MOLECULAR PHARMACOLOGY, 14, 24-37

Modulation of Intracellular Adenosine Cyclic 3',5'-Monophosphate and Contractility of Rat Uterus by Prostaglandins and Polyunsaturated Fatty Acids

MARIE-FRANÇOISE VESIN, LIEN DO KHAC, AND SIMONE HARBON

Institut de Biochimie, Université Paris-Sud, 91405 Orsay Cedex, France

(Received April 29, 1977)

(Accepted July 19, 1977)

SUMMARY

VESIN, MARIE-FRANÇOISE, DO KHAC, LIEN & HARBON, SIMONE (1978) Modulation of intracellular adenosine cyclic 3',5'-monophosphate and contractility of rat uterus by prostaglandins and polyunsaturated fatty acids. *Mol. Pharmacol.*, 13, 24-37

Prostaglandins E_1 (PGE_1) and E_2 stimulated contractions of estrogen-treated rat uteri. They also caused the accumulation of adenosine cyclic 3',5'-monophosphate (cAMP) in the isolated myometrium and endometrium. Both stimulations were also induced by arachidonic ($C_{20:4}$) and homo-γ-linoleic ($C_{20:3}$) acids, which are direct precursors of prostaglandins. Other unsaturated fatty acids, including oleic, linoleic, γ-linolenic, and eicosadienoic, were without effect. The stimulations of contraction and adenylate cyclase activity by arachidonic and homo-γ-linolenic acids were very rapid, detectable at 30 sec, dose-dependent, and abolished by the specific cyclooxygenase inhibitors indomethacin, meclofenamic acid, and eicosatetraynoic acid. Hence the conversion of $C_{20:3}$ and $C_{20:4}$ fatty acids to prostaglandin or prostaglandin-like material occurred in both myometrium and endometrium and was responsible for the observed activations. The levels of cAMP can therefore be modulated by local prostaglandin effectors in a manner similar to that produced by exogenous PGE compounds. The stimulatory effects of arachidonic acid were followed by an unresponsive phase, as shown by the subsequent inhibited responses of the myometrium to contractile agents such as prostaglandins and carbamylcholine as well as by the suppression of adenylate cyclase activation by either epinephrine or PGE_2 in both endometrium and myometrium. The latter inhibition was not readily reversible. It did not involve the prostaglandin synthetase system, inasmuch as indomethacin failed to prevent the subsequent inhibitory effect of arachidonic acid. Furthermore, similar marked inhibition was exerted by linoleic, γ-linolenic, and eicosatetraynoic acids. These results suggest the possible alteration by fatty acids of a membrane architecture crucial for adenylate cyclase activation and signal transmission during hormone-induced contraction.

This work was supported by Grant ERA 33, LA 272 from the Centre National de la Recherche Scientifique, Grant 77.7.0250 from the D.G.R.S.T., a contribution from the Fondation pour la Recherche Médicale Française, and the participation of the Commission de l'Energie Atomique in the purchase of radioactive compounds.

INTRODUCTION

In an attempt to clarify the role of adenosine cyclic 3',5'-monophosphate as an intracellular mediator of the relaxation of uterine smooth muscle (1-7), particular attention has been given in our laboratory

0026-895X/78/0141-0024$02.00/0

to the interactions of epinephrine and prostaglandins with the adenylate cyclase–cAMP[1] system of rat myometrium (3, 7, 8). As documented in our previous studies with estrogen-treated rat myometrium, there seems to be a causal relationship between the relaxing effect of epinephrine and the ability of the *beta* adrenergic agonist to raise intracellular cAMP levels. On the other hand, PGE_1 and PGE_2, in spite of elevating cAMP levels, exert an effect opposite to that of epinephrine on rat uterus motility—contraction. In addition, it has been shown recently (9) that for a given concentration of intracellular cAMP elicited by either the relaxing agents epinephrine and theophylline or the contracting agent PGE_1, an identical degree of saturation of intracellular cAMP receptor proteins and equal activation of myometrial cAMP-dependent protein kinases[2] are obtained. Hence the hypothesis of cAMP compartmentalization (7) in order to discriminate between the effects of epinephrine and PGE_1 on myometrial activity was invalidated.

These observations rendered the role of cAMP on uterine relaxation more complex and raised a question concerning the significance of the dual, unrelated effects of PGE_1 and PGE_2 on contraction and adenylate cyclase activation. The foregoing prompted us to investigate the extent to which the same situation might prevail with endogenously synthesized prostaglandins, which are presumed to be the local physiological effectors. The aim of the present work is to demonstrate that arachidonic acid ($C_{20:4}$) and homo-γ-linolenic acid ($C_{20:3}$) which are precursors of prostaglandins of series 2 and 1, respectively, can evoke myometrial contractility and induce an increase in intracellular cAMP in both the myometrium and endometrium. These stimulations seem to result from the ability of both uterine tissues to convert the two fatty acids to prostaglandin or prostaglandin-like material. Hence it appears that in rat myometrium the endogenously synthesized prostaglandins are equally effective in inducing contractions and cAMP accumulation. Furthermore, an interesting problem became apparent during the course of this investigation. It concerns the delayed unresponsive phase elicited by added unsaturated fatty acids. During this inhibitory phase, adenylate cyclase activation by epinephrine and PGE_2, as well as the contractile response to a series of agonists, was markedly impaired. These latter inhibitory effects are shared by a series of unsaturated fatty acids that do not give rise to any prostaglandin-like material, and may well be the result of lipid interactions at the membrane level. Some of these results have appeared in a preliminary report (10).

[1] The abbreviations used are: cAMP, adenosine cyclic 3′,5′-monophosphate; PGE, prostaglandin E; PGF, prostaglandin F; TYA, eicosa-5, 8, 11, 14-tetraynoic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; PPP, polyphloretin phosphate.

[2] L. Do Khac and S. Harbon, manuscript in preparation.

MATERIALS AND METHODS

Chemicals. cAMP was obtained from P-L Biochemicals; theophylline, from Merck (Darmstadt); L-epinephrine bitartrate, from Calbiochem; and β-estradiol 3-benzoate, from Sigma. All fatty acids were purchased from Nu Chek Prep, Inc. Solutions of the ammonium salts of all fatty acids were prepared by dissolving the acid at a concentration of 9 mg/ml in NH_4OH for neutralization with agitation under a stream of N_2.

Cellulose ester membrane filters (HA, 0.45 μm, 24 mm) were obtained from Millipore Corporation. [^{3}H]cAMP (10 Ci/mmole) was a product of the Commissariat à L'Energie Atomique, Saclay, France. All reagents used were products of Prolabo (reagent grade). Prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ were generous gifts from Dr. J. E. Pike of the Upjohn Company. Compound D-600, a methoxy derivative of verapamil, was a gift from Knoll A.G. (Ludwigshafen, West Germany) to Dr. G. Vassort. Indomethacin was kindly supplied by Merck Sharp & Dohme, and meclofenamic acid (Meclomen), by Parke, Davis and Company. Polyphloretin phosphate was obtained through the courtesy of Dr.

