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EFFECT OF PROSTAGLANDINS ON STEROID BIOSYNTHESIS

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SUMMARY

The role of prostaglandins (PGs) as agents interfering in steroidogenesis is contradictory *in vivo* and *in vitro*. PGs may affect steroid production by (1) altered utilization of cholesterol via changes in esterase or synthetase enzyme levels, (2) by affecting levels of adenyl cyclase enzyme system and cAMP, (3) by increasing 20α-hydroxysteroid dehydrogenase levels. PGs may also indirectly influence steroid synthesis by modulating the secretion of gonadotropins. The more completely investigated action of PGs has been that of induced luteolysis on the corpus luteum of the luteal phase and of pregnancy. The *in vivo* luteolytic properties of $PGF_{2\alpha}$ have been, on occasion, contradicted by *in vitro* studies in which progesterone synthesis has been stimulated. The mechanism of action of PGs in luteolysis seems to be that of increasing production of inactive progestins. Other roles of PGs, such as stimulation of cAMP, estrogen production and testosterone secretion, are still a matter of continuous investigation. In bovines, $PGF_{2\alpha}$ increases release of this C_{19} steroid, while in the mouse, the peripheral testosterone levels are reduced, probably by inhibition of cholesterol esterase.

INTRODUCTION

Compounds of the unsaturated 20 carbon fatty acids known as prostaglandins (PGs) have been suggested and documented to play a role in modulating certain physiologic phenomena in animals and human beings. Recently, a little more stress has been placed on the role of PGs in reproductive physiology in general and in steroidogenesis in particular. PGs have been documented to influence ovulation [1–3], implantation [4], pseudopregnancy and pregnancy [5], parturition [6–7], lactation [8–10] in females, and testosterone production in males [11–13]. In addition to this, PGs have been reported to influence the secretion of hormones of the anterior and posterior pituitary [14–17] in general and, specifically, of pituitary gonadotropins[18–20], Batta, Niswender and Brackett, 1974, unpublished data. The prostaglandins which have been studied most extensively are $F_{2\alpha}$, E_1 and E_2; less information is available insofar as the other compounds of E, F, A and B series are concerned.

Recently, more stress has been placed on the luteolytic role of $PGF_{2\alpha}$ toward the development of a new contraceptive. $PGF_{2\alpha}$ has been shown to be the physiologic luteolytic agent in sheep [21]. In other animals, namely mares [22], cows [23], pseudopregnant and pregnant rats [24], rabbits [25], pseudopregnant and pregnant mice [26, 5], monkeys [27] and guinea-pigs [28], PGs seem to act as luteolytic agents, since they caused lowered plasma progestin levels in pregnant monkeys, rats and hamsters, caused visible regression of the corpora lutea in rabbits and guinea pigs, and induced ovulation in cows [29], mares [30] and guinea pigs [5].

In this report, an effort has been made to review briefly the effect of prostaglandins on steroidogenesis in animals *in vivo*. For this purpose, extensive use of the work done by various investigators has been made.

STEROIDOGENESIS

Steroidogenesis or steroid biosynthesis is a complex process; for the successful and continued production of steroid hormones, cell metabolism requires a sensitive balance among the various requisites. Basically, the following requirements should be met for steroid biosynthesis:

1. Adequate stores of precursors; namely, cholesterol, with efficient enzymes for utilizing the precursor pool.
2. Metabolic tools for generating substrates and cofactors required for steroid synthesis.
3. Stimulatory influences such as hormones must remain at a steady level.
4. Biotransformation and inhibitory factors must always be minimal.

Interference with any one or more of these processes might be sufficient to terminate or reduce steroid production. The skeletal process by which steroids are synthesized is as follows: The precursor of all steroids is cholesterol, which is of dietary origin or is synthesized by the organism from acetyl coezyme A. Steroid producing cells seem to rely mainly on the circulating sterol pool for their cholesterol. The basic conversion processes involved are given in Fig. 1.

ACETYL CO A (C2)
ACETOACETYL CO A (C4)
MEVALONIC ACID (C6)
ISOPENTENYL PYROPHOSPHATE (C5)
GERANYL PYROPHOSPHATE (C10)
FARNESYL PYROPHOSPHATE (C15)
SQUALENE (C20)
LANOSTEROL (C29)
CHOLESTEROL (C27)

Fig. 1. Intermediates in *de novo* synthesis of cholesterol.

Most of the cholesterol ready for conversion into steroids within steroid forming cells exists as esters of unsaturated fatty acids, presumably because the free sterol might readily diffuse from the cells into the intercellular fluid.

