsson (24). Incubations with [^{14}C]arachidonic acid, extractions and TLC were proceeded as above. It is clear that in these radiochromatographic experiments, it was not attempted to trap the unstable PGI_2 but rather to characterize and quantify its stable end-product, 6-keto-$PGF_{1\alpha}$.

RESULTS

Comparative effects of different prostaglandins and derivatives on the contractility of rat myometrium. The characteristic contractile activities exhibited by arachidonic acid and different prostaglandin derivatives are depicted in Figure 1. In addition to the previously described stimulatory effects of PGE_2 and $PGF_{2\alpha}$ (4), the two stable endoperoxide analogues, U44069 and U46619, whose structures are closely related to that of the natural endoperoxide PGH_2 generated during prostaglandin biosynthesis, both caused contractions of the estrogen-dominated rat uterus. Prostacyclin and its stable end-product, 6-keto-$PGF_{1\alpha}$, are also shown to stimulate uterine contractility. Comparative results of the dose-related activities of different prostaglandins and their metabolic precursor, arachidonic acid, are depicted in Figure 2. Of all the tested compounds, $PGF_{2\alpha}$ was the

more potent activator of rat uterus contractions, followed by PGE_2 and PGI_2 (half-maximal activations at 0.15, 0.5 and 1 μM, respectively). Our observations, that PGI_2 was still more active (10-fold) than its metabolized end-product, agree with the recent data of Omini *et al.* (3). Additional experiments indicated that both endoperoxide analogues (dose-response curves not shown) were less active than any of the analyzed prostaglandin-compounds or even arachidonic acid. This situation is quite different from that described with other smooth muscle preparations, where the endoperoxide analogues, whose activities have been considered to mimic the naturally occurring endoperoxides, were equipotent, if not more potent than $PGF_{2\alpha}$ (25, 26). Thus, although endoperoxide formation seems a prerequisite step in the arachidonic acid-induced contractions of the rat myometrium (7), it seems unlikely that these endogenously produced labile substances are involved in the contractile response without further conversion to a more active prostaglandin-like compound(s). Based on the dose-response curves for arachidonic acid and the various prostaglandins (Fig. 2), it was possible to obtain a rough evaluation of the quantity of each prostaglandin material that had to be generated, assuming it was involved in the arachidonic acid contractile effect. Conversion of arachidonic acid to $PGF_{2\alpha}$, PGE_2

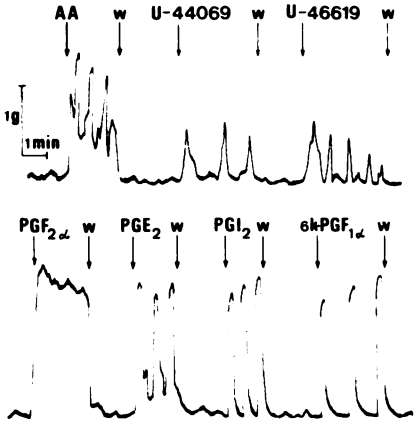

FIG. 1. *Tracing of isometric contractions of isolated rat uterus in the presence of arachidonic acid, stable endoperoxide analogues and various prostaglandins*

Uterine contractions were recorded as described under EXPERIMENTAL PROCEDURES. The concentration of arachidonic acid (AA) was 25 μM, U 44069 15 μM, U 46619 15 μM, $PGF_{2\alpha}$ 0.5 μM, PGE_2 1.5 μM, PGI_2 3 μM and 6-keto-$PGF_{1\alpha}$ (6-kPGF$_{1\alpha}$) 15 μM. W = washing with buffer solution (recording was stopped during the washing period).

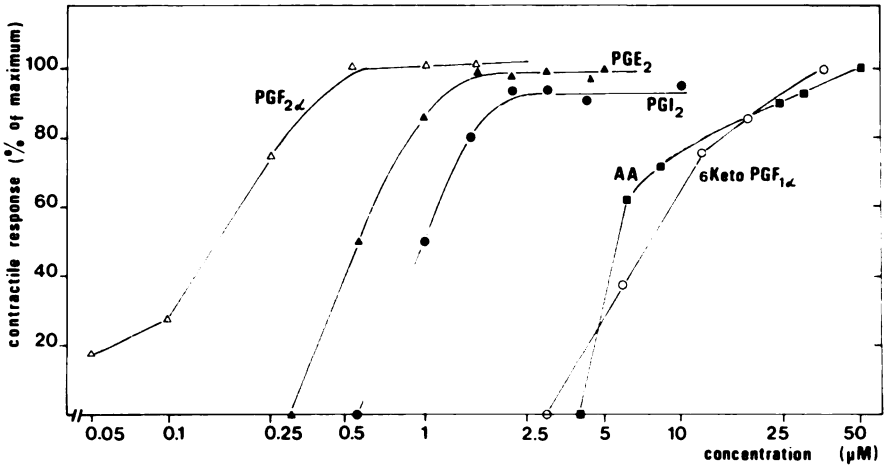

FIG. 2. *Comparison of the contractile response of the isolated rat uterus to increasing concentrations of arachidonic acid and various prostaglandins*

Uterine contractile responses were recorded as described under EXPERIMENTAL PROCEDURES. The degree of the contractile response to each agent was expressed as the percentage of the response to a maximal effective concentration of carbamylcholine (20 μM). The maximal response elicited by each contractile agent refers to the force of the first contractions.

or PGI_2 could thus be estimated roughly as 2.5%, 8% or 15%, respectively. Our previous estimations of a 0.03% conversion of arachidonic acid to PGE_2, based on the dose-response curves for arachidonic acid and PGE_2 on cAMP accumulation in the myometrium (7) seemed to preclude PGE_2 as the major component involved in the arachidonic acid contractile activity.