J. Ahlin (Leolab), and eicosa-5,8,11,14-tetraenoic acid was generously provided by Dr. E. Scott, Hoffmann-La Roche.

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°. After the organs had been freed from adhering fat and connective tissue, the myometrium was separated by stripping away the endometrium as previously described (7). Myometrial preparations thus obtained consist almost exclusively of longitudinal muscles, while the endometrium is variably contaminated with some circular muscle.

Incubation and assay of cAMP levels. Myometrial and endometrial preparations from about 20 rat uteri were cut into two or three segments, which were pooled and divided into equal parts. Incubations were carried out in Krebs 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°, followed by the addition of theophylline (final concentration, 10 mM). The various agents to be tested were then added, and incubation was carried out for varying periods of time. Reactions were stopped by immersing the tissue strips in 2 ml of cold 7% trichloracetic acid, followed by immediate homogenization of the samples at 4° and centrifugation for 30 min at 30,000 × g. cAMP was estimated in the trichloracetic acid-soluble extracts according to Gilman (11), as previously described (7). The centrifuged pellets were dissolved in 2 ml of 1 N NaOH for protein determination (12). cAMP levels were expressed as picomoles per milligram of protein.

Methods for recording uterine responses. The contractile activity of isolated uterine strips was measured with an isometric transducing device as described previously (7). The segments were loaded at a basal tension of 0.2–0.3 g and bathed at 37° in 15 ml of Krebs bicarbonate buffer (95% O_2–5% CO_2) with the same salt composition as used for the above incubations. 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. Sensitivity was adjusted electronically so that the maximum contraction yielded about half-scale deflection of the pen.

RESULTS AND DISCUSSION

Effects of variable concentrations of PGE_2 and arachidonic acid on cAMP levels in rat myometrium and endometrium. Figure 1 shows that increasing concentrations of added PGE_2 stimulated cAMP accumulation in both tissues. The accumulation in the endometrium began at lower concentrations of PGE_2 than in the myometrium. Half-maximal accumulation occurred at 2.3 μM PGE_2 in the myometrium and at 1.2 μM in the endometrium. At maximal effective concentrations of PGE_2, the cyclic nucleotide level in the endometrium was always about 20% higher than in the myometrium. Figure 1 also shows the response of both endometrium and myometrium to exogenous arachidonic acid. In both tissues, arachidonic acid induced a dose-dependent rise in cAMP level. This response was comparable to that obtained with exogenous PGE_2; however, much higher concentrations of arachidonic acid were required. At 3 mM arachidonic acid, the rise in cAMP was equivalent to the stimulation evoked by 0.3 μM PGE_2 in endometrium and by 0.8–1 μM PGE_2 in myometrium. As with PGE_2, endometrium was more responsive than myometrium to lower concentrations of arachidonic acid.

In our previous study (7) PGE_1 and PGE_2 repeatedly yielded identical dose-response curves for cAMP accumulation in myometrium. The findings in Table 1 indicate that PGE_1 can also stimulate the accumulation of cAMP in endometrium. Table 1 also shows that homo-γ-linolenic acid, a direct precursor of prostaglandins

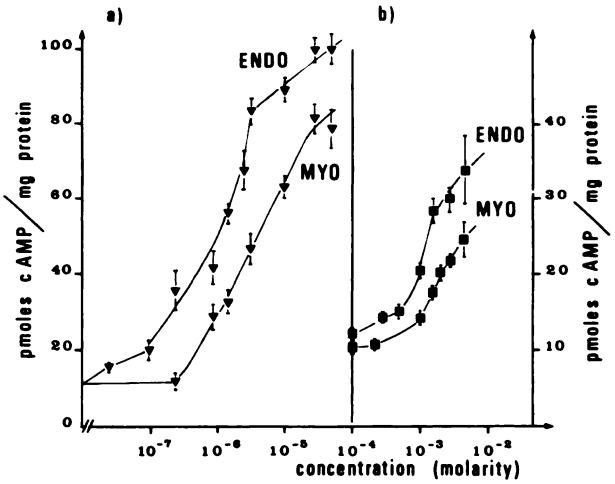

FIG. 1. *Effects of various concentrations of PGE_2 and arachidonic acid on cAMP level in rat myometrium and endometrium*

Myometrial or endometrial strips (50 mg) were incubated in 1.5 ml of Krebs bicarbonate buffer for 30 min at 37°; then theophylline (10 mM) was added as well as different concentrations of PGE_2 (a) or arachidonic acid (b). After 5 min of further incubation, tissue was extracted for cAMP determination as described in the text. Values represent the means ± standard errors of 6–10 different experiments.

of series 1, induced an identical rise in cAMP in both myometrium and endometrium and that this effect appears to be dose-dependent.

Time course of arachidonic acid- and homo-γ-linolenic acid-induced cAMP accumulation in rat myometrium and endometrium. The rise in cAMP in response to the two fatty acids was rapid in onset (Fig. 2), being detectable at 1 min and reaching a maximum at about 5 min. The cAMP level then declined slowly and remained slightly higher than the control after 15 min. The decline in cAMP occurred in spite of the presence of theophylline in the incubation medium. This observation differs from those reported in our previous studies (7) with rat myometrium, in which the rise in cAMP induced by exogenous PGE_1 or PGE_2 was sustained and never decreased with time up to 30 min, in both the absence and presence of theophylline. When similar kinetic studies were performed with oleic acid (3 mM), no effect on cAMP accumulation could be observed in incubations as long as 30 min.

Effects of inhibitors of prostaglandin synthesis. The possible correlation between the stimulation of adenylate cyclase by arachidonic and homo-γ-linolenic acids with the conversion of these precursors to PGE or PGE-like material was investigated with endometrial and myometrial perparations. Experiments were performed in the presence of the well-known

TABLE 1

Effects of various concentrations of PGE_1 and homo-γ-linolenic acid on cAMP level in rat myometrium and endometrium

See the legend to Fig. 1. After a 30-min incubation, myometrial and endometrial strips were incubated for 5 min in the presence of 10 mM theophylline and the indicated concentrations of either PGE_1 or homo-γ-linolenic acid. Means ± standard errors of three different experiments are shown.

