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Interaction between prostaglandins and gonadotrophins in the rabbit ovary

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Summary

1. It has been suggested that prostaglandins function as feedback modulators of hormonal actions which are mediated by adenosine 3′,5′-monophosphate (cyclic AMP). This hypothesis has been tested on the rabbit ovary, whose steroidogenic response to luteinizing hormone (LH) is mediated by the cyclic nucleotide.

2. Prostaglandin E_1 (1–100 μg/ml) reduced the production of progesterone by rabbit ovaries incubated in the presence of a submaximal concentration of LH, but had no effect on the formation of this steroid when exogenous cyclic AMP was used as the stimulating agent. The prostaglandin was without effect on the formation of 20α-hydroxypregn-4-en-3-one in the presence of either LH or cyclic AMP.

3. Prostaglandin E_2 (1 μg/ml) was without effect on ovarian steroidogenesis in the presence of LH.

4. There was no evidence that exogenous prostaglandin E_1 was inactivated during incubation with rabbit ovaries in the presence of LH.

5. Prostaglandin E-like compounds were detected in homogenates of incubated rabbit ovaries. However, concentrations of LH sufficient to stimulate steroidogenesis did not stimulate the synthesis of these compounds by the ovary *in vitro*, nor their release from the ovary *in vivo*.

6. It is concluded that the prostaglandin-like compounds detected in the ovary are unlikely to play a role in the regulation of steroidogenesis. The results of this investigation do not support the hypothesis that prostaglandins function as general modulators of hormonal actions which are mediated by cyclic AMP.

Introduction

Despite our knowledge of the diverse pharmacological actions of the prostaglandins, little is known of the physiological roles of these compounds. One attractive hypothesis is that prostaglandins of the E series are involved in local feedback control mechanisms, modulating the responses of tissues to hormones whose actions are mediated by adenosine 3′,5′-monophosphate (cyclic AMP). This hypothesis was proposed by Ramwell & Shaw (1967) to correlate the inhibitory effects of these prostaglandins on adipose tissue lipolysis (Steinberg, Vaughan, Nestel & Bergström, 1963) and on vasopressin-induced water permeability (Orloff, Handler & Bergström,

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1965) with their release from fat pads and other tissues in response to nervous or hormonal stimuli (Ramwell & Shaw, 1967; Shaw & Ramwell, 1967).

The work described here was carried out to investigate the applicability of the negative feedback hypothesis of Ramwell & Shaw (1967) in another biological system: steroidogenesis in the rabbit ovary. There is good evidence that the steroidogenic effect of luteinizing hormone (LH) on this organ is mediated by activation of adenyl cyclase (Dorrington & Kilpatrick, 1967; Dorrington & Baggett, 1969). The occurrence of prostaglandins in the rabbit ovary has not been investigated previously, but Euler & Hammarström (1937) found evidence of prostaglandin-like substances in ethanol extracts of cow and pig ovaries. If prostaglandins function as general feedback modulators of hormones which act *via* cyclic AMP, they should antagonize the effect of LH on steroidogenesis, and be released from the ovary in response to this hormone.

Methods

Incubation of rabbit ovaries

Ovaries were taken from mature New Zealand white rabbits, made pseudopregnant by the injection of 500 IU of human chorionic gonadotrophin into the marginal ear vein 6–8 days previously. The ovaries were chopped up with scissors and distributed among tared incubation flasks containing 10 ml of Krebs-Ringer bicarbonate buffer, of the following composition (g/l.): NaCl, 6·9; KCl, 0·35; $CaCl_2$, 0·28; Mg SO_4,$7H_2O$, 0·29; $NaHCO_3$, 2·1; KH_2PO_4, 0·16; dextrose 2·0. Approximately 0·4 g of tissue was weighed accurately into each flask.

In experiments to investigate the effects of prostaglandins on steroidogenesis, four flasks were used. One contained Krebs solution only. To the other three were added submaximal concentrations of LH (0·5 μg/ml) or cyclic AMP (5 mM). Two of the flasks containing the stimulating agent contained in addition the prostaglandin under investigation, a different concentration being used in each flask.

Investigations into the effects of LH on the production of prostaglandin-like material *in vitro* involved incubations of ovarian tissue in 'control' flasks, containing Krebs solution only, and 'test' flasks to which LH had been added to give a final concentration of 2 μg/ml.