Effects of PGI_2 and the stable endoperoxide analogues on cAMP accumulation in the rat myometrium and endometrium. PGE_2 stimulates cAMP accumulation in both the myometrium and endometrium, while $PGF_{2\alpha}$ is without effect (4). Hence PGE_2 was initially suspected to be one possible compound involved in the arachidonic acid-induced rise in cAMP observed in both uterine tissues (7). However, PGI_2 also is a potent activator of the adenylate cyclase system (27–29) and in some clonal cell lines in culture, the stable endoperoxide analogues may as well contribute to the elevation of intracellular cAMP levels (29). Results of Table 1 show that compound U_{44069} has a slight stimulatory effect on cAMP accumulation in myometrium as well as endometrium and that this effect appeared dose-related. Compound U_{46619} was inactive in both tissues. It is clear that even though U_{44069} seemed more active than the cyclooxygenase substrate, arachidonic acid, it was at least 100 times less active than PGE_2 (Fig. 3). The data of Figure 3 also demonstrate that PGI_2 caused a marked increase in the cAMP content of both the myometrium and endometrium incubated in the presence of MIX. PGI_2 stimulations were dose-dependent with a maximal effect being obtained at 1 μM and half-maximal activation at 0.4 μM. Similar concentration dependent curves were obtained during 3 min incubation in the absence of MIX (not shown), although under these conditions, PGI_2-induced cAMP elevations were less pronounced (Fig. 4). Both uterine tissues appeared more sensitive to PGI_2 than to PGE_2 with regard to cAMP accumulation. Maximal responses were however similar in magnitude with both PGI_2 and PGE_2. 6-Keto-$PGF_{1\alpha}$ did not significantly affect total cAMP levels even at concentrations as high as 100 μM. When the time course of PGI_2 effect on cAMP was analyzed (Fig. 4), it was noted that in the absence of the phosphodiesterase inhibitor, the response to 1.0 μM PGI_2 was very rapid in onset with a peak at 90 sec. The cyclic nucleotide content then declined with time and at 10 min remained slightly higher than the control value. In the presence of MIX, there was a potentiation of the PGI_2 response which was already detectable at 30 sec, with a maximum reached at 4 min; but even in this case, and particularly with submaximal concentrations of PGI_2, the plateau could not be maintained for more than 10 min. When the same kinetic experiments were performed in the presence of MIX and PGI_2 at a supramaximal concentration (10 μM), no decline in cAMP could be noted for incubation periods as long as 25 min. Similar observations were made with both myometrium and endometrium. The decline in cAMP observed with PGI_2 could easily be explained by the great lability (1/2 life of 5–7 min at pH 7.4) of PGI_2 and its conversion during the incubation to the inactive 6-keto-$PGF_{1\alpha}$. Consistent with this interpretation is the fact that when myometrial strips were incubated with 1.5 μM PGI_2

TABLE 1

Effect of various concentrations of the stable endoperoxide analogues on total cAMP level in rat myometrium and endometrium

Myometrial or endometrial strips (50 mg) were incubated in 1.5 ml of Krebs-Ringer bicarbonate buffer for 25 min at 37°. Then MIX (276 μM) was added as well as the indicated concentrations of U44069 and U46619. After 10 min of further incubation, tissue was extracted for cAMP determination as described under EXPERIMENTAL PROCEDURES. Values represent the mean ± S.E. of 6 different experiments.

Addition	cAMP	
	Myometrium	Endometrium
	pmol/mg protein	
None	11.32 ± 1.0	10.8 ± 0.8
U44069		
8.8 μM	16.4 ± 2.0	16.7 ± 1.5
26.4 μM	21.4 ± 1.9	21.1 ± 1.0
88.0 μM	25.9 ± 2.1	35.4 ± 1.4
U46619		
8.8 μM	10.0 ± 1.6	8.7 ± 0.7
26.4 μM	10.3 ± 1.0	8.7 ± 0.8
88.0 μM	11.3 ± 0.8	11.8 ± 1.0

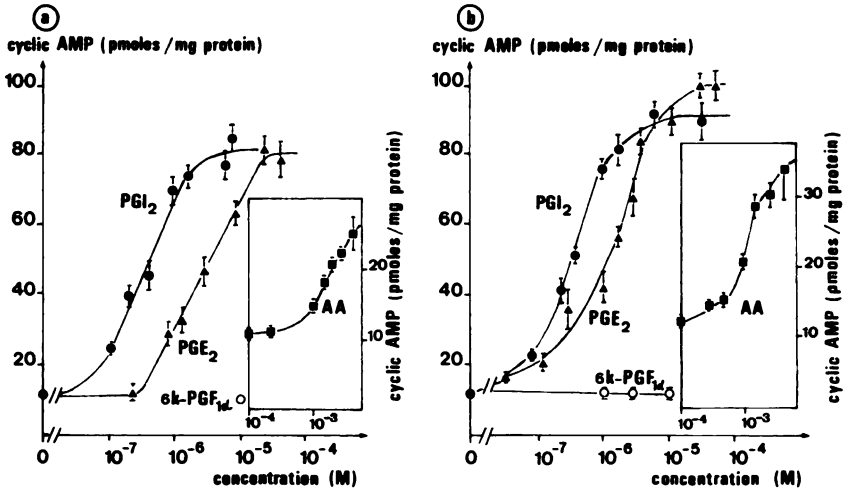

FIG. 3. *Comparative dose-response curves of PGI_2, PGE_2 and arachidonic acid on total cAMP level in rat myometrium and endometrium*

Myometrial (a) or endometrial (b) strips were incubated in 1.5 ml Krebs-Ringer bicarbonate buffer for 25 min. Then MIX (276 μM) was added as well as different concentrations of PGE_2, PGI_2, 6-keto-$PGF_{1\alpha}$ (6-k$PGF_{1\alpha}$) or arachidonic acid (AA). After 5 min of further incubation, tissue was extracted for cAMP determination as described under EXPERIMENTAL PROCEDURES. Values represent the mean ± S.E. of 6 different experiments.

for 20 min and the incubation medium was subsequently tested on a second batch of uterine strips, the rise in cAMP in the fresh tissue preparation averaged 16.0 pmol/mg protein, a value usually obtained with 0.14 μM authentic PGI_2.

By analyzing the dose-response curves for PGI_2 and arachidonic acid both at the level of contractility (Fig. 2) and cAMP accumulation (Fig. 3), it clearly appears that PGI_2 seems to be more potent in activating the adenylate cyclase system than in inducing uterine contractions. This was not the case for arachidonic acid, whose stimulatory activity on uterine contractions could be demonstrated within a concentration range (10–50) μM) that did not detectably alter the tissue cAMP content. These findings would tend to suggest that the causative factor in the arachidonic acid-induced contractions could not be ascribed to PGI_2. However in view of the marked sensitivity of the myometrial adenylate cyclase to PGI_2, it was tempting to speculate that PGI_2 might be the endogenous prostaglandin material which mediates the arachidonic acid stimulatory effect on cAMP accumulations.