Addition	cAMP	
	Myometrium	Endometrium
	pmoles/mg protein	
None	11.73 ± 0.9	10.52 ± 1.0
PGE_1, 1.65 μM	35.0 ± 2.5	50.0 ± 3.5
PGE_1, 3.3 μM	40.0 ± 3.2	66.3 ± 5.1
PGE_1, 10 μM	61.5 ± 2.9	
PGE_1, 50 μM		117.0 ± 6.5
Homo-γ-linolenic acid, 1.5 mM	14.8 ± 1.4	15.6 ± 0.6
Homo-γ-linolenic acid, 3 mM	21.3 ± 1.1	21.2 ± 1.0

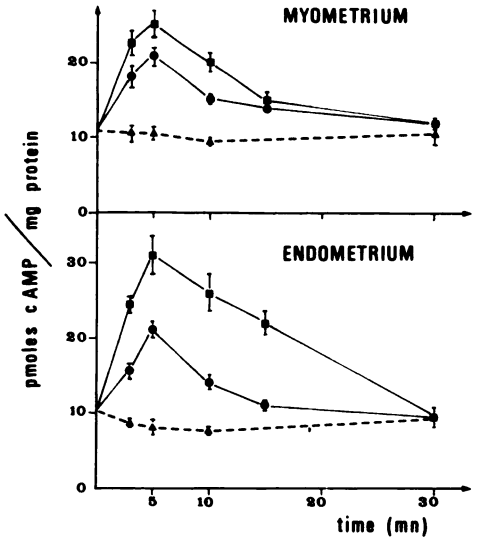

FIG. 2. *Time course of effects of arachidonic, homo-γ-linolenic, and oleic acids on cAMP level in rat myometrium and endometrium*

Myometrial or endometrial strips were incubated in Krebs bicarbonate buffer for 30 min at 37°. Incubations were then continued in the presence of 10 mM theophylline, with the addition of 3 mM arachidonic acid (■), homo-γ-linolenic acid (●), or oleic acid (▲). Reactions were stopped at the indicated times, and cAMP was estimated as described in the text. Values represent the means ± standard errors of five to seven different experiments.

inhibitors of the cyclooxygenase step in the prostaglandin synthesis pathway (13, 14). The Anti-inflammatory drugs indomethacin and meclofenamic acid, both at 15 μM, almost completely abolished the rise in cAMP level normally evoked by arachidonic acid or homo-γ-linolenic acid (Fig. 3). Under the same conditions these antagonists were without effect on basal cAMP levels and did not detectably alter the response of the myometrium or endometrium to exogenous PGE_2, epinephrine, or isoproterenol (results for the last two are not shown). However, at higher concentrations, indomethacin (100 μM) and meclofenamic acid (50 μM) inhibited by 50–60% the rise normally induced by 1.5 μM PGE_2. Nonspecific effects of anti-inflammatory drugs at doses above those inhibiting the response to prostaglandin precursors have been described (15–17). These observations point out the difficulty of interpreting the effects of high concentrations of these compounds in terms of the physiological role of prostaglandins. Nevertheless, the specific inhibition observed at the lower concentrations (15 μM) of indomethacin and meclofenamic acid can be interpreted as indirect evidence that these fatty acids exert their effect through their conversion to prostaglandin-like material. This was further supported by the results obtained with eicosa-5,8,11,14-tetraynoic acid, which strongly inhibited the conversion of arachidonic acid to PGE_2 (18). Incubation of both myometrium and endometrium with 3 mM TYA almost completely counteracted the rise in cAMP level normally evoked by 3 mM arachidonic acid. Under the same con-

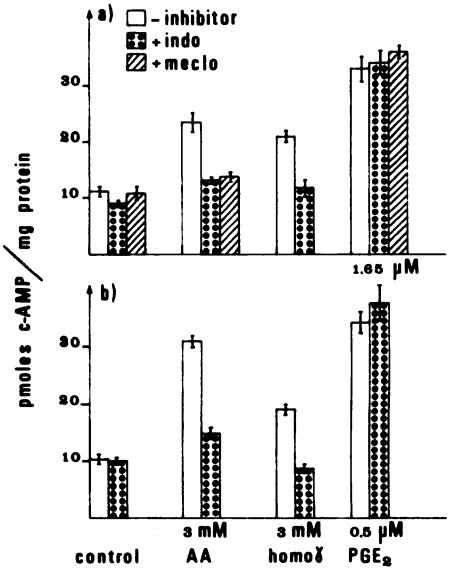

FIG. 3. *Inhibition by indomethacin and meclofenamic acid of cAMP accumulation induced by arachidonic and homo-γ-linolenic acids in rat myometrium and endometrium*

After a 10-min incubation at 37°, myometrial (a) or endometrial (b) strips were incubated in the absence and presence of 15 μM indomethacin (indo) or 15 μM meclofenamic acid (meclo) for 20 min before the subsequent addition of 10 mM theophylline. Incubation was continued for 5 min with and without the addition of arachidonic acid (AA), homo-γ-linolenic acid (homo γ), or PGE_2 at the indicated concentrations. Values represent the means ± standard errors of six (indomethacin) and two (melofenamic acid) experiments.

ditions TYA had no effect on the basal cAMP level, nor did it affect the response of either tissue to exogenous PGE_2 (Table 2).

Comparative effects of fatty acids on cAMP accumulation in rat myometrium and endometrium. Extensive studies reported by Beerthuis *et al.* (19) and by Samuelsson (20) have clearly demonstrated that the main active substrates for prostaglandin-synthesizing activity in the vesicular gland homogenate are the C_{20} polyunsaturated fatty acids. While minor activity can be observed with C_{19} and C_{21} polyunsaturated fatty acids, the C_{18} compounds, such as oleic and linoleic acids, which are the natural intracellular metabolic precursors of the corresponding C_{20} unsaturated fatty acids, can in no case be direct substrates for the cyclooxygenase involved in prostaglandin synthesis. In addition, these authors pointed to a stringent requirement for *cis* double bonds in position 8, 11, and 14; 5, 8, 11, and 14; or 5, 8, 11, 14, and 17. Table 3 shows the effects of a series of C_{18} and C_{20} fatty acids (3 mM) on cAMP accumulation in rat myometrium and endometrium after a short incubation time of 3 min. Of all the fatty acids tested, only the direct precursor of PGE_2, arachidonic acid, and that of PGE_1, homo-γ-linolenic acid, produced any significant accumulation of cAMP in both tissues. Similar observations were reported by Bergeron and Barden (21) in rat anterior pituitary gland.

Effect of Ca^{2+} ions. It has been shown previously that PGE_2-induced contractions in rat myometrium require the presence of Ca^{2+} while the effect of PGE_2 on cAMP accumulation shows no requirement for this divalent cation (8). The results of Table 4 depict the usual rise in the level of cAMP in myometrium in response to

TABLE 2

Inhibition by TYA of arachidonic acid-induced cAMP accumulation in rat myometrium and endometrium

Tissues were incubated and theophylline (10 mM) was added as described in the legend to Fig. 1. Arachidonic acid (or PGE_2) and TYA, combined or individually, were added in the indicated concentrations, and incubation was continued at 37° for 5 min. Means ± standard errors of three different experiments are shown.