Esterification of cholesterol is catalyzed by two enzymes: Acyl CoA synthetase and sterol acyl transferase. Acyl CoA synthetase converts free fatty acids to CoA esters in the presence of CoA and ATP, while sterol acyl transferase directs the esterification of the fatty acid moiety to cholesterol. Once esterified, cholesterol is stored in cytoplasmic droplets along with smaller amounts of other lipids. But before this precursor pool can be utilized, the cholesterol must be liberated and that is accomplished by a sterol esterase. The esterol esterase hydrolyzes the cholesterol ester and cholesterol is thereby liberated for further utilization (Fig. 2). The whole process of conversion and reversion of cholesterol to and from esters is rather obscure, and the factors regulating this process need further intensive study for proper resolution of the whole chain of events responsible for intracellular transfer of sterols and steroid esters from one organelle to another and for keeping free cholesterol ready for conversion into steroid hormones.

TESTICULAR STEROID AND PGs: TESTOSTERONE

As indicated previously, PGs tend to influence the secretion of pituitary gonadotropins [2, 4, 13, 16, 17]. It is known that LH is the stimulator of testosterone but the biochemical mode of stimulation is not clearly elucidated. The LH released possibly by the PGs may influence the secretion of testosterone from the testes. This seems to be the case in the report of Eik-Nes[12] who found that compounds of the PGE series stimulated testosterone when perfused through the testes of the dog. On the other hand, through the injection of PGs in male mice, Bartke, Musto, Caldwell and Behrman[11] showed a fall in the plasma testosterone level. These investigators treated the male mice chronically and measured the peripheral plasma testosterone by radioimmunoassay. Administration of $PGF_{2\alpha}$ for a $3\frac{1}{2}$ week period did not affect the weight of the testes but lowered the blood testosterone level and elevated esterified cholesterol in the testes. Bartke and co-workers[11] suggested that this increase in testicular concentration of esterified cholesterol was due to inhibition of the utilization of esterified cholesterol, which could have been due to interference in enzyme involvement in cholesterol turnover or in the blood flow through the testes. However, it seems that when $PGF_{2\alpha}$ is given to male rat pups within 24 h of birth, the testicular weight increased at Day 45 but was not evident at Day 15 or Day 30 (Batta, unpublished data). This could be explained by the observation that in adult male rats, PGs cause sustained release of FSH [2, 4, 19] which might affect the weight of the testes. Further studies are evidently needed to elucidate the function of PGs in the testes. It will be of great interest if the levels of pituitary gonadotropins are also measured in the serum of the animals which are being treated with PGs and are simultaneously observed for their effect on testosterone secretion.

PGs AND ADRENAL STEROIDS

Neuroendocrine effect

Evidence that PGs of the E and F series tend to modulate the secretion of ACTH (adrenocorticotropic hormone) from the pituitary controlling the functioning of steroid production by the adrenals was provided by Peng *et al.*[15], Hedge[31], Coudert and Faiman[32], and de Wied *et al.*[33]. This was shown by *in vivo* studies on normal and hypophysectomized rats in which an increased ACTH release was noted, as determined by increase in plasma corticosterone levels and depletion of adrenal ascorbic acid and cholesterol after administration of PGE_1 but not after PGA_1 or $PGF_{2\alpha}$. Since this effect of PGE_1 was not observed in hypophysectomized rats or in those treated with morphine and pentobarbitone, Peng and colleagues[15] suggested that PGE_1 stimulated

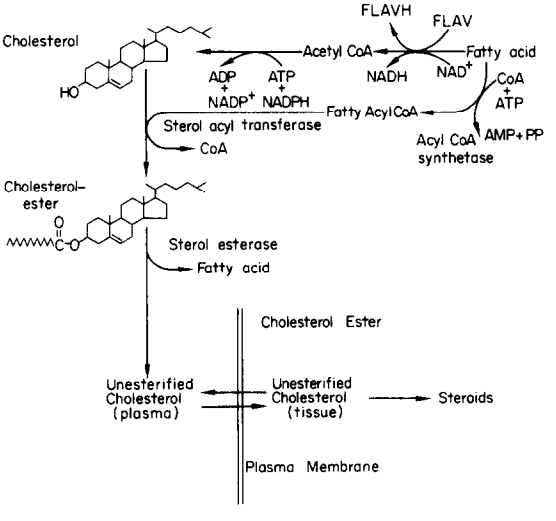

Fig. 2. Various steps involved in conversion of simple fatty acids to cholesterol. Esterification and deesterification of cholesterol for conversion to steroids is also shown.