Effects of inhibitors of prostacyclin synthesis. In order to evaluate whether the stimulation of the adenylate cyclase by arachidonic acid was due to conversion of the endoperoxides generated *in situ* to PGI_2, experiments were performed with both myometrium and endometrium, using two inhibitors of PGI_2 synthesis, *viz.* tranylcypromine and 15-hydroperoxy arachidonic acid (30). Results of Figure 5 show that when the tissues were incubated for 3 min with 10 μM HP-AA, the rise in cAMP induced by the subsequent addition of arachidonic acid was markedly reduced and averaged 25–30% of the response observed in the absence of inhibitor. At the same concentration, 15-hydroxy arachidonic acid exhibited a lower inhibitory activity. Tranylcypromine (50 μg/ml) also counteracted the rise in cAMP normally caused by arachidonic acid in both myometrium and endometrium. Under the same conditions, tranylcypromine and HP-AA did not detectably alter the response to exogenous PGE_2 or PGI_2. Gryglewski *et al.* (30) have pointed to the fact that tranylcypromine at concentrations up to 0.5 mg/ml and HP-AA acid up to 30 μM exhibited a rather selective inhibition of PGI_2 generation but that at higher concentrations both could block the syn-

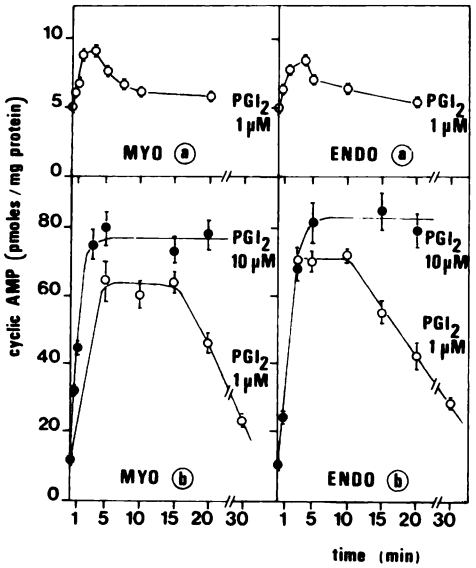

FIG. 4. *Time course of the effect of PGI_2 on cAMP accumulation in rat myometrium and endometrium*

Myometrial (Myo) or endometrial (Endo) strips were incubated in 1.5 ml Krebs-Ringer bicarbonate buffer for 25 min at 37°. Incubations were then continued in the absence (a) or the presence (b) of 276 μM MIX, with the addition of 1 μM or 10 μM PGI_2. Reactions were stopped at the indicated times and cAMP assayed as described under EXPERIMENTAL PROCEDURES. Values represent the mean ± S.E. of 5 different experiments.

thesis of all cyclooxygenase products. We have also noted nonspecific effects of tranylcypromine, which at concentrations above 100 μg/ml inhibited by 50% the rise in cAMP evoked by exogenous PGI_2 in the intact myometrium. Nevertheless, the specific inhibition obtained at the lower concentrations of both tranylcypromine and HP-AA (Fig. 5) can reasonably be interpreted as an indirect evidence that arachidonic acid exerts its effect on cAMP accumulation through its local conversion to PGI_2. Interestingly, the contractile activity exerted by 15 μM arachidonic acid, which could be completely abolished by prior treatment of the myometrium with indomethacin (7), was not appreciably affected by the presence of tranylcypromine (results not shown). This observation favors our interpretation (see above) that arachidonic acid stimulates uterine contractions through its conversion to a prostaglandin material that presumably is not PGI_2. It also supports the fact that at 50 μg/ml, tranylcypromine does not affect the cyclooxygenase reaction in the intact myometrium.

Inhibition of platelet aggregation by PGI_2 generated in the intact myometrium and endometrium. Quantitative evaluation and correlations with uterine cAMP content. The ability of PGI_2 to prevent platelet aggregation constitutes the basis of a biological assay currently used for the

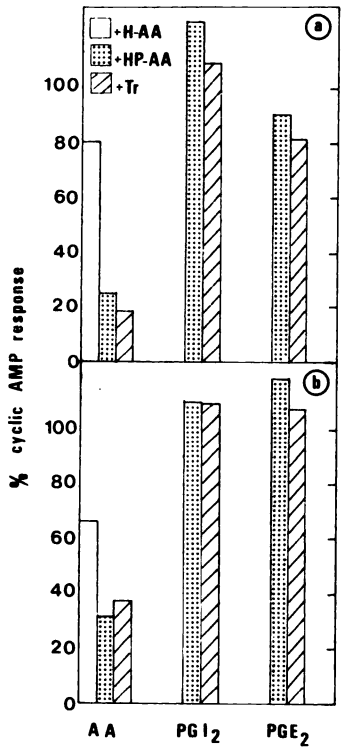

FIG. 5. *Effect of PGI_2 synthesis inhibitors on the arachidonic acid-induced cAMP accumulation in rat myometrium and endometrium*

After 25 min incubation at 37°, myometrial (a) or endometrial (b) strips were incubated in the absence or presence of 15-hydroperoxy arachidonic acid (HP-AA) or 15-hydroxy arachidonic acid (H-AA) both at 4 μg/ml and tranylcypromine (tr) at 50 μg/ml for 3 min before the subsequent addition of 276 μM MIX. Incubation was continued for 5 min with either 3 mM arachidonic acid (AA), 1 μM PGI_2 or 10 μM PGE_2. cAMP was estimated as described under EXPERIMENTAL PROCEDURES. Results are expressed as percentage of the normally evoked response for each specific agent in the absence of inhibitors.

identification of this relatively unstable prostaglandin derivative. A similar approach was also adopted in order to characterize in more detail the production of PGI_2 and to assess to what degree the locally generated PGI_2 could participate in the arachidonic acid stimulatory effects on uterine tissue cAMP levels. To test for the production of PGI_2, either myometrial or endometrial strips, after a short incubation of 3 min at 37° in the absence or presence of 3 mM arachidonic acid, were immediately transferred into 0.5 ml Tris-saline buffer pH 8.0 for 2 min at 37°. Following a rapid centrifugation at 0°, subsamples of the corresponding supernatants were assayed for their ability to affect platelet aggregation induced by a subsequent addition of arachidonic acid. Typical results of such experiments are depicted in Figure 6. It is evident that myometrial preparations treated with arachidonic acid released in the supernatant fluid appreciable amounts of antiaggregatory activity. The potency of the inhibitory activity was dose-dependent. Almost complete inhibition of the arachidonic acid-induced platelet aggregation could be obtained with 0.2 ml of the supernatant fluid (equivalent to 13 pmoles authentic PGI_2 (a)). The anti-aggregatory activity was markedly reduced in the supernatant fluids obtained from myometrial samples which have been preincubated with 15 HP-AA before arachidonic acid addition. Tissue control, untreated with arachidonic acid, also released easily detectable antiaggregatory activity, although in smaller amounts as compared to the arachidonic acid-stimulated myometrium. The inhibitory activity of the control samples was also reduced by prior incubation of the tissue with HP-AA. Similar inhibitory patterns of platelet aggregation were obtained with arachidonic acid-treated and untreated endometrial preparations (tracings not shown).