Additions	cAMP	
	Myometrium	Endometrium
	pmoles/mg protein	
None	10.1 ± 0.6	10.3 ± 0.8
Arachidonic acid (3 mM)	19.8 ± 1.2	28.5 ± 1.2
PGE_2 (1.5 μM)	31.7 ± 2.9	50.0 ± 3.3
TYA (3 mM)	11.5 ± 1.4	10.2 ± 0.6
TYA (3 mM) + arachidonic acid (3 mM)	10.0 ± 0.6	8.6 ± 0.4
TYA (3 mM) + PGE_2 (1.5 μM)	31.2 ± 2.7	49.0 ± 4.4

TABLE 3

Comparative effects of various fatty acids on cAMP accumulation in rat myometrium and endometrium

Tissues were incubated and theophylline (10 mM) was added as described in the legend to Fig. 1. Incubation was continued for 5 min in the absence and presence of the indicated fatty acids at a concentration of 3 mM. When oleic acid was used, Ca^{2+} was omitted from the incubation medium in order to avoid fatty acid precipitation. Values represent the means ± standard errors of six different experiments, except for eicosadienoic acid, for which the individual values of two different experiments are listed.

Fatty acid	Double bond positions	cAMP	
		Myometrium	Endometrium
		pmoles/mg protein	
None		11.45 ± 0.8	9.3 ± 0.7
Oleic ($C_{18:1}$)	9	11.0 ± 0.8	10.9 ± 0.6
Linoleic ($C_{18:2}$)	9, 12	11.4 ± 0.7	11.2 ± 0.9
γ-Linolenic ($C_{18:3}$)	6, 9, 12	11.8 ± 0.9	12.5 ± 0.7
Eicosadienoic ($C_{20:2}$)	11, 14	10.4, 9.2	10.0, 9.2
Eicosatrienoic ($C_{20:3}$)	11, 14, 17	12.6 ± 1.3	
Homo-γ-linolenic ($C_{20:3}$)	8, 11, 14	21.3 ± 1.0	21.2 ± 0.9
Arachidonic ($C_{20:4}$)	5, 8, 11, 14	23.0 ± 0.7	30.4 ± 3

TABLE 4

Effect of Ca^{2+} deprivation and compound D-600 on the PGE_2- and arachidonic acid-induced rise in cAMP level of rat myometrium

Myometrial strips were incubated for 30 min in Krebs bicarbonate buffer in the presence and absence of Ca^{2+} with or without 4 mM EGTA. Theophylline (10 mM) was then added to all media, and compound D-600 was added where indicated. Incubation was continued in the absence and presence of PGE_2 or arachidonic acid for 5 min. Numbers in parentheses designate the number of determinations, each carried out in duplicate.

Addition	cAMP		
	Ca^{2+} (2.5 mM)	$-Ca^{2+}$, +EGTA (4 mM)	Ca^{2+} (2.5 mM) + D-600 (2.5 μM)
	pmoles/mg protein		
None	9.7 ± 1.1 (6)	8.5 ± 0.4 (6)	9.0 ± 1.5 (6)
PGE_2, 1.5 μM	32.6 ± 2.9 (4)	33.2 ± 2.6 (4)	31.3 ± 3.2 (4)
Arachidonic acid, 3 mM	21.3 ± 1.2 (5)	20.4 ± 0.8 (3)	20.7 ± 0.9 (3)

either PGE_2 or arachidonic acid despite calcium deprivation. The removal of Ca^{2+} from the medium, or the presence of EGTA or compound D-600, a derivative of verapamil known to antagonize specific Ca^{2+} movement (22), did not affect the response of the tissue to either arachidonic acid or PGE_2.

Effects of fatty acids on uterine contractility. The contractile activity of isolated uterine strips was measured in the same physiological buffer and with dose ranges of active substances and inhibitors identical with those used for evaluating the levels of cAMP. Arachidonic or homo-γ-linolenic acid induced contractions of the myometrium very rapidly (Figs. 4 and 5). Prior incubation of the muscle strips in 15 μM indomethacin prevented this response. However, at this concentration indomethacin did not appreciably affect the contractile response to PGE_2 or $PGF_{2\alpha}$. By contrast, higher concentrations (100 μM) of indomethacin reduced in a nonspecific manner the contractile effects of PGE_1 and $PGF_{2\alpha}$ as well as that of carbamylcholine (not shown). Again these high concentrations of indomethacin have to be avoided. Nevertheless, our present findings with the lower inhibitory dose suggest that the effects of arachidonic and homo-γ-linolenic acid may be due to their conversion to prostaglandin-like material through the cyclooxygenase. The same conclusion has been reached by Aiken (23) with regard to the arachidonic acid-stimulated contractility of the pregnant rat uterus. This is also supported by the present finding that a

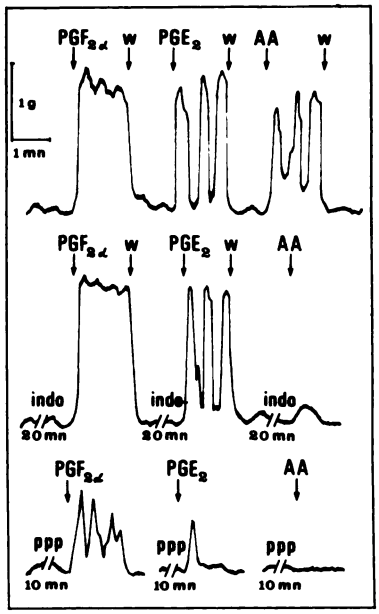

FIG. 4. *Tracing of isometric contractions of isolated rat uterus in the presence of PGE_2, $PGF_{2\alpha}$, and arachidonic acid: effects of indomethacin and PPP.*

The concentration of $PGF_{2\alpha}$ was 0.5 μM; PGE_2, 1.5 μM; arachidonic acid (AA), 100 μM; indomethacin (indo), 3 μM; PPP, 200 μg/ml. *w* = washing with buffer solution (recording was stopped during the washing period). In this and the following figures, the abbreviation mn stands for minutes.

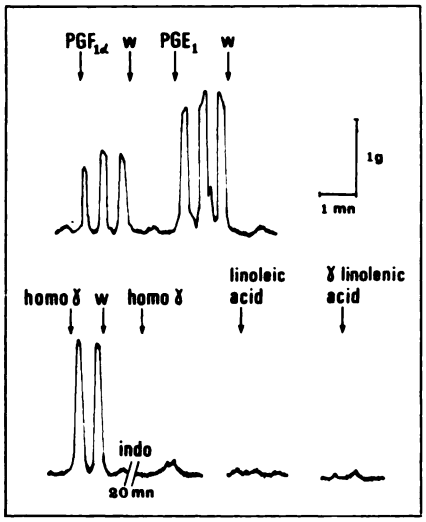

FIG. 5. *Tracing of isometric contractions of isolated rat uterus in the presence of homo-γ-linolenic, linoleic, and γ-linolenic acids: effect of indomethacin*

The concentration of PGE_1 was 1.5 μM; $PGF_{1\alpha}$, 3 μM; homo-γ-linolenic (homo γ), linoleic, and γ-linolenic acids, 100 μM; indomethacin, (indo), 3 μM. *w* = washing with buffer solution (recording was stopped during the washing period).

prostaglandin antagonist, polyphloretin phosphate (7, 24), at 200 μg/ml, inhibited arachidonic acid-induced contractions in much the same manner and extent as it affected the contractions generated by PGE_2 and $PGF_{2\alpha}$. Under these conditions PPP is without effect on the contractile response to carbamylcholine (7). It can be assumed, therefore, that the final site of action of arachidonic acid is at the level of prostaglandin. The specificity of the effects of arachidonic and homo-γ-linolenic acids was confirmed by the observation that those fatty acids which do not form prostaglandins did not affect contractility. Thus repeated attempts to induce myometrial contractions by linoleic or γ-linolenic acids were singularly unsuccessful (Fig. 5).

