ADVANCES IN

STEROID BIOCHEMISTRY AND PHARMACOLOGY

VOLUME 5

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Edited by

M. H. BRIGGS
School of Applied Sciences,
Gordon Institute of Technology,
Geelong,
Victoria, Australia

and

G. A. CHRISTIE
Syntex Research Centre,
Research Park,
Heriot-Watt University,
Edinburgh, Scotland

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CONTRIBUTORS

MAXINE BRIGGS *Kingston Centre, Cheltenham, Melbourne, Australia.**

J.-J. HICKS *Sección Bioquímica de la Reproducción, Departamento de Investigación Científica, Instituto Mexicano del Seguro Social, Mexico City, Mexico.*

A. ROSADO *Sección Bioquimica de la Reproducción, Departmento de Investigación Científica, Instituto Mexicano del Seguro Social, Mexico City, Mexico.*

J. R. STOCKIGT *Ewen Downie Metabolic Unit, Alfred Hospital, Melbourne, Australia.*

T. J. VECCHIO *Medical Research, Upjohn International Inc., Kalamazoo, Michigan, U.S.A.*

H. WATANABE *Health Protection Branch, Health and Welfare Canada, Ottowa, Ontario, Canada.*

* **Present address:** *Belmont Clinic, Geelong 3216, Australia*

EDITORIAL

This is the first editorial to be included in this series. It is planned that all future volumes will contain a brief comment on an area of current research in steroid biochemistry or pharmacology. This comment will not be a comprehensive review, but will draw attention to recent significant findings, or to promising areas of research.

STEROID HORMONES AND PROLACTIN IN HUMAN CANCER

It has been known for many years that the anterior pituitary gland of most mammals secretes a lactogenic hormone, which is responsible, primarily, for the initiation and maintenance of lactation. These 'prolactins' are secreted by specific acidophilic cells and are distinct from gonadotrophins and growth hormones. The prolactins of certain species also possess gonadotrophic ('luteotrophic') activity, e.g. rodent prolactin, while many prolactins also have some anabolic properties similar to growth hormones. The existence of a human prolactin was uncertain up until about 1972. The status of human pituitary hormones was complicated by the fact that human growth hormone (HGH) has potent lactogenic properties. A further difficulty is the unusual acidity of HGH, which migrates on electrophoresis ahead of human prolactin (HPL). In almost all other species, prolactin is more acidic than growth hormone, so that electrophoretic migration is the opposite of the human hormones.

Once the existence of HPL was firmly established, the question of its significance, if any, in human breast cancer was immediately raised. Pituitary ablation is known to induce objective responses in recurrent breast cancer in some patients. Cases of response to this procedure have been recorded in occasional patients in whom the ovaries had been removed previously. Could these responses be due to removal of prolactin which was stimulating growth of the carcinoma?

An alternative approach provided supportative evidence. With the recognition of HPL as an independent hormone, specific radioimmunoassays were developed to measure the concentration of HPL in blood. The first interesting result from such studies was the discovery that men and women had similar plasma concentrations and that women showed no significant fluctuation in plasma HPL during a normal menstrual cycle. Very high values were found during late pregnancy and in nursing mothers. The effect of drugs was then investigated. Compounds which deplete hypothalamic catecholamines, e.g. chlorpromazine, reserpine, etc., were found to stimulate prolactin release. A large intravenous infusion of tryptophan has a similar action. In contrast, *L*-dopa (*L*-dihydroxy-

phenylalanine) inhibits prolactin release, presumably by supplementing hypothalamic catecholamines.

Unlike gonadotrophins and corticotrophin, which are secreted in response to hypothalamic releasing factors, HPL is regulated by an inhibiting factor: prolactin inhibiting factor (PIF). Presumably the pituitary cells which make HPL do so with little or no stimulus, but are blocked by PIF. Combining this information with the effects reported above of various drugs on prolactin release, the control of prolactin can be understood if hypothalamic dopamine stimulates formation and release of PIF (so that HPL is blocked), while hypothalamic serotonin inhibits formation and release of PIF (so that HPL is secreted). It is possible that PIF-producing cells respond to the *balance* between dopamine and serotonin concentrations in their immediate environment.