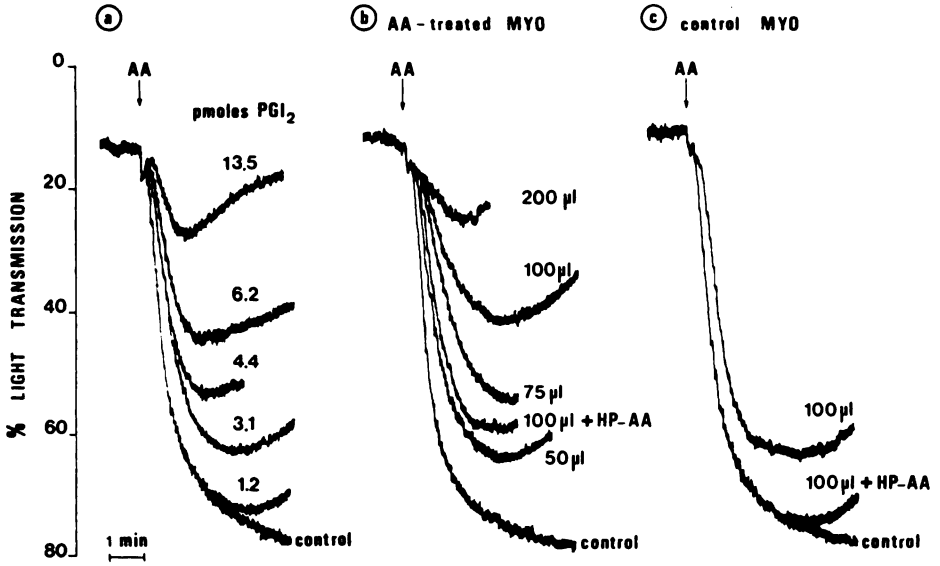

FIG. 6. *Inhibition of platelet aggregation by PGI_2 generated in the myometrium*

Test samples of PRP (0.3 ml) were diluted with 0.9% NaCl to a final volume of 0.5 ml. Aggregation induced by 130 µM arachidonic acid (AA) was monitored at 37° in a Chrono-log aggregometer by following changes in light transmission. To test for the production of the antiaggregatory activity, myometrial strips were conditioned for 25 min in Krebs-Ringer bicarbonate buffer and further incubated in the absence (c) or presence of 3 mM arachidonic acid (b) at 37° for 3 min. In experiments with 15-hydroperoxy arachidonic acid (HP-AA), the inhibitor was added during the last 5 min of the preincubated period. At the end of various treatments, myometrial strips were immediately transferred in 0.5 ml Tris saline buffer pH 8 for 2 min at 37°. Following a rapid centrifugation (3000 × *g*, 5 min, 0°), subsamples of the corresponding supernatant were added to PRP 1 min before challenging with the aggregating agent (130 µM AA). The generated anti-aggregatory activity was compared to that of an authentic solution of PGI_2(a).

Generation of the antiaggregatory activity was further impaired by prior treatment of the myometrium and endometrium with indomethacin or tranylcypromine (Table 2). These observations strongly suggest that the appearance of an antiaggregatory activity in the supernatant fluid of uterine tissue preparations was the result of local synthesis and release of PGI_2. This interpretation was reinforced by the findings that the properties exhibited by the inhibitory substance were also suggestive of its being PGI_2. Indeed, when the supernatant fluid, adjusted to pH 7.4, was either incubated at 37° for 12 min, boiled for 1 min or acidified at pH 3.5 with 0.1 N HCl, its antiaggregatory potency was abolished or markedly reduced in parallel with the decline of the antiaggregatory potency of a solution of authentic PGI_2 under the same conditions (30, 31).

Table 2 summarizes the results of a series of experiments in which myometrial and endometrial preparations were separately submitted to various incubation conditions, in the absence or presence of 3 mM arachidonic acid. The resulting inhibition of aggregation induced by the corresponding tissue supernatant fluids was quantitated against the antiaggregatory effect of known concentrations of PGI_2. In each particular case, variations in the amount of PGI_2 were analyzed in parallel with the intracellular concentrations of cAMP. It clearly appeared that both myometrium and endometrium were able to generate, from endogenous precursors, almost equivalent amounts of PGI_2-like material and that this production was stimulated (2-fold) by the addition of 3 mM arachidonic acid. The release of PGI_2 in the latter stimulatory conditions averaged 130 pmoles/g wet tissue. The values of PGI_2 obtained for the control untreated tissue (about 60 pmol/g wet tissue) may represent basal line levels of PGI_2, which were continuously synthetized together with some additional PGI_2-material which might be artefactually released during the centrifugation step due to

TABLE 2

Correlation of total cAMP level with the amount of PGI_2 released by myometrium and endometrium

Results are expressed as pmol cAMP/mg protein and pmol PGI_2/g wet tissue. Values represent the mean ± S.E. of 4–6 different experiments

Incubation Conditions[a]	− arachidonic acid		+ 3 mM arachidonic acid	
	PGI_2 pmol/g tissue	cAMP pmol/mg protein	PGI_2 pmol/g tissue	cAMP pmol/mg protein
Myometrium				
no inhibitor	64.5 ± 3.5	9.3 ± 0.8	122.0 ± 7.6	23.6 ± 1.4
+ indomethacin 15 μM	40.0 ± 3.0	7.5 ± 0.6	62.4 ± 5.3	11.8 ± 0.7
+ HP-AA 4 μg/ml	35.0 ± 4.2	8.8 ± 0.7	67.9 ± 6.2	13.0 ± 1.5
+ tranylcypromine 50 μg/ml		8.5 ± 0.4	66.0 ± 5.7	13.3 ± 1.1
Endometrium				
no inhibitor	72.0 ± 5.9	10.3 ± 1.0	132.0 ± 9.9	31.1 ± 1.5
+ indomethacin 15 μM		9.0 ± 0.8	69.5 ± 8.4	9.6 ± 0.7
+ HP-AA 4 μg/ml	40.0 ± 3.8	8.7 ± 0.7	82.1 ± 6.6	17.1 ± 2.3
+ tranylcypromine 50 μg/ml		7.7 ± 0.3		18.6 ± 1.5
Without incubation[b]				
Myometrium	200.0 ± 22.5	43.8 ± 5.0		
Endometrium	205.0 ± 16.3	44.0 ± 5.8		

[a] After 25 min preincubation at 37°, myometrial or endometrial strips were incubated in the absence or presence of 3 mM arachidonic acid for 3 min. Potential inhibitors, when used, were added as described in legends to fig. 5 and 6. The incubated tissues were then either extracted with trichloroacetic acid for total cAMP estimation or immediately transferred in 0.5 ml Tris-saline buffer, pH 8.0, 2 min at 37° for determination of antiaggregatory activity in the corresponding incubation fluids treated as described under EXPERIMENTAL PROCEDURES. The resulting inhibition of aggregation was quantitated against the antiaggregatory activity of known concentrations of PGI_2.