Flasks were incubated in a metabolic shaker at 37° C for 3 h, with continuous gassing with 5% CO_2 in O_2. Incubation was terminated by rapid chilling, followed by homogenization of the tissue in the incubation fluid using a Teflon glass homogenizer.

Collection of ovarian venous blood

New Zealand white rabbits, 8–10 days pseudopregnant, were anaesthetized by intravenous injections of urethane, the initial dose being 1·25—1·5 g/kg. Cannulae were inserted into the trachea, a carotid artery and a jugular vein. The abdomen was opened by a midline incision, and heparin (1,000 U/kg) injected intravenously.

Prominent branches of one ovarian vein were ligated and a short catheter (I.D. 0·6 mm) inserted close to the ovary. The catheter was joined to a length of polythene tubing (I.D. 1·0 mm), the tip of which was positioned above the collecting vessel and approximately 30 cm below the level of the animal.

Blood was collected into beakers kept surrounded by ice during successive periods of 45 min before and after the injection of LH (500 μg, i.v.). To achieve an adequate blood flow (>0·5 ml/min) it was necessary to maintain the blood pressure of the animal by continuous infusion of whole rabbit blood at a rate equivalent to that of outflow from the ovarian vein.

The plasma was separated from each blood sample by centrifugation, and the cells were washed twice with 0·9% saline (0·75 vol.). The combined plasma and saline washings were stored at −20° C before steroid and prostaglandin estimations.

Steroid assays

Ovarian homogenates or plasma samples were titrated to pH 11·0 with 1 N sodium hydroxide. The two principal progestational steroids produced by the rabbit ovary, progesterone and 20 α-hydroxypregn-4-en-3-one (20 α-HP), were extracted by the procedure of Dorrington & Kilpatrick (1966), and isolated by two-dimensional thin layer chromatography (Armstrong, O'Brien & Greep, 1964). The steroids were located on the chromatograms by examination under ultraviolet light (254 nm), and the appropriate areas of silica gel eluted with 2·5 ml of methanol. Quantitation was by light absorption at 240 nm, using a Unicam SP800B double-beam recording spectrophotometer.

The authenticity of the extracted steroids was confirmed using an LKB 9000 gas chromatograph-mass spectrometer. Retention times and mass spectra of extracted samples were indistinguishable from those of authentic samples of progesterone and 20 α-HP.

Prostaglandin extractions

1. From ovarian homogenates

Ovarian homogenates were titrated to pH 3 with 1 N HCl, and subjected to solvent extractions, silicic acid chromatography and thin layer chromatography in the AI and AII systems of Gréen & Samuelsson (1964). Details of these procedures have been described by Horton & Main (1967).

2. From plasma samples

The method was based on the procedure described by Hickler (1968). The final residue from the solvent extractions was subjected to silicic acid chromatography followed by thin layer chromatography, as above.

Prostaglandin assays

Prostaglandin-like material eluted from column and thin layer chromatograms was assayed biologically. Preparations used were the rat fundus, guinea-pig ileum, rabbit jejunum, ovariectomized rat uterus, spinal cat blood pressure and jird colon. Details of the methods have been described previously (Horton & Jones, 1969; Horton & Main, 1967).

Drugs

Progesterone was purchased from Koch-Light Laboratories Ltd., and 20 α-HP was kindly donated by the Upjohn Company, Kalamazoo, Michigan. Ovine LH

(NIH-LH-S11) was a gift from the National Institutes of Health, Bethesda, U.S.A., and cyclic AMP was purchased from Boehringer Corporation Ltd. Prostaglandins were kindly supplied by Dr. J. E. Pike of the Upjohn Company, and Professor D. A. van Dorp of the Unilever Research Laboratories, Vlaardingen, Netherlands.

Results

Effects of prostaglandins E_1 and E_2 on progesterone and 20 α-HP production stimulated by LH and by cyclic AMP

Both LH (0·5 μg/ml) and cyclic AMP (5 mM) caused a large stimulation of progesterone and 20 α-HP production in all experiments. This was shown by the much greater amounts of steroids produced in the flasks containing the stimulating agents compared with control flasks containing Krebs solution only.