If HPL is involved in human breast cancer, it follows that inhibition of HPL secretion by *L*-dopa should be helpful. A few objective responses to this preparation have been reported, but its widespread trial in carcinoma of the breast has not yet been reported.

The major paradox in HPL regulation must now be discussed. This is the relationship to steroid hormones, especially estrogens.

It is well known that estrogens inhibit or prevent lactation, and oral or depot synthetic estrogens are widely used for this purpose. An obvious explanation would be that estrogens inhibit prolactin secretion, but there is clear experimental evidence that the reverse is true. The plasma concentration of prolactin in an estrogen-treated patient is usually very significantly raised. The effect appears to be dose-related and there is no inhibition of prolactin release even at very high estrogen doses. It has been shown that estrogen implants into the hypothalamus of experimental animals causes a stimulation of lactation and release of prolactin. This strongly suggests that systemically administered estrogens have a double action related to lactation. The first is stimulation of prolactin release: the second is inhibition of prolactin *action* on the breasts.

Like most protein hormones, HPL appears to act via activation of adenyl cyclase in outer membranes of its target cells, so that cytoplasmic 3′,5′-cyclic adenosine monophosphate (cAMP) is formed as a 'second messenger'. The hormone is presumably bound to specific membrane receptors, so that the HPL-receptor complex can interact with a nearby inactive adenyl cyclase molecule to induce a conformational change and release an enzymically active site. Presumably estrogens in a dose sufficient to inhibit lactation, also disrupt some aspect of HPL action on breast cells. A likely site of action would be inhibition of prolactin-receptor formation, so that HPL was unable to bind its target cells.

This finding of stimulation of prolactin release by estrogens, yet simultaneous inhibition of breast stimulation, could also be relevant to breast cancer. Estrogens are widely used in post-menopausal women with advanced or recur-

rent carcinoma of the breast, yet are without value in younger women, where they are more likely to stimulate breast tumour growth. This observation is soundly based on clinical reports from many centres. It suggests that, if the clinical response to estrogen administration is mediated via HPL, then HPL is of importance only in post-menopausal patients. This could make biological sense if estrogens are able to block the formation of HPL receptors, for post-menopausal women have an estrogen-deprived internal milieu and could, therefore, have maximum HPL-receptors in their breast cells.

Further evidence has accumulated to reinforce the picture outlined above of molecular antagonism between estrogens and prolactin on the breast. It has been shown in mice that cortisol is able to counteract the inhibitory effect of estrogens on lactation. Apparently this action occurs within the breast and is not due to suppression of prolactin secretion by cortisol. Interestingly, it has been known for some time that lactation inhibited in rodents cannot be restored by administration of prolactin alone, but a combination of prolactin and corticotrophin is very effective.

In terms of hormone receptors, this could be interpreted as promotion of prolactin receptors by corticosteroids, but inhibition of their formation by estrogens.

Aside from *L*-dopa, other fairly specific drugs for the inhibition of HPL secretion have been developed. One of these is 2-bromo-α-ergocryptine (Sandoz CB 154), which suppresses plasma HPL concentrations of lactating women to less than the basal level seen in non-lactating subjects. This drug may prove to be of use in post-menopausal women with recurrent breast cancer and trials are awaited.

So far in this discussion control of HPL secretion has been assumed to be primarily under the control of hypothalamic PIF. It is, in fact, uncertain that this is true under even normal physiological conditions, for thyrotrophin releasing hormone (TRH) of the hypothalamus has been shown unequivocally to stimulate secretion of *both* HPL and thyrotrophin. It is possible, therefore, that the amount of HPL secreted by the pituitary is controlled by a balance between PIF and TRH. An attempt to represent these influences is shown in Fig. 1.

It seems likely that thyroid status could be of considerable importance in determining the rate of prolactin secretion. Plasma HPL concentration rises markedly following the administration of TRH (200 μg i.v.). The HPL response is poor in patients with Graves disease, but is exaggerated in hypothyroidism. This suggests that thyroid hormones modify the action of TRH on prolactin releasing cells of the pituitary.