[b] cAMP and PGI_2 contents were estimated in myometrial and endometrial strips immediately after tissue preparation, without further incubation.

slight mechanical damage of the tissue (32, 33). Moreover, the findings reported in the table seem to establish a causal relationship between the ability of arachidonic acid to induce an increased formation of PGI_2 and its stimulatory effect on the tissue cAMP content. The generation of PGI_2, both in myometrium and endometrium, either form endogenous or exogenous substrate, as well as the rise in cAMP induced by exogenous arachidonic acid, were markedly and similarly reduced by a pretreatment with indomethacin, HP-AA or tranylcypromine. The results can be taken as evidence that both the cyclooxygenase and PGI_2 synthetase pathways are involved in the generation from arachidonic acid of both antiaggregatory and adenylate cyclase stimulatory activities.

It is pertinent to point out that with lower concentrations of arachidonic acid (50 μM), which already induced maximal contractions (Fig. 2), there was little or no additional release of PGI_2 as compared to the control (72 pmoles PGI_2/g wet tissue and 80 pmol g wet tissue in the case of myometrium and endometrium, respectively). At the same time, basal levels of cAMP were not detectably affected in both uterine tissues (Fig. 3). Furthermore, the data reported in Table 2 provide suggestive evidence for a quantitative correlation between the production of PGI_2 and the effect observed at the level of cAMP formation. Assuming the amount of locally generated PGI_2 to reflect an intracellular concentration of PGI_2 at a specific time of incubation, tissue concentrations of PGI_2 expressed as molar concentrations, are then found to average 0.07 μM for the control and to increase to 0.122 μM and 0.132 μM for the arachidonic acid-treated myometrium and endometrium, respectively. On the other hand, from the dose-response curves of PGI_2 (Fig. 3) it could roughly be estimated that the rise in cAMP induced by 3 mM arachidonic acid was comparable to that caused by 0.1 μM authentic PGI_2. Hence, challenging the myometrium or endometrium with 3 mM arachidonic acid provided a concentration range of PGI_2 which appears fairly compatible with the accompanied 2-fold increment in uterine tissue cAMP content. In another series of experiments, the initial incubation medium was also tested for the presence of antiaggregatory activity that could have been released from the tissue preparations during the 3 min incubation with arachidonic acid. It was found that the amount of PGI_2-like activity in these incubation media (about 20 pmol PGI_2 in a total volume of 2.0 ml buffer in which was incubated 200 mg of either myometrial or endometrial strips) was almost equivalent to that of PGI_2 present in the tissue, which was subsequently released during a 2 min further incubation in a fresh NaCl-Tris HCl buffer (Table 2). It is clear that due to a 10-fold dilution, PGI_2 concentrations in the incubation medium (0.01 μM) would be inadequate to account for the observed elevations in tissue cAMP contents. This would tend to imply that local PGI_2 presumably interacts with the membrane adenylate cyclase at or near its site of synthesis rather than after its release from the tissue in the medium.

A clear relationship between the concentration of locally synthetized PGI_2 and the corresponding levels of cAMP was also evidenced by additional data from experiments conducted with myometrial and endometrial strips immediately after tissue preparation omitting the 25 min preincubation period. The preincubation step has routinely been introduced in order to allow tissue samples to equilibrate and to recover from the different manipulations during the isolation and stripping processes. As shown in Table 2, the nonpreincubated preparations released an increased amount of PGI_2-like activity as compared to the corresponding preincubated tissue, either untreated or treated with 3 mM arachidonic. A plausible explanation for this elevated PGI_2-like activity would be that of an increased substrate availability due to phospholipase activation and liberation of arachidonic acid from the membrane phospholipids. It has frequently been observed that the rate of formation of PGI_2 or other prostaglandins might be accelerated by some trauma to the cells (scraping, stretching...) (32, 33). Our results showing that basal generation of PGI_2 declined, after the preincubation step, support this interpretation. Obviously,

the major interest was that with these non preincubated preparations, the increased production of PGI_2-like activity was accompanied by a concomitant elevation in intracellular cAMP content. In these myometrial and endometrial preparations the four fold increment in the cAMP level which could be attributable to 0.2 μM endogenously synthesized PGI_2 was comparable to that produced by the addition of a similar concentration of authentic PGI_2. The latter observations added to those obtained with the arachidonic acid-treated preparations are consistent with the idea that endogenous PGI_2 synthesis is a major contributing factor to uterine tissue cAMP content.

Synthesis of 6-keto-$PGF_{1\alpha}$ by rat myometrial and endometrial homogenates. Following incubation of both myometrial and endometrial homogenates with [1-^{14}C]arachidonic acid, the synthesis of 6-keto-$PGF_{1\alpha}$, the stable end-product of PGI_2, and other possible prostaglandin derivatives was investigated by TLC of lipid extracts derived from the incubation mixture. The ability of the uterine tissues to metabolize arachidonic acid was compared with that of the model system of ram seminal vesicle (RSV) microsomes, whose main prostaglandin products, when incubated in the absence of exogenous cofactors, have recently been identified as PGE_2 and 6-keto-$PGF_{1\alpha}$ (34–36). Figure 7 shows the characteristic chromatographic pattern (solvent A) of the labeled compounds obtained with each tissue preparation. The different areas of radioactivity developed by the autoradiographic procedure were further cut from the chromatographic plates and radioactivity associated with each prostaglandin compound was then determined. In the case of RSV microsomes, a 3 min incubation at 37° with 20 μM [^{14}C]arachidonic acid retained about 50% of unconverted substrate which migrated as a radioactive peak close to the solvent. Other radioactive peaks which migrated close to the solvent front were probably monohydroxy fatty acids. The remainder [^{14}C]arachidonic acid has been converted to more polar compounds showing two major peaks of radioactivity which corresponded to the location of standard 6-keto-$PGF_{1\alpha}$ (Rf 0.27) and PGE_2 (Rf 0.6),