Prostaglandin E_1 inhibited the production of progesterone in the presence of LH. This effect was seen consistently with prostaglandin concentrations of 1, 10, 50 and 100 μg/ml. The extent of the inhibition increased with increasing concentrations of the prostaglandin (Fig. 1). No consistent effect was observed with this prostaglandin at a concentration of 0·1 μg/ml.

Quantities of 20 α-HP produced in response to LH were little affected by prostaglandin E_1. A mean increase of 9·5% ($P<0·05$, analysis of variance) was observed at the 10 μg/ml dose level, but at concentrations of 50 and 100 μg/ml there was no indication of such an effect.

Unlike prostaglandin E_1, prostaglandin E_2 (1 μg/ml) did not consistently reduce the concentrations of progesterone produced by rabbit ovaries incubated in the presence of LH (mean increase 1·5%, ±S.E. 10·7, $n=5$). This prostaglandin also had no effect on the concentrations of 20 α-HP produced by the ovaries (mean increase 0·9%, ±S.E. 4·1, $n=5$).

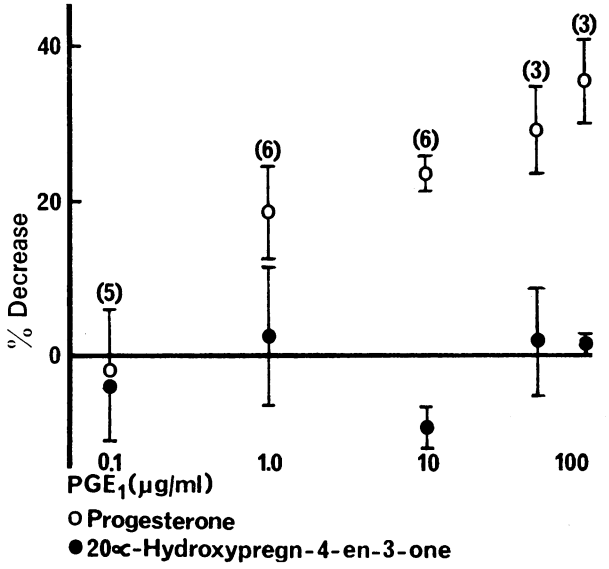

FIG. 1. Effect of prostaglandin E_1 on the production of progesterone and 20 α-hydroxypregn-4-en-3-one by rabbit ovaries incubated in the presence of LH (0·5 μg/ml). Vertical lines represent standard errors of the means.

When cyclic AMP was used as the stimulating agent, prostaglandin E_1 (10 μg/ml) had no consistent effect on the production of progesterone (mean increase 5·6% ± S.E. 8·7, n=9) or of 20 α-HP (mean increase 6·9% ± S.E. 8·4, n=9). Thus, prostaglandin E_1 did not affect the steroidogenic response of the rabbit ovary to exogenous cyclic AMP.

Metabolism of prostaglandin E_1 by rabbit ovaries in vitro

An experiment was carried out to investigate whether any of the prostaglandin E_1 added in the above experiments had been metabolized or otherwise removed from the incubation medium, during the 3 h incubation period.

Ovarian tissue was distributed among incubation flasks containing Krebs solution to which had been added LH (0·5 μg/ml) and prostaglandin E_1, in concentrations of 0–10 μg/ml. Before adding the tissue, 0·5 ml of the incubation fluid was removed from each flask, and kept as the standard against which to assay the prostaglandin remaining in each respective solution after incubation. At the end of incubation, samples of supernatant fluid were withdrawn, diluted as appropriate, and assayed on the rat fundus.

No biological activity (<5 ng of prostaglandin E_1) was found in 0·5 ml of supernatant taken from the control flasks after incubation. The biological assays showed that prostaglandin concentrations remaining in the other flasks were the same as those added initially. There was no indication, therefore, that any of the prostaglandin E_1 had been inactivated during the 3 h incubation.

Effect of LH on the formation of prostaglandin-like material by rabbit ovaries in vitro

Two experiments were carried out to investigate the effects of LH on the production of steroids and prostaglandin-like material by the rabbit ovary. After incubation and homogenization of the tissue, an aliquot of the homogenate was assayed for progesterone and 20 α-HP. The remainder of the homogenate was extracted for prostaglandins.