ACTH release from the pituitary by acting on the higher centers in the brain and not directly on the pituitary or adrenal glands. However, Flack *et al.*[34], suggested that PGE_1 might not have any stimulatory effect on ACTH release but that the corticosteroidogenic effect may be due to direct adrenal stimulation. These authors demonstrated that corticosterone secretion was doubled when adrenal glands from acutely hypophysectomized rats were perfused *in vitro* with PGE_2. This stimulation was not observed with adrenals from intact rats; and, furthermore, the response to PGE_2 was insignificant 12 h after hypophysectomy. Injection of PGE_2 into acutely hypophysectomized rats also caused a significant rise in plasma and adrenal concentrations of corticosterone. However, 24 h after hypophysectomy, the effect was at its nadir.

The neuroendocrine factor in PG stimulation of ACTH secretion for corticosteroidogenic effect has thus apparently been minimized, though further studies are clearly indicated for a clearcut answer as to the role played by PGs in ACTH release.

Effect of PGs on corticosterone

PGs have been shown to directly stimulate the secretion of adrenal steroids [35]. In their elegant set of experiments, Funder and co-workers showed that PGE_2 was able to increase the concentration of corticosterone in the adrenal gland and in the perfusion medium. Furthermore, this increase was inhibited by a protein synthesis inhibitor, cycloheximide. The rise in plasma cortisol in men after an infusion of PGA_1 for 2–4 h was significant, and inhibition of the increased secretion was induced by dexamethasone [36].

As for the mechanism by which PGs may stimulate corticosterone secretion, several hypotheses can be put forward. The more attractive ones are:

(a) PGs may directly stimulate the adrenal glands and alter corticosterone secretion. This may be by regulation of intracellular cyclic AMP levels or by controlling the blood flow through the adrenal vasculature [34]. However, Zor and his colleagues[37] demonstrated that in rats, PGE_2 did not alter the cyclic AMP in adrenals from hypophysectomized animals, but a significant alteration in corticosterone resulted. Thus, the increase in corticosterone production by PGE_2 may be independent of cyclic AMP formation.

(b) Similarly, the hypothesis of blood flow regulation has not been given a higher degree of credence, since Funder, *et al.*[35], demonstrated that PGE_1 increased the flow of blood through the adrenals without affecting steroid secretion.

(c) PGs, due to their other pharmacological properties, are associated with alteration of cellular calcium movement [38]; and, since ACTH is known to affect cation transport and calcium uptake in the adrenal gland [39], prostaglandin may possibly induce corticosteroid production through such a mechanism.

PGs and aldosterone

Fichman *et al.*[36], recently reported that PGA_1 could selectively stimulate the adrenal cortex and increase aldosterone levels in the plasma of men. This seemed to be the result of a direct effect of PGA_1, since, under those conditions, there were no significant alterations in plasma renin activity, cortisol or serum electrolytes. These authors also showed that the same dose employed for a longer period not only increased aldosterone to three-fold the pretreatment value but also released significant amounts of cortisol.

The antagonistic properties of different PGs were supported by the studies of Blair-West *et al.*[40]. These investigators showed that PGE_1 was able to inhibit aldosterone secretion from transplanted adrenals while A_1 stimulated aldosterone release.

The physiological role of PGA_1 in the regulation of aldosterone secretion seems a paradoxical and controversial issue in man. There is a dichotomy in that PGA_1, on one hand, acts as a proximal natriuretic factor; and, on the other hand, it stimulates aldosterone secretion which leads to distal sodium reabsorption. Further investigations are required to resolve the apparently contradictory actions of PGA_1.

PROSTAGLANDINS AND OVARIAN STEROIDS

There have been increasing numbers of studies on prostaglandins and reproductive systems in recent years. The main reason for the interest seems to have been a desire to understand their role in reproductive biology and their future as potential contraceptives. To this purpose, recently, several reviews have appeared with varying degree of emphasis on different aspects of reproduction [41–45].