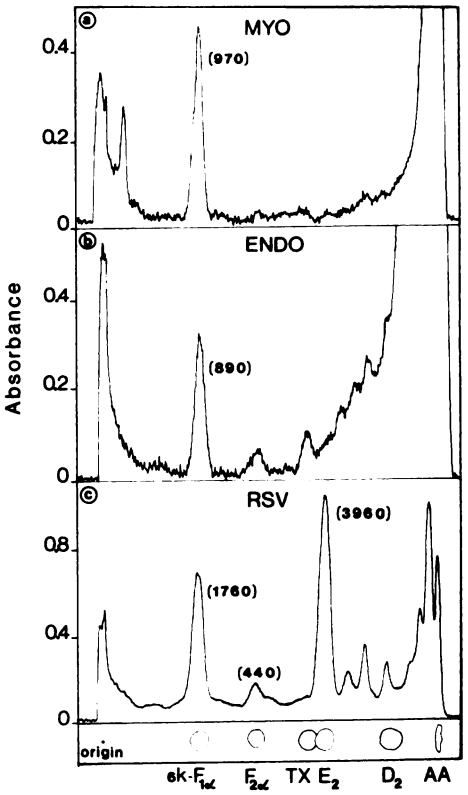

FIG. 7. *Difference scan of autoradiogram of the lipid extracts obtained from myometrial and endometrial homogenates and RSV microsomes*

Tissue preparation, incubations with [^{14}C]arachidonic acid and lipid extractions were as described under EXPERIMENTAL PROCEDURES. Aliquots of the lipid extracts in chloroform/methanol (2:1) were submitted to TLC on silica gel plates with double development in system A: ethyl acetate/acetic acid/isooctane/H_2O (110:20:50:100). Positions of standard prostaglandins and arachidonic acid, visualized with iodine vapor are noted. Radioactive zones were located using Kodak X-ray films and intensity of the different areas on the developed films was monitored with a densitometer. Figures in parentheses represent the radioactivity associated with the prostaglandin compounds as determined by liquid scintillation counting of the corresponding areas on the chromatographic plates. AA = arachidonic acid, 6 k-$F_{1\alpha}$ = 6-keto-$PGF_{1\alpha}$, $F_{2\alpha}$ = $PGF_{2\alpha}$, E_2 = PGE_2, D_2 = PGD_2, TX = thromboxane B_2.

together with trace amounts of $PGF_{2\alpha}$ (Rf 0.45). Analysis of the myometrial and endometrial homogenates treated under identical conditions, yielded a chromatographic pattern with most of the radioactivity on

the TLC plates being due to unconverted arachidonic acid (85–90%). Furthermore in both uterine tissues, the predominant peak of radioactivity comigrated with standard 6-keto-$PGF_{1\alpha}$ without demonstrable PGE_2. In some cases, trace amounts of $PGF_{2\alpha}$ have been tentatively identified on the autoradiochromatogram of myometrial or endometrial homogenates. Comparable observations were made when the chromatographic plates of the tissue extracts were developed in ethyl acetate/acetic acid (99:1, v/v) System (solvent B): a predominant radioactive peak with a mobility similar to that of 6-keto-$PGF_{1\alpha}$ was detected in the case of myometrial and endometrial homogenates, while two major peaks corresponding to PGE_2 (Rf 0.78) 6-keto-$PGF_{1\alpha}$ (0.42) and a minor component in the $PGF_{2\alpha}$ zone (Rf 0.56) could be revealed for RSV microsomes. The radioactivity ratio 6-keto-$PGF_{1\alpha}$/PGE_2 in the latter system was fairly similar to that obtained with solvent A (data not shown). In order to appreciate any possible generation of PGD_2 in the uterine tissue homogenates, TLC was conducted in a third solvent (chloroform/methanol/acetic acid, 180:10:10, v/v) which provides a suitable resolution of PGD_2 (Rf = 0.52) from the bulk of unconverted arachidonic acid moving well up the plate. In contrast, PGE_2 has a motility similar to that of 6-keto-$PGF_{1\alpha}$ (Rf 0.41). Analysis of such radiochromatograms (not illustrated) derived from myometrial and endometrial homogenates could in no case reveal the presence of any radioactive peak which corresponded to PGD_2. Moreover the amount of radioactivity in the predominating product located in the chromatographic area, where PGE_2 and 6-keto-$PGF_{1\alpha}$ co-migrated, was similar to the radioactivity detected in the 6-keto-$PGF_{1\alpha}$ area when the chromatographic plates were developed in either solvent A or B which adequately separated PGE_2 from 6-keto-$PGF_{1\alpha}$. These observations tend to suggest that, under the described incubation conditions, both myometrial and endometrial homogenates did not detectably synthetize any PGE_2 or PGD_2 material but that they were able to generate from [^{14}C]arachidonic acid a major metabolite with a chromatographic mobility, in different solvent systems, identical to that of 6-keto-$PGF_{1\alpha}$.

From the knowledge of the specific activity of [^{14}C]arachidonic acid at the onset of the incubation and taking into account recovery of total radioactivity (see METHODS), the amount of each radioactive prostaglandin compound generated by the uterine and seminal gland preparations could further be evaluated (Fig. 8). It is clear that on the basis of tissue weight, the uterine homogenates (both myometrium and endometrium) generated several times less prostaglandin material than did the RSV microsomes. Under the 3 min incubation conditions, 6-keto-$PGF_{1\alpha}$, the predominating prostaglandin-material generated by the myometrial and the endometrial extract, averaged 0.5 nmol/g wet tissue while the vesicular gland preparation yielded 7 times more 6-keto-$PGF_{1\alpha}$ (3.8 nmoles) in addition to 7 nmoles of PGE_2 whose synthesis was almost undetectable in the uterine preparations. The reported data also illustrate the expected marked reduction in the formation of all prostaglandin products (75–80% inhibition) when the tissue preparations were preincubated with 15 μM indomethacin prior to the addition of [^{14}C]arachidonic acid. In contrast, a pretreatment with HP-AA resulted in an appreciable inhibition (75–80%) for the ability of the different tissue preparations to generate 6-keto-$PGF_{1\alpha}$, while under the same conditions, there was no more than 25–30% decrease in the formation of PGE_2 or $PGF_{2\alpha}$ by the RSV microsomes. The latter findings provide additional support for our interpretation, based on chromatographic migrations, that the major labeled prostaglandin material, detected in both myometrial and endometrial homogenates, is very much likely to be 6-keto-$PGF_{1\alpha}$.