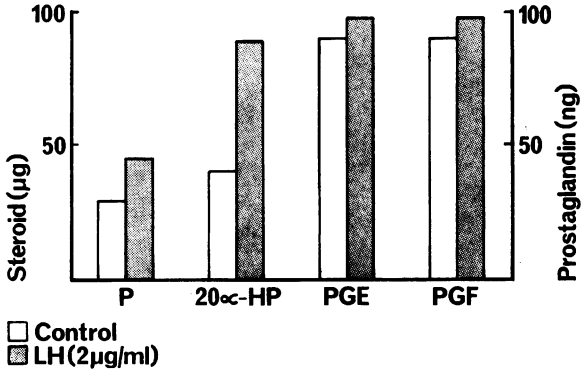

FIG. 2. Production of steroids and prostaglandin-like material by rabbit ovaries incubated in the presence and absence of LH, Expt. 1. Prostaglandin concentrations are based on results from assays on the rat fundus in terms of PGE_1, or on the rabbit jejunum in terms of $PGF_{2\alpha}$.

The results of the first experiment are shown in Fig. 2. Prostaglandin concentrations were estimated in 40% and 80% ethyl acetate in benzene eluates from silicic acid chromatograms. 'PGE' refers to material eluted with 40% ethyl acetate, assayed in terms of prostaglandin E_1, on the rat fundus. 'PGF' refers to material eluted with 80% ethyl acetate, assayed in terms of prostaglandin $F_{2\alpha}$ on the rabbit jejunum. In each case, the difference between the concentrations found in 'test' and 'control' extracts was small, and well within the estimated limits of error of the extraction and assay procedures. Measurements of steroid concentrations confirmed that the concentration of LH used (2 μg/ml) had been effective in stimulating steroidogenesis.

In the second experiment, prostaglandin E-like material eluted from the silicic acid columns was subjected to thin layer chromatography in the AI system. Material with smooth muscle stimulating activity was found in the zones of the plates corresponding with the position of prostaglandin E_1 on a marker plate run concurrently. This material was rechromatographed in the AII system. Zones corresponding with the positions of prostaglandins E_1 and E_2 on the marker plate were eluted and extracted (Holmes & Horton, 1968), together with zones of equal width immediately above and below the prostaglandin zones. Biological activity in the extracts was assayed against prostaglandins E_1 and E_2 on up to five different preparations (Table 1).

The results of the thin layer chromatographic investigations and parallel biological assays were compatible with the prostaglandin-like material extracted from the ovarian incubates consisting mainly of prostaglandin E_2. From the results of assays on the rat fundus, it appeared that the production of this prostaglandin had been increased slightly in the presence of LH. However, when the extracts were assayed on the other tissues, the differences between prostaglandin concentrations in 'test' and 'control' extracts were smaller, and are considered to be within the limits of error of the biological assays.

Small quantities of material with smooth muscle stimulating activity were also found in the zones of the AII thin layer chromatograms corresponding with the position of prostaglandin E_1 on the marker plate. The results of biological assays of this material, in terms of prostaglandin E_1, showed considerable variation between different tissues (greatest index of discrimination=2·4). The concentration of this material present in the 'test' extract showed no increase over that present in the 'control' extract.

TABLE 1. *Biological assays of material extracted from PGE_2 and PGE_1 zones of AII thin layer chromatograms of extracts of rabbit ovaries incubated in the presence and absence of LH*

		Biological activity		PGE_2 or E_1 equivalents ((ng/g)/3 h)		
		Rat fundus	Guinea-pig ileum	Jird colon	Rat uterus	Spinal cat blood pressure
PGE_2	Control	169	188	197	207	*
	LH (2 μg/ml)	223	215	223	179	179
PGE_1	Control	47	94	113	*	*
	LH (2 μg/ml)	45	72	54	*	*

* Not assayed.

The concentration of LH used in this experiment increased the production of progesterone from (24·0 μg/g)/3 h to (44·8 μg/g)/3 h, and that of 20 α-HP from (24·0 μg/g)/3 h to (103·8 μg/g)/3 hours. Thus, the LH was effective in stimulating steroidogenesis, but had no convincing effect on the production of prostaglandins.