Our previous studies (7) definitively indicated that in myometrium the effects of exogenous PGE_1 and PGE_2 on contractions and cAMP accumulation are not causally related. These two distinct activities have now been reproduced with prostaglandin-like material synthesized *in situ*. Although the two prostaglandin effects are mimicked by arachidonic acid when the tissue is exposed to the fatty acid for a maximum of 5 min, the similarity ceases with longer incubations. Figure 2 has shown that the rise in cAMP evoked by arachidonic acid was not sustained but declined with time in spite of the presence of theophylline. In contrast, the cyclic nucleotide level remained constant following PGE_2 stimulation under the same conditions (7). In a parallel manner the intensity of contractions induced by arachidonic acid also declined with time, and almost none were observed after 10 min. Further addition of the prostaglandin precursor failed to elicit new contractile activity (experiment not shown). Figure 6 shows a typical experiment in which a uterine horn was exposed for 3 min to arachidonic acid. After basal activity was restored by washing, further addition of arachidonic acid yielded a much weaker response than the initial one. Further washings did not improve the situation, and arachidonic acid gave rise to even smaller responses. This phenomenon was never observed with added PGE_2 or $PGF_{2\alpha}$. Thus the decline in cAMP level parallel to the diminution of the contractile response prompted further investigation of this "delayed inhibitory" state that appeared after prolonged incubations with arachidonic acid.

Delayed inhibitory effects of arachidonic and other fatty acids on contractile response of myometrium. The delayed effect of arachidonic acid rendered the myometrium relatively unresponsive not only to

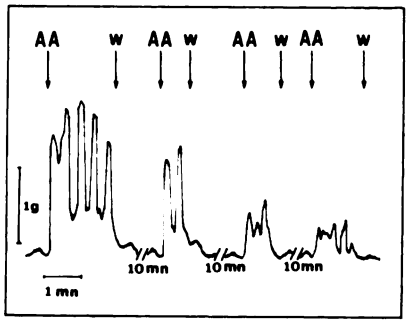

FIG. 6. *Tracing of isometric contractions of isolated rat uterus in response to successive additions of arachidonic acid*

Each addition of arachidonic acid (AA) was made at a concentration of 100 μM. *w* = washing with buffer solution.

arachidonic acid itself but also to other contractile stimulants (Fig. 7). After exposure to arachidonic acid for 10–15 min, the contractile response to added PGE_2, $PGF_{2\alpha}$, or carbamylcholine was strongly inhibited: washing of the uterine tissue with large volumes of buffer was not sufficient to recover a normal response to any of the contractile agents. This inhibitory effect of arachidonic acid could still be observed in the presence of 15 μM indomethacin. Similar inhibition was found with homo-γ-linolenic acid (data not shown). It is interesting that linoleic acid, which has no action on uterine motility, exhibited also a strong inhibitory effect on the contractions elicited by PGE_2, $PGF_{2\alpha}$, or carbamylcholine. Results of further experiments, not described in this paper, indicated that this inhibition can also be induced by prior exposure to γ-linolenic acid, while two other fatty acids, eicosadienoic and oleic, were without effect. These results suggest that the unresponsive state demonstrated with arachidonic and homo-γ-linolenic acids cannot be related to their metabolism through the prostaglandin-synthesizing system. The same state of inhibition can still be observed after indomethacin treatment and can be reproduced by other fatty acids that are not prostaglandin precursors under these conditions.

Delayed inhibitory effects of arachidonic acid on PGE_2- and epinephrine-induced accumulation of cAMP. The decline in cAMP level following its augmentation by arachidonic acid was not due to extrusion of cAMP from the tissue to the medium. Measurements of cAMP level in the medium were negative (data not shown). Furthermore, the decline was not due to the disappearance of arachidonic acid from the medium. This was verified by incubating arachidonic acid with myometrial strips for 30 min and subsequently testing the stimulatory capacity of the incubation medium on a second batch of myometrial strips. Arachidonic acid was still present in sufficient concentrations to effect a normal increase in cAMP level (data included in experiments of Table 8). Further experiments were performed by adding epinephrine or PGE_2 to myometrial or endometrial strips previously incubated for 30 min in the presence of 3 mM arachidonic acid (Table 5). The stimulatory effect of PGE_2 on cAMP accumulation was inhibited 60–75% by incubation with arachidonic acid. The response to epinephrine was also impaired (66% and 30% inhibition for endometrium and myometrium, respectively).

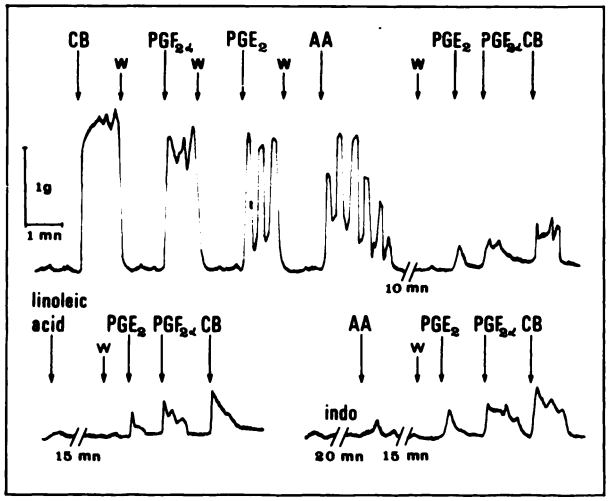

FIG. 7. *Tracing of effects of arachidonic and linoleic acids on contractile response of isolated uterus to carbamylcholine, $PGF_{2\alpha}$, and PGE_2*

Agents were used at the concentrations described in the legends to Figs. 4 and 5. The concentration of carbamylcholine (CB) was 20 μM. AA, arachidonic acid; indo, indomethacin. *w* = washing with buffer solution.

TABLE 5

Effect of prior incubation with arachidonic acid on epinephrine- and PGE_2-induced rise in cAMP of rat myometrium and endometrium

Myometrial and endometrial strips were conditioned for 30 min in Krebs bicarbonate buffer without or with indomethacin (15 μM) present during the last 20 min. Theophylline (10 mM) was then added, and a second preliminary incubation was performed in the absence and presence of arachidonic acid for 30 min. Incubations were then continued with and without PGE_2 or epinephrine for 5 min at 37°. Means ± standard errors of six different experiments are shown.