In further experiments, it was attempted to detect the formation of the labile biologically active PGI_2, rather than its breakdown-product, in the incubates of the different tissue extracts. For such purpose, following a 3 min incubation with 20 μM arachidonic acid, the uterine homogenates and RSV microsomes were rapidly chilled and centrifuged. Aliquots of the corresponding supernatants were directly tested

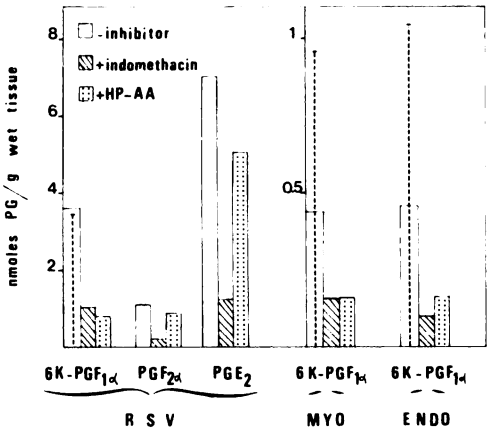

FIG. 8. *Quantitative evaluation of [^{14}C] 6-keto-PGF$_{1\alpha}$ formation by myometrial and endometrial homogenates and RSV microsomes. Effect of indomethacin and 15-hydroperoxy arachidonic acid*

Tissue preparation, incubations with [^{14}C]arachidonic acid, lipid extractions were as described under EXPERIMENTAL PROCEDURES. When indicated, indomethacin (15 μM), HP-AA (4 μg/ml) were added 10 min and 3 min prior to the addition of [^{14}C]arachidonic acid. Chromatography of the lipid extracts was proceeded as in the legend to figure 7. The radioactivity associated with the prostaglandin compounds was determined by liquid scintillation counting of the corresponding areas on the chromatographic plates. The amount of [^{14}C]PGE$_2$, PGF$_{2\alpha}$, 6-keto-PGF$_{1\alpha}$ (6-PGF$_{1\alpha}$) formed during the various incubations was estimated on the basis of the initial [^{14}C]arachidonic acid specific activity (40.000 cpm/nmole) and after taking into account the recovery of total radioactivity. Results are expressed as nmol PG/g wet tissue. In alternate experiments, following incubation, the different extracts were rapidly chilled, centrifuged and the amount of PGI$_2$ present in the corresponding supernatants was assayed for antiaggregatory activity as described in figure 6 (dotted lines). Results are expressed as nmol PGI$_2$/g wet tissue. For the myometrium and endometrium, the figures depict the amount of PGI$_2$ generated in the homogenates incubated in the presence of 20 μM arachidonic acid after subtraction of the basal values of PGI$_2$ formed in the same extract in the absence of arachidonic acid (about 0.5 nmol/g wet tissue). Values are the mean of three experiments and agreed within 7%.

for their ability to inhibit platelet aggregation. The assays were usually conducted within 7–10 min following the arrest of incubations. At that time, no appreciable breakdown of PGI$_2$ (pH 8.0, 4°) should be expected to occur (30, 31). Results of these experiments demonstrated (Fig. 8) that the amount of antiaggregatory activity with a lability identical to that of PGI$_2$ generated by the vesicular gland microsomes was in good agreement with the values determined for 6-keto-PGF$_{1\alpha}$ through the radiolabelling experiments. This was not the case for uterine tissue homogenates. In fact, it was found that both myometrial and endometrial homogenates, when incubated in the absence of exogenous arachidonic acid, already produced an appreciable amount of PGI$_2$-like anti-aggregatory activity (0.5 nmol/g wet tissue) which could further be increased to 1.5 nmol/g wet tissue by the addition of 20 μM arachidonic acid. It clearly appeared that the amount of PGI$_2$-like activity generated by the myometrial or endometrial homogenates, and attributable to exogenous arachidonic acid, was twice higher than the amount determined for [^{14}C]6-keto-PGF$_{1\alpha}$. The latter apparent discrepancy may be accounted for by the presence, in the tissue homogenates, of a pool of endogenous free arachidonic acid which would contribute to the dilution of the labeled arachidonic acid resulting in an underestimation of [^{14}C]6-keto-PGF$_{1\alpha}$ biosynthesis.

DISCUSSION

Our previous experiments (7) have shown that arachidonic acid can elicit contractions of the estrogen-treated rat uterus and induce a significant increase in intracellular cAMP in myometrium as well as endometrium. These effects were the result of fatty acid conversion to prostaglandin-like material. It was also known (4–7) that PGE$_2$ and PGF$_{2\alpha}$ induced contractions while PGE$_2$ alone was able to increase intracellular cAMP levels in both uterine tissues. The results reported in the present work demonstrate that another prostaglandin material, prostacyclin, but not its stable metabolite 6-keto-PGF$_{1\alpha}$, is a potent activator of the adenylate cyclase system of the myometrium and endometrium, which appeared rather more sensitive to PGI$_2$ than to PGE$_2$. Thus at the early stage of this study both PGI$_2$ and PGE$_2$ were considered possible candidates for the arachidonic acid stimulatory effects on cAMP levels. Our observations that the rises in cAMP induced by arachidonic acid were markedly

decreased when the tissues were treated with tranylcypromine or HP-AA, the well known inhibitors of PGI_2 synthetase, were indicative of a potential role for endogenous PGI_2 in mediating the cAMP effect of arachidonic acid. This interpretation was further confirmed by the findings that the arachidonic acid treatments which cause elevations of cAMP levels in the myometrium and endometrium also stimulate the concomitant synthesis and release of an active principle that inhibits platelet aggregation. The basal release of this antiaggregatory material, which consistently occurred with the untreated tissue preparation, could not be further increased with lower concentrations of arachidonic acid that were ineffective at the cAMP level. Several pieces of evidence suggested that the antiaggregatory activity generated by both uterine tissues was prostacyclin and not a nonspecific factor as reported for some tissues (37). First, the substance exhibited a lability identical to that of authentic PGI_2. Secondly, the generation of the antiaggregatory activity was abolished by prior treatment of the myometrium or endometrium with indomethacin, tranylcypromine or HP-AA. Thirdly, homogenates of freshly prepared myometrium and endometrium, when incubated with [^{14}C]-arachidonic acid, yielded a major radioactive product identified by thin layer chromatography in multiple solvent systems as 6-keto-$PGF_{1\alpha}$, the stable metabolite end-product of PGI_2. Although some minor prostaglandin peaks could be observed, no PGE_2 or PGD_2 was detected. The latter observation eliminates any possible contribution of PGD_2 to the noted antiaggregatory activity (27). It gives also further support to the idea that PGI_2 and not PGE_2 is the endogenous prostaglandin-material generated in the myometrium and endometrium and responsible for the arachidonic acid effect on the uterine tissue cAMP content.