Effect of LH on the release of steroids and prostaglandin-like material into rabbit ovarian venous blood

The results from four experiments with LH *in vivo* are shown in Fig. 3. 'PGE' represents smooth muscle-stimulating material detected in eluates from silicic acid chromatograms of plasma extracts, which behaved like a prostaglandin E in AI thin layer chromatography and/or parallel biological assays on the rat fundus, guinea-pig ileum and jird colon. Values are based on results from assays on the rat fundus, in terms of prostaglandin E_1.

In each experiment, the dose of LH (500 μg) was effective in stimulating the release of progesterone and 20 α-HP from the ovary. Only in one experiment (Expt. 4) was there any evidence for the presence in the venous effluent of biologically active material which behaved like a prostaglandin. Amounts present in the samples of blood collected after the injection of LH showed no increase over the concentration found in the initial control sample. There was no evidence, therefore, that LH stimulated the release of prostaglandins into ovarian venous blood.

Discussion

Prostaglandin E_1 decreased the production of progesterone by rabbit ovaries incubated in the presence of LH. The prostaglandin had no consistent effect on progesterone production when steroidogenesis was stimulated by exogenous cyclic AMP. This suggests that prostaglandin E_1 inhibits the action of LH on the rabbit ovary at some stage before the induction of increased tissue concentrations of cyclic AMP.

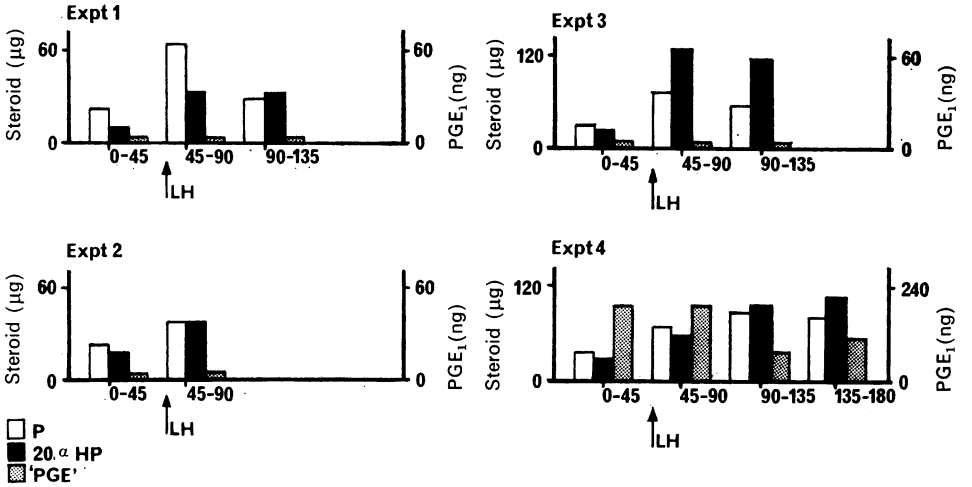

FIG. 3. Effect of LH on the output of progesterone (P), 20 α-hydroxypregn-4-en-3-one (20 α-HP) and 'prostaglandin E' (see text) into rabbit ovarian venous blood. Abscissa: time (min); 500 μg of LH injected at 45 minutes.

It was surprising that the prostaglandin did not cause a simultaneous decrease in the LH-stimulated production of 20 α-HP, as it is generally assumed that this steroid is formed only by the reduction of progesterone. It is unlikely that the prostaglandin affected the interconversion of the two steroids, for example by stimulating 20 α-hydroxysteroid dehydrogenase activity, since the decreased progesterone production in each experiment was not associated with a concomitant rise in 20 α-HP production. The results are more suggestive of a separate pathway to 20 α-HP, not involving progesterone as a precursor. Such a pathway, in which pregn-5-ene-3β, 20 α-diol is an intermediate, has been discovered recently in the rat ovary (Kuhn & Briley, 1970).

An alternative explanation for the lack of effect of prostaglandin E_1 on 20 α-HP production is that the activity of the enzyme 20 α-hydroxysteroid dehydrogenase is the limiting factor in the conversion of progesterone to 20 α-HP, rather than availability of the substrate. Under these conditions, the concentration 20 α-HP produced might be independent of the amount of progesterone available, above a particular substrate concentration.