Incubations with		cAMP					
		Myometrium			Endometrium		
Indomethacin, 15 μM	Arachidonic acid, 3 mM	No addition	PGE_2, 4 μM	Epinephrine, 0.5 μM	No addition	PGE_2, 3 μM	Epinephrine, 0.5 μM
		pmoles/mg protein			*pmoles/mg protein*		
−	−	11.7 ± 0.8	53.4 ± 6.5	73.8 ± 5.8	9.9 ± 0.7	77.95 ± 5.6	60.8 ± 9.5
−	+	12.5 ± 0.6	30.7 ± 1.2	55.8 ± 5.6	12.5 ± 1.3	32.9 ± 3.0	27.0 ± 2.7
+	+	10.9 ± 0.9	31.5 ± 1.8	55.1 ± 4.4	10.3 ± 1.1	32.7 ± 3.2	28.6 ± 3.4
Inhibition			57%	30%		70%	66%

This inhibition of the PGE_2- and epinephrine-induced rise in cAMP could still be observed when the tissue was treated with indomethacin before the addition of arachidonic acid in order to prevent any possible prostaglandin synthesis. The inhibitory effect of arachidonic acid toward PGE_2 (or epinephrine) activation of adenylate cyclase was not observed early in the incubation with the fatty acid. When endometrium or myometrium was incubated with submaximal concentrations of PGE_2 (or epinephrine) together with arachidonic acid for the short interval of 3 min, the stimulatory effects of PGE_2 (or epinephrine) and that of arachidonic acid on cAMP accumulation were found to be additive (Table 6). Average cAMP levels in both myometrium and endometrium were approximately the same as the calculated values for effects induced by arachidonic acid plus PGE_2 or by arachidonic acid plus epinephrine at the corresponding concentrations. In the presence of indomethacin the stimulatory effect of arachidonic acid was again abolished, and only the PGE_2 or epinephrine stimulation persisted.

Inhibitory effects of various unsaturated fatty acids on rise in cAMP elicited by epinephrine and PGE_2. The unresponsive state effected by arachidonic acid could be reproduced by homo-γ-linolenic acid and also by a series of fatty acids that are not direct prostaglandin precursors and that did not alter basal cAMP levels in either myometrium or endometrium (Table 7). After the tissues had been incubated for 30 min in the presence of 3 mM linoleic or γ-linolenic acids, there was a significant alteration in the subsequent PGE_2- or epinephrine-induced activation of adenylate cyclase in both tissues, to the extent of about 100% inhibition with linoleic acid and about 70% with γ-linolenic acids. Interestingly, the $C_{18:2}$ and $C_{18:3}$ fatty acids exhibited an even more pronounced inhibitory effect than that exerted by arachidonic acid or homo-γ-linolenic acid. Preliminary incubation under the same conditions in the presence of oleic acid ($C_{18:1}$) or eicosadienoic acid ($C_{20:2}$) resulted in about 50% inhibition of the endometrial response to PGE_2 or epinephrine but had no effect on myometrium. With all fatty acids tested, inhibition of the epinephrine or PGE_2 response was usually more pronounced in endometrium than in myometrium. Table 7 also shows that the C_{20} polyacetylenic acid TYA, a known inhibitor of prostaglandin synthetase (18) as well as of platelet lipoxygenase (25), by itself had an inhibitory effect on PGE_2-induced increase in cAMP in myometrium. When both TYA and arachidonic acid were added together, inhibition of the PGE_2 response was even more pronounced.

TABLE 6

Effect of simultaneous combination of arachidonic acid and PGE_2 on cAMP levels in rat myometrium and endometrium

Myometrial and endometrial strips were conditioned in Krebs buffer for 30 min with and without 15 μM indomethacin present during the last 20 min. PGE_2 (or epinephrine) and arachidonic acid, combined or individually, were then added at the indicated concentrations and incubation was continued at 37° for 3 min in the presence of 10 mM theophylline. Means ± standard errors of three different experiments are shown.

Additions	cAMP					
	Myometrium			Endometrium		
	−Indomethacin	+Indomethacin	Calculated (if additive)	−Indomethacin	+Indomethacin	Calculated (if additive)
	pmoles/mg protein			*pmoles/mg protein*		
None	9.9 ± 0.7			9.8 ± 0.7		
Arachidonic acid (3 mM)	20.2 ± 1.2	11.8 ± 0.7		27.2 ± 1.4	9.6 ± 0.7	
PGE_2 (1.65 μM)	25.8 ± 1.0			28.7 ± 2.8		
Arachidonic acid + PGE_2	35.3 ± 2.7	24.1 ± 1.5	36.0	43.4 ± 3.2	25.3 ± 1.5	46.0
Epinephrine (0.2 μM)	26.0 ± 2.4			40.1 ± 3.9		
Arachidonic acid + Epinephrine	35.7 ± 2.6	24.6 ± 3.4	36.2	55.7 ± 3.4	39.0 ± 4.1	57.4

TABLE 7

Comparative effects of incubation with various fatty acids on epinephrine- and PGE_2-induced rises in cAMP of rat myometrium and endometrium

Myometrial and endometrial strips were incubated as described in the text. After the addition of theophylline (10 mM), incubation was continued for 30 min in the absence and presence of the indicated fatty acid at a concentration of 3 mM. PGE_2 (4 μM) or epinephrine (0.5 μM) was then added, and the incubation was continued for 5 min at 37°. Results are expressed as inhibition of the PGE_2 or epinephrine response obtained in the absence of fatty acid.

Addition	Inhibition				
	Myometrium		Endometrium		*n*
	PGE_2	Epinephrine	PGE_2	Epinephrine	
	%	%	%	%	
Arachidonic Acid	56	27	68.1	66.7	12
Homo-γ-linoic acid		43.5	81.7	78	4
Eicosadienoic acid	0		58.3	32.2	2
γ-Linolenic acid	63	67.4	79	78	3
Linoleic acid	69.4	97	100	100	4
Oleic acid	0	0	47.7	51.2	8
TYA	55				3
Arachidonic acid + TYA	80				3

This conforms with the proposal that the inhibitory effect of arachidonic acid is not due to its metabolism through either of the two enzymatic reactions mentioned above.

Additional experiments performed with different concentrations of linoleic acid revealed that the inhibition was dose-dependent (40% inhibition of the PGE_2 response in endometrium with 0.3 mM, 62% inhibition with 1 mM, and almost 90% inhibition with 3 mM linoleic acid). Furthermore, inhibition was almost complete after a 5-min preliminary incubation in the presence of the fatty acid, followed by 5 min of incubation with PGE_2 (results not shown).