Furthermore, our data demonstrate that the arachidonic acid-induced elevations in cAMP are generally proportional to the increments noted in the generation of prostacyclin. It was found that under basal conditions myometrium and endometrium released an almost identical amount of PGI_2 (about 60 pmol/g wet tissue), which was increased to a value of 122 ± 7.6 pmol and 132 ± 9.0 pmol PGI_2/g wet tissue when the myometrium and endometrium, respectively, were stimulated with 3 mM arachidonic acid. Based on the dose-response curve for the stimulatory effect of exogenous PGI_2 on uterine tissue cAMP, it clearly appeared that the amounts of PGI_2, expressed as pmol/ml, in the incubate fluids were substantially lower than those required to account for the arachidonic acid response. It was however tempting to assume that after addition of arachidonic acid, there may be an initial rise in the cellular content of PGI_2 before its release in the medium. Then such local PGI_2 levels, expressed as tissue molar concentrations, would be sufficient to account for the concomitant increases in the cAMP content recorded with the arachidonic acid-treated preparations. A quantitative causal relationship, PGI_2 level versus cAMP content, could also be provided under conditions of increased local PGI_2 synthesis from the endogenous precursor, e.g., during tissue isolation and stripping of the myometrium. These observations would tend to imply—although they do not prove—that endogenous PGI_2 presumably interacts with the adenylate cyclase via its putative receptor, at or near its site of synthesis, rather than after its release in the medium. A similar interpretation was also proposed by Burstein *et al.* (38) as a plausible explanation for the increments in cAMP content induced by arachidonic acid in primary epithelial cell cultures of a mouse mammary tumor, as compared to the relatively low amount of PGE_2 detected in the incubation fluid. PGE_2 was shown to raise cAMP levels in that particular cell line, but any possible effect of PGI_2 was not investigated. Several reports have recently revealed that PGI_2 is a potent activator of the adenylate cyclase system in a variety of tissues (27, 28). To our knowledge, only one earlier study with $3T_3$ fibroblasts (39) has provided an indirect evidence that locally synthesized PGI_2 may play an active role as a modulator of intracellular cAMP content, similar to that which has been conclusively demonstrated

herein for both myometrium and endometrium.

Consistent with the observations of Omini *et al.* (3), the present data indicate that PGI_2 is also able to induce contractions of the estrogen-treated rat uterus. However, it is less potent than $PGF_{2\alpha}$ or PGE_2. In contrast to the stimulatory effect on cAMP accumulation, which was restricted to PGI_2, contractions were also elicited by 6-keto-$PGF_{1\alpha}$ which was only 1/10 as active as its labile precursor PGI_2. Furthermore, PGI_2 appeared more potent in activating the adenylate cyclase system than in inducing uterine contractions. Thus during 3 min incubation with 0.5 μM PGI_2, 50% of the cAMP response was obtained with almost undetectable effect on contraction. One example of a biphasic response to PGI_2, *viz.*, relaxation at low doses that changed with high doses to a contractile response, was recently provided with the human umbilical artery (40). Such was not the case for the myometrium, since PGI_2, at low concentrations (0.1–0.5 μM), did not produce any relaxation, nor did it antagonize the contractions elicited by $PGF_{2\alpha}$ or oxytocin (data not shown). This confirms our previous findings obtained with PGE_1 and PGE_2, which clearly indicated that increases in intracellular cAMP content are not necessarily accompanied by relaxation of the myometrium (4, 5). Still more interesting were the experimental data indicating that PGI_2 is unlikely to be the local prostaglandin-material implicated in the arachidonic acid-evoked contractions. This interpretation was first derived from the dose-response curve of arachidonic acid which, in contrast to PGI_2, was much more effective in inducing contractions than in stimulating cAMP accumulation. It was finally substantiated by the failure of tranylcypromine to prevent the arachidonic acid-induced contractions which were nevertheless abolished in the presence of cyclooxygenase inhibitors (7). The nature of the prostaglandin-material generated in the myometrium and mediating the arachidonic acid contractile response remains to be elucidated. The differential potencies of arachidonic acid and PGE_2 in activating cAMP accumulation and contractions would not be in favor of PGE_2. $PGF_{2\alpha}$ might be considered as a possible candidate. Of all the tested prostaglandins, it appeared the more potent contractile effector. Moreover, trace amounts of $PGF_{2\alpha}$ have in some cases been identified on the autoradiochromatograms from myometrial homogenates. It is still possible to speculate that other cyclooxygenase products, including thromboxane A_2 (11, 41) might mediate the arachidonic acid contractile activity. Studies in progress are aimed at answering this question.

As documented in recent reports, 6-keto-$PGF_{1\alpha}$ is the major prostaglandin product of homogenates (15) and myometrial microsomes (42) of the pseudopregnant rat uterus. The present results indicate that it is also the predominant prostaglandin material synthetized by the homogenates of both the myometrium and endometrium of the nonpregnant estrogen-dominated rat uterus. Furthermore, our findings confirm that the appearance of 6-keto-$PGF_{1\alpha}$ did actually reflect the generation of its active precursor PGI_2 whose antiaggregatory activity could easily be demonstrated in such uterine preparations, omitting extraction and chromatographic steps. It also appeared that the capacity to generate PGI_2, whether measured in the intact tissue or its homogenate, was similar for both myometrium and endometrium. This is of particular interest since Williams *et al.* (14) recently reported that in the rat uterus, as pregnancy progressed, there was a marked enhancement of PGI_2 formation in myometrium as compared to the decidua and to the extrinsic blood vessels. Thus at the time of parturition, the myometrium produced 10 nmol PGI_2/g tissue (i.e., 10 times more than the decidua), while the nonpregnant, estrogen-treated myometrium, as shown presently, generated 0.2 nmol PGI_2/g tissue. Similarly, Downing *et al.* (42) observed that myometrial microsomes of the pregnant uterus produced mainly 6-keto-$PGF_{1\alpha}$ which, for the decidual preparation, did not account for more than 12% of the total prostaglandin material, the latter including mainly PGE_2 and $PGF_{2\alpha}$ together with trace amounts of thromboxane B_2. These data taken together indicate that the myometrium largely contributes to the local gen-

eration of PGI_2 in the uterus and that its synthesizing capacity strikingly increases during pregnancy, although it remains mainly restricted to this sole compound. Conversely, the endometrium, which appears to synthesize only PGI_2 in the estrogen-dominated organ evolves a capacity to generate a much larger spectrum of prostaglandin-like compounds during pregnancy. It still remains to establish the physiological significance of myometrial PGI_2 production. Although the exact role for PGI_2 in the uterus is presently not as yet known, it is conceivable, in view of the above described stimulatory effects on intracellular cAMP levels, that its function may be mediated or controlled by mechanisms that involve interaction with the cAMP system.

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