Prostaglandin E_2 (1 μg/ml) had no consistent effect on steroidogenesis in the presence of LH. Since 1 μg/ml was the lowest concentration of prostaglandin E_1 which reduced the production of progesterone under these conditions, it would appear that prostaglandin E_2 is either less potent than E_1, or is without effect on the steroidogenic response of the rabbit ovary to LH. In an earlier series of experiments in which steroidogenesis was stimulated by a combination of human chorionic gonadotrophin and pregnant mare's serum, prostaglandin E_2 appeared to potentiate the production of 20 α-HP, without having any effect on progesterone formation (Bedwani & Horton, 1968).

Speroff & Ramwell (1970) have investigated the effects of prostaglandins on steroidogenesis in bovine ovarian tissue. In contrast to the results obtained in our present work with the rabbit ovary, prostaglandins E_1 and E_2 stimulated net progesterone synthesis and the incorporation of labelled acetate into progesterone. Prostaglandin E_2 also stimulated bovine luteal adenyl cyclase activity (Marsh, 1970). Thus, the prostaglandin mimicked the action of LH. Prostaglandin E_2 was reported to have no additive effect on progesterone synthesis when given in the presence of a maximal concentration of LH.

Prostaglandins E_1 and E_2 also stimulate progesterone production and adenyl cyclase activity in the mouse ovary, and it was suggested that prostaglandins might play an essential role in mediating the action of LH (Kuehl, Humes, Tarnoff, Cirillo & Ham, 1970). No measurements of prostaglandin release in response to LH were undertaken.

The concentrations of prostaglandin E_1 which were required to antagonize the action of LH on progesterone production in our experiments were rather high. In other systems where prostaglandins are thought to act by inhibiting the formation of cyclic AMP, effects have been seen with much lower concentrations. For example, hormonally-induced lipolysis was inhibited by 20 ng/ml of prostaglandin E_1 (Steinberg *et al.*, 1963). Inhibition of vasopressin-induced water permeability in the toad bladder was seen with concentrations of prostaglandin E_1 as low as $1{\cdot}7 \times 10^{-9}$M (0·6 ng/ml) (Orloff *et al.*, 1965).

The possibility was considered that some of the prostaglandin added may have been metabolized, or otherwise removed from the medium, during the incubation

period. Experiments involving the biological assay of incubation medium containing prostaglandin E_1, before and after incubation with ovarian tissue, indicated that this was not so. Although it was not confirmed that the biological activity detected was due to unchanged prostaglandin E_1, it was unlikely to have been due to known metabolites which are considerably less active than the prostaglandin itself on isolated smooth muscle preparations (Änggård, 1966).

If the hypothesis that prostaglandins function as feedback modulators of hormonal actions is true, they should be synthesized in, and released from, the ovary in response to LH. In neither the *in vitro* nor the *in vivo* experiments reported here was there evidence that concentrations of LH sufficient to stimulate steroidogenesis had any effect on the synthesis or release of prostaglandins from the ovary. It is possible that a local release could occur within the ovary, in which case prostaglandins might not appear in the venous effluent. If this were the case, it would be expected that evidence of increased prostaglandin concentrations would be found in ovarian tissue incubated in the presence of LH. In other situations where prostaglandins are released, this release appears to be preceded by a rapid synthesis within the tissues of origin (Ramwell, Shaw, Douglas & Poisner, 1966; Davies, Horton & Withrington, 1968; Coceani, Pace-Asciak & Wolfe, 1968). It is possible that prostaglandins may have been formed during the *in vitro* experiments, and subsequently metabolized during the course of the incubation, but exogenous prostaglandin E_1 did not appear to be metabolized during incubation with rabbit ovaries in the presence of LH. Furthermore, in one of the *in vitro* experiments, biologically active material was detected following the complete extraction and identification procedure, and substantial evidence was obtained for its identification as a mixture of prostaglandins E_1 and E_2. This showed that detectable concentrations of prostaglandin-like substances could occur in ovarian incubates, and any effect of LH on these concentrations should have been apparent in this experiment.

In view of the relatively high concentrations of prostaglandin E_1 which were required to inhibit the effect of LH on progesterone production, and the finding that prostaglandins are not apparently released from the rabbit ovary in response to LH, it seems unlikely that the prostaglandin E-like substances detected in the ovary have a physiological role in the regulation of steroidogenesis. Our results lend no support to the hypothesis that prostaglandins function as general modulators of hormonal actions which are mediated by cyclic AMP.

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