A series of experiments was performed in order to determine whether the inhibitory effects of arachidonic acid and other fatty acids were associated with continuous contact of the fatty acid with the tissue

preparation. Myometrial strips were incubated with either 3 mM arachidonic acid or linoleic acid for 30 min. The tissue was then separated from the medium, washed, and further incubated for 5 min in fresh medium containing theophylline and epinephrine or PGE_2. Inhibition persisted under these conditions in all cases. The response to epinephrine and PGE_2 was significantly altered in comparison with tissue incubated under the same conditions but in the absence of fatty acids (Table 8).

It is noteworthy that when the medium containing arachidonic acid was separated from the tissue after the 30-min preliminary incubation and added to fresh myometrial strips for a 3-min incubation, cAMP accumulation was stimulated to the same extent as in fresh arachidonic acid-containing medium. This indicates that under these conditions arachidonic acid was not appreciably metabolized or inactivated. Medium separated from the tissue after 30 min of incubation with linoleic acid, then added to a fresh tissue preparation, did not affect the subsequent epinephrine- or PGE_2-mediated increases in cAMP (Table 8). The latter results indicate that the inhibitory effect may not be due to the accumulation of some inhibitory factor in the medium. The fatty acids seem to alter the myometrium in some way that renders it unresponsive to further stimulation by epinephrine or PGE_2. This effect is not readily reversible, but persists even after washing of the tissue.

CONCLUSION

We have reported earlier that prostaglandins E and F induce contractions of estrogen-treated rat uterus and that PGE_1 and PGE_2, but not $PGF_{1\alpha}$ or $PGF_{2\alpha}$ are also able to elevate intracellular cAMP levels in the isolated myometrium and endometrium (7, 8). The results of the present study demonstrate that arachidonic acid ($C_{20:4}$) and homo-γ-linolenic acid ($C_{20:3}$), both PG precursors, elicit contractions of rat myometrium and evoke a significant increase in intracellular cAMP in myometrium as well as endometrium. These effects, which are dose-dependent and very rapid in onset, appear to be the result of fatty acid conversion to prostaglandin or prostaglandin-like material. This conclusion is supported by the inhibitory action exerted by indomethacin, meclofenamate, and eicosatetraynoic acid and is further strengthened by the observation that stimulation of contraction and of cAMP accumulation could not be achieved with a series of fatty acids that are not direct substrates of the prostaglandin synthetase system. Based on the dose-response curve for cAMP accumulation obtained with exogenous PGE_2 and that of arachidonic acid, it is possible to obtain a rough evaluation of the quantity of PGE_2-

TABLE 8

Localization of fatty acid-induced inhibitory effect in treated myometrium and incubation medium

Myometrial strips were first incubated for 30 min as described in the text. After the addition of 10 mM theophylline, incubation was continued for 30 min in the absence and presence of 3 mM arachidonic or linoleic acid. The treated tissue was then separated from the medium and washed twice. To the incubated tissue was added fresh medium containing 10 mM theophylline, and to the previous incubation medium were added fresh myometrial strips. PGE_2 (4 μM) or epinephrine (0.5 μM) was then added where indicated, and incubation was continued for 5 min before tissue extraction for cAMP determinations. In these experiments the value for cAMP in the control tissue incubated with 3 mM arachidonate for 5 min averaged 30 pmoles/mg of protein. Values are the means ± standard errors of the number of experiments designated in parentheses.

Test material	cAMP		
	No addition	PGE_2	Epinephrine
	pmoles/mg protein		
Untreated tissue (control)	11.7 ± 0.4	64 ± 1.86	60.8 ± 4.12
Arachidonic acid (6)			
Treated tissue + fresh medium		26.6 ± 1.57	44.6 ± 3.49
Incubation medium + fresh tissue	33.04 ± 2.19		
Linoleic acid (3)			
Treated tissue + fresh medium	11.4 ± 0.6	14.9 ± 0.72	13.7 ± 1
Incubation medium + fresh tissue	10.8 ± 0.7	59.9 ± 2.56	54.6 ± 2.16

like material that is synthesized. Conversion of arachidonic acid to PGE_2-like material can be estimated roughly as 0.3% and 0.1% for myometrium and endometrium, respectively. This relatively low estimate of arachidonic acid transformation to PGE-like material is not very surprising, since recent studies *in vitro* with the uterine prostaglandin-synthesizing system indicated a low capacity of uterine tissue for producing prostaglandins from either endogenous or exogenous precursors (26–29) compared with the active vesicular gland system. Prostaglandin levels in such instances were estimated by bio- and radioimmunoassays or through conversion to products from [^{14}C]arachidonic acid. Our findings suggest that the apparent activation of adenylate cyclase can also be taken as an indirect measure of active fatty acid transformation to PGE or PGE-like material. Whether the stimulation is due to the final end product PGE, to its intermediate endoperoxide (30), or to the novel prostaglandin compound prostacyclin (31–33) was not investigated in the present study. Previous work on prostaglandin synthesis in the uterus (26-29) was done either with the intact organ (endometrium plus myometrium) or with the isolated endometrium. To our knowledge, the present paper describes for the first time active prostaglandin synthesis in the isolated rat myometrium. Furthermore, it is evident that in rat myometrium the endogenously synthesized prostaglandins are effective in inducing contractions, probably as PGE plus PGF, and in stimulating adenylate cyclase, in this case as PGE-like material. No definite answer could be provided for the role of cAMP in the regulation of uterine contractility (7, 8). However, it is clear that the level of the cyclic nucleotide could be modulated by active effectors *in situ* in a manner similar to that produced by the addition of exogenous PGE_1 and PGE_2 compounds.

Moreover, an interesting phenomenon was observed with various fatty acids when the effects of arachidonic acid or homo-γ-linolenic acid on cAMP accumulation and contraction were studied as a function of time. It was noted that the stimulatory effects that occurred via prostaglandin synthesis appeared almost immediately but were followed within 3–5 min by a prolonged, unresponsive late phase. During this late inhibitory phase, the responses of the myometrium to different contractile agents such as prostaglandins, oxytocin, or carbamylcholine were severely altered. Under these conditions arachidonic acid also elicited a dramatic decrease in epinephrine- and PGE_2-induced accumulation of cAMP in both myometrium and endometrium. The failure of indomethacin to prevent this inhibition indicated that it was not due to any prostaglandin-like material arising from arachidonic acid metabolism through the prostaglandin synthetase system. Furthermore, a series of fatty acids that are not direct substrates for this enzyme complex nevertheless precipitated a phase of unresponsiveness toward both adenylate cyclase activation and hormone-induced contraction. The greatest inhibition was observed with linoleic ($C_{18:2}$) and γ-linolenic ($C_{18:3}$) acids, while oleic ($C_{18:1}$) and eicosadienoic ($C_{20:2}$) acids were less effective. The relative irreversibility of this phenomenon is suggested by the finding that washing out the fatty acid prior to measurement of cAMP accumulation or contractile activity in the presence of various agonists did no eliminate inhibition in either myometrial or endometrial cells. Additional experiments (not described here) seem to exclude any toxic effect produced via β-oxidation of the fatty acids: inhibition was similar whether the tissue was incubated in the absence or presence of glycerol, which has been shown to minimize the β-oxidation route significantly in isolated hepatocytes (34). On the other hand, incorporation of [^{14}C]arachidonic acid into phospholipid components of the myometrium has been observed.[3] Hence our data seem to suggest that inhibition of adenylate cyclase activation by epinephrine or PGE_2 in the myometrium and endometrium and of hormone-induced myometrial contractility may be the result of a direct effect of the fatty acid, as such or after esterification, through its insertion into the membrane lipid components. This