Progesterones

A surge of interest stemmed from the studies of Pharriss *et al.*[46], who demonstrated that $PGF_{2\alpha}$ caused regression of rat corpora lutea. Studies since then have been extended to primates [27], guinea-pigs [28], hamsters [47], rabbits [48], mice [5], sheep [49], cattle [50], and mares [30]. In all these species, $PGF_{2\alpha}$ was found to be luteolytic. Pharriss and Wyngarden[51] reported that in pseudopregnant rats, intrauterine infusion of $PGF_{2\alpha}$ for 2 days resulted in a sharp decrease in ovarian progesterone content and a rise in 20α-dihydroprogesterone; while Blatchley and Donovan[28] observed that $PGF_{2\alpha}$ injections caused morphological degeneration of corpora lutea of hysterectomized guinea-pigs. In addition to the reservation expressed below, the only species in which $PGF_{2\alpha}$ does not seem to effect progesterone secretion and pregnancy is the dog [52]. Evidence for the luteolytic effects of prostaglandins in primates and particularly in humans is less persuasive [27, 53, 54]. Despite the concerted efforts of several investigators, clear luteolytic effects have not been shown in the human. Although premature bleeding can be induced by prostaglandins in humans [42, 55], it is, however,

not always associated with a deline in circulating progesterone levels [55, 56].

The difficulty in demonstrating unequivocal luteolytic effects in primates and humans may be a failure to achieve a high enough concentration of prostaglandins in the ovary following systemic administration and not that prostaglandins have no luteolytic effects.

Mechanism of action of PGs

Morphological degeneration of corpus luteum is preceded by progesterone secretion diminution. The corpus luteum eventually shows profound degenerative changes in hamsters [25], guinea-pigs [28] and mice [5]. Okamura *et al.*[57], suggested that the ultrastructural luteolysis induced by $PGF_{2\alpha}$ in rats may not be similar to that occurring naturally.

The mechanism by which $PGF_{2\alpha}$ turns off secretion of progesterone by the corpus luteum is cloudy. To elucidate this phenomenon, various attractive hypotheses have been offered in the past. Pharriss[58] suggested vascular interference, while Labhsetwar[59] suggested alteration in gonadotropin secretion; and Behrman *et al.*[60, 61] and Strauss and Stambaugh[62] recommended local antigonadotropin actions of $PGF_{2\alpha}$ in the rat.

None of the above-mentioned theories accounts for the luteal degeneration in all species and under all conditions. These suggestions seem to indicate that the different changes associated with prostaglandin-induced luteolysis may vary among species. It may be possible that some or all of them could be involved at some levels of luteal degeneration under appropriate conditions.

The most favored theory is that of Behrman *et al.*[60, 61], and Strauss and Stambaugh[62]. The basic concept of this theory is that $PGF_{2\alpha}$ affects specific enzymes as a result of antagonism of luteotrophins at the pre-luteal level. Behrman and his colleagues suggested that $PGF_{2\alpha}$ in rats acts directly on the corpus luteum by neutralizing prolactin activity. $PGF_{2\alpha}$ depressed ovarian cholesterol ester turnover by an induced loss in cholesterol ester synthetase and, to some extent, in sterol esterase activity which would decrease the availability of cholesterol for conversion to progesterone. On the other hand, Strauss and Stambaugh[62] suggested that $PGF_{2\alpha}$ acted on rat corpora lutea to induce 20α-OH-SDH activity by preventing the luteotropic action of prolactin. The induction of 20α-OH-SDH by $PGF_{2\alpha}$ could be reversed by LH, HCG and prolactin under appropriate conditions (Fig. 3).

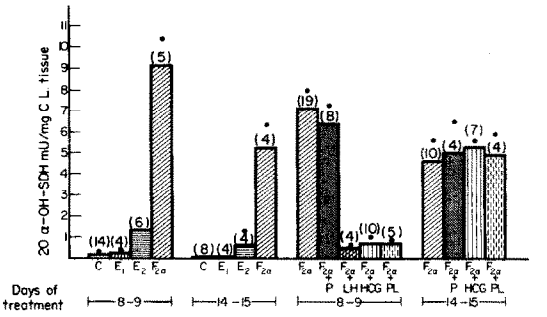

Fig. 3. Effect of treatment with various prostaglandins (E_1, E_2, $F_{2\alpha}$) and combination of various gonadotropins (P-progesterone, LH-luteinizing hormone, HCG-human chorionic gonadotropin, PL-prolactin) on induction of 20α-OH-SDH in pregnant rats.

PROSTAGLANDINS AND RECEPTORS

Recently Rao[63] working on bovine corpora lutea, showed that there are definite receptor sites for PGE_1, PGE_2 and HCG. The receptor sites seemed to be different for PGs and HCG, as demonstrated by inhibition of binding of the different molecules. The PG antagonist, 7-oxa-13-prostynoic acid (PY1), has been shown to inhibit the activation of adenyl cyclase by both PGE_1 and LH [64]. In addition, Rao demonstrated that PY1 affected the binding of only PGE_1 and not HCG to the receptors. PY1 was shown to be 160,000 times less active than PGE_1 in inhibiting the 3H-PGE_1 binding to the receptors. Rao suggested, on the basis of his observations, that, as labeled PGE_1 and HCF did not bind to the same site, thus they did not compete with each other for binding. This led to the hypothesis that PGE_1 and HCG bind to different sites on the same receptor or that each binds different receptor molecules. Similarly, Powell and his colleagues[65] detected the presence of receptor sites for $PGF_{2\alpha}$ in bovine corpora lutea. They further elaborated the necessity of various structural entities important for binding of different PGs. The affinity between the receptors and $PGF_{2\alpha}$ was found to be stronger as compared to PGE_1, PGE_2, PGF_1, PGA_2 and PGB_2. They went on to show that the carboxyl group, the three hydroxyl groups, the 5, 6-*cis* double bond, but not the 13, 14 *trans* double bond of $PGF_{2\alpha}$, were important for binding to the receptor. Powell *et al.*[65] suggested the nature of $PGF_{2\alpha}$ receptor to be that of lability to digestion with trypsin, protease and phospholipase A, as well as to treatment with N-ethylmaleimide.

PROSTAGLANDINS AND THE ADENYL CYCLASE SYSTEM

The involvement of adenyl cyclase-cyclic AMP system in the mechanism of action of prostaglandins has long been debated and is still controversial. Kuehl[66] has compiled literature recently both in favor and against this hypothesis. Prostaglandins of the E series are known to stimulate cyclic AMP formation in a dose-related manner [36a, 67]. The mechanism of action of prostaglandins of the E series was proposed based on this evidence and their affinity for a membraneous receptor [63]. However, the same was not proven true for the prostaglandins of the F series.

Goldberg and his associates[68] proposed a novel hypothesis on the basis of inverse relationship shown

to exist between cyclic AMP and cyclic GMP in response to stimulators. These workers, rather than attributing control of cell function solely to bidirectional changes in cyclic AMP levels (i.e., increase or decrease), suggested an equally important role for cyclic GMP. Kuehl *et al.*[69], found that 10^{-5} M $PGE_{2\alpha}$ caused a four-fold increase in cyclic GMP levels in the rat uterus within 45 seconds. More recently, Dunham and his colleagues[70] have shown that in bovine and canine veins, the cyclic GMP/cyclic AMP ratio increases under the influence of $PGF_{2\alpha}$ whereas the reverse situation was obtained for PGE_2. Since $PGF_{2\alpha}$ causes contraction and PGE_2 relaxation in this tissue, this finding is consistent with the concept that the opposing actions of the E and F prostaglandins are expressed at the cyclic nucleotide level.

Based on the documented evidence, it is tempting to speculate on the mechanism of luteolytic action of $PGF_{2\alpha}$ as follows:

$PGF_{2\alpha}$ gains relatively high concentrations in the ovarian artery by a counter-current phenomenon[21], which in turn constricts the ovarian arterioles and venules[58, Batta and Martini, unpublished data]. The reduced blood supply cuts off the nutrition of the corpus luteum. At the same time, $PGF_{2\alpha}$ gains access into the cell through the specific receptors [65] and induces stimulation of cyclic GMP [69]. $PGF_{2\alpha}$, either through cyclic GMP or directly, inhibits the enzymes involved in steroid synthesis and thereby nullifies the luteotropic effect of gonadotropins [60–62]. Simultaneously, $PGF_{2\alpha}$ may, at the hypothalamo-hypophyseal level, inhibit the release of pituitary gonadotropins [59] and prevent further support of the corpora lutea, and thus hastens its early demise.

Estrogen

While studies on the effect of prostaglandins on progesterone synthesis and secretion have been extensive, the estradiol secretion in response to these compounds has almost been neglected. Nevertheless, a few papers have appeared reporting the levels of plasma estrogens after administration of prostaglandins. In cows, Hixon *et al.*[71], demonstrated significant elevation in plasma estrone and estradiol-17β levels during the 24-h period following treatment. This elevation of plasma estrogen did not cause behavioral estrus. The authors suggested that increased estrogen secretion induced by $PGF_{2\alpha}$ may be from the follicular elements of the ovary.

However, there seems to be unanimous accord that, in man, infusion of $PGF_{2\alpha}$ for induction of abortion does not induce elevated levels of plasma estrogen [72–74].

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