[3] M. F. Vesin and S. Harbon, unpublished observations.

would result in a modification of the adenylate cyclase environment or of the receptor-adenylate cyclase interaction domain. Modifications by fatty acids of the adenylate cyclase system and its activation by catecholamines (35), PGE_1 (36, 37), and cholera toxin (37) have recently been described. The importance of selected lipids and lipid-protein interactions for adenylate cyclase activity and its hormonal stimulation in these various membrane preparations has been suggested. This may likewise prevail for the initial, yet unidentified membrane interactions and for signal transmission occurring with the different agonists stimulating contraction of the myometrium. These membrane interactions deserve further investigation, which is outside the scope of the present report.

ACKNOWLEDGMENTS

We are indebted to Professor H. Clauser for his support and helpful discussions. We wish to thank Professor V. Najjar for valuable comments and critically reviewing the manuscript. A special acknowledgment is extended to Mrs. Robichon for technical assistance and Mrs. Crosnier for typing the manuscript.

REFERENCES

1. Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1970) in *Fundamental Concepts in Drug-Receptor Interaction*, pp. 59–91, Academic Press, New York.
2. Triner, L., Overweg, N. I. A. & Nahas, G. G. (1970) *Nature*, **225**, 282–283.
3. Harbon, S. & Clauser, H. (1971) *Biochem. Biophys. Res. Commun.*, **44**, 1496–1503.
4. Polacek, I. & Daniel, E. E. (1971) *Can. J. Physiol. Pharmacol.*, **49**, 988–998.
5. Bhalla, R. C., Sanborn, B. M. & Korenman, S. G. (1972) *Proc. Natl. Acad. Sci. U. S. A.*, **69**, 3761–3764.
6. Angles d'Aurjac, G. & Meyer, P. (1972) *Life Sci.*, **11**, 631–641.
7. Vesin, M. F. & Harbon, S. (1974) *Mol. Pharmacol.*, **10**, 457–473.
8. Harbon, S., Vesin, M. F. & Do Khac, L. (1975) *Colloq. INSERM*, **50**, 83–100.
9. Harbon, S., Do Khac, L. & Vesin, M. F. (1976) *Mol. Cell. Endocrinol.*, **6**, 17–34.
10. Vesin, M. F. & Harbon, S. (1975) *Adv. Prostaglandin Thromboxane Res.*, **2**, 847.
11. Gilman, A. (1970) *Proc. Natl. Acad. Sci. U. S. A.*, **69**, 3761–3764.
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265–275.
13. Vane, J. R. (1971) *Nat. New Biol.*, **231**, 232–235.
14. Smith, J. B. & Willis, A. L. (1971) *Nat. New Biol.*, **231**, 235–237.
15. Andersson, K. E., Hedner, P. & Persson, C. G. A. (1974) *Acta Physiol. Scand.*, **90**, 657–663.
16. Lindner, H. R., Zor, U., Bauminger, S., Tsafriri, A., Lamprecht, S. A., Koch, Y., Antebi, S. & Schwartz, A., (1974) in *PG Synthetase Inhibitors* (Robinson, H. J. & Vane, J. R., eds.), p. 271, Raven Press, New York.
17. Sorrentino, L., Capasso, F. & Di Rosa, M. (1972) *Eur. J. Pharmacol.*, **17**, 306–308.
18. Ahern, D. G. & Downing D. T. (1970) *Biochim. Biophys. Acta*, **210**, 456–461.
19. Beerthuis, R. K., Nugteren, D. H., Pabon, H. J. I. & Van Dorp, D. A. (1968) *Rec. Trav. Chim.*, **87**, 461–479.
20. Samuelsson, B. (1972) *Fed. Proc.*, **31**, 1442–1450.
21. Bergeron, L. & Barden, N. (1975) *Mol. Cell. Endocrinol.*, **2**, 253–260.
22. Fleckenstein, A., Grun, G., Tritthart, H. & Byron, K. (1971) *Klin. Wochenschr.*, **49**, 32–41.
23. Aiken, J. W. (1974) in *Prostaglandin Synthetase Inhibitors* (Robinson, H. J. & Vane, J. R., eds.), pp. 289–301, Raven Press, New York.
24. Eakins, K. E., Karim, S. M. & Miller, J. D. (1970) *Br. J. Pharmacol.*, **39**, 556–563.
25. Downing, D. T., Ahern, D. G. & Bachta, M. (1970) *Biochem. Biophys. Res. Commun.*, **40**, 218–223.
26. Poyser, N. L. (1972) *J. Endocrinol.*, **54**, 147–159.
27. Ham, E. A., Wrillo, V. J., Zanetti, M. E. & Kuehl, F. A., Jr. (1975) *Proc. Natl. Acad. Sci. U. S. A.*, **72**, 1420–1424.
28. Wlodarver, P., Kindahl, H. & Hamberg, M. (1975) *Biochim. Biophys. Acta*, **431**, 602–614.
29. Mitchell, S., Poyser, N. L. & Wilson, N. H. (1977) *Br. J. Pharmacol.*, **59**, 107–113.
30. Hamberg, M., Svensson, J., Wakabayashi, T. & Samuelsson, B. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, **74**, 345–349.
31. Moncada, S., Gryglewski, R., Bunting, S. & Vane, J. R. (1976) *Nature*, **263**, 663–665.
32. Isakson, P. C., Raz, A., Denny, S. E., Pure, E. & Needleman, P. (1977) *Proc. Natl. Acad. Sci. U. S. A.*, **74**, 101–105.
33. Fenwick, L., Jones, R. L., Naylor, B., Poyser, N. L. & Wilson, N. H. (1977) *Br. J. Pharmacol.*, **59**, 191–199.
34. Ontko, J. A. (1972) *J. Biol. Chem.*, **247**, 1788–1800.
35. Orly, J. & Schramm, M. (1975) *Proc. Natl. Acad. Sci. U. S. A.*, **72**, 3433–3437.
36. Engelhard, V. H., Esko, J. D., Storm, D. R. & Glaser, M. (1976) *Proc. Natl. Acad. Sci. U. S. A.*, **73**, 4482–4486.
37. Zenser, T. V., Petrela, V. J. & Huyes, F. (1976) *J. Biol. Chem.*, **251**, 7431–7436.