To add a further complication, thyroxine administration has been shown to lead to very high plasma concentrations of prolactin. As thyroxine appears to suppress the action of TRH on the pituitary, the increase in prolactin secretion must be mediated other than by an action via THR. Suppression of PIH action

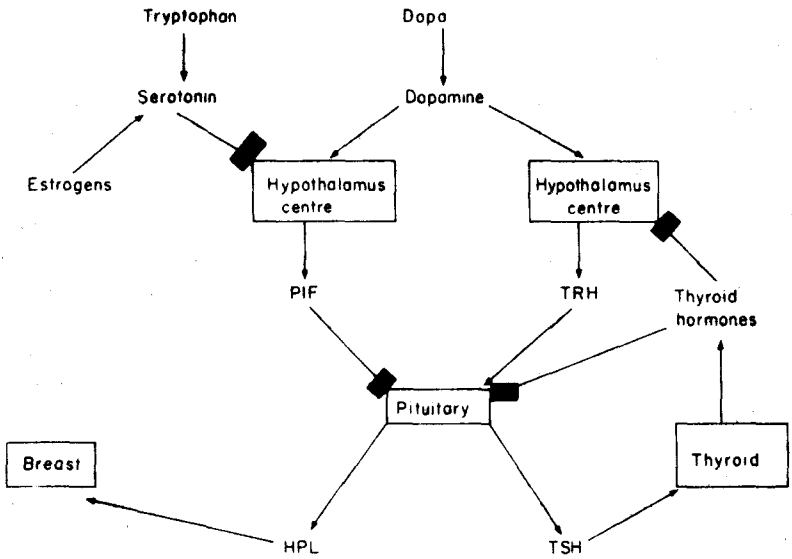


Fig. 1.

on the pituitary, or a direct stimulation of prolactin secretion, are two possibilities.

Interestingly, the rise in plasma thyroid hormones that follows administration of TRH is modified by estrogens and corticosteroids in a manner analogous to their modifying actions on lactation.

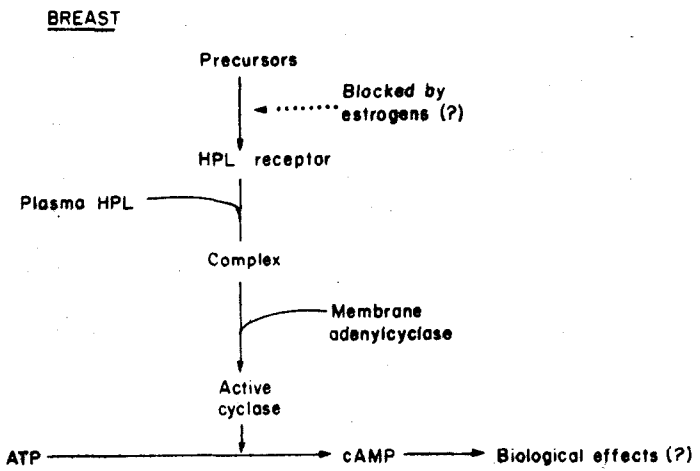
Several studies have now been undertaken to measure plasma HPL in women with breast cancer. There seems general agreement that plasma concentrations are similar to those seen in normal women. If HPL is involved in breast carcinoma, the latter observation implies that normal plasma HPL is sufficient to induce abnormal growth of the tumour cells, and that hypersecretion of the hormone does not occur. No study appears to have been reported of HPL receptors in human breast cancer.

The relevance of thyroid status may be important, for the clinical association between various hypothyroid conditions and breast carcinoma is well known. Patients with breast cancer could have an exaggerated prolactin response to TRH, though this does not appear to have been investigated.

Breast cancer patients treated with estrogen show an increased plasma prolactin, even when an objective response occurs during this treatment. In contrast, patients receiving nafoxidine (a non-steroidal antiestrogen) show no increase in plasma HPL, though occasional remissions associated with a decrease in plasma HPL following hypophysectomy have also been reported.

The present situation is tantalizing. It may indeed prove to be the long-sought for key to the successful treatment of advanced or recurrent breast cancer.

Mention must also be made of prostate gland carcinoma. Plasma HPL concentration is the same in men and women. Men with gynaecomastia, other than in association with drugs known to stimulate prolactin release (e.g. chlorpromazine) have normal plasma HPL. There has been considerable speculation as to whether males possess target cells for HPL and attention has centred on the prostate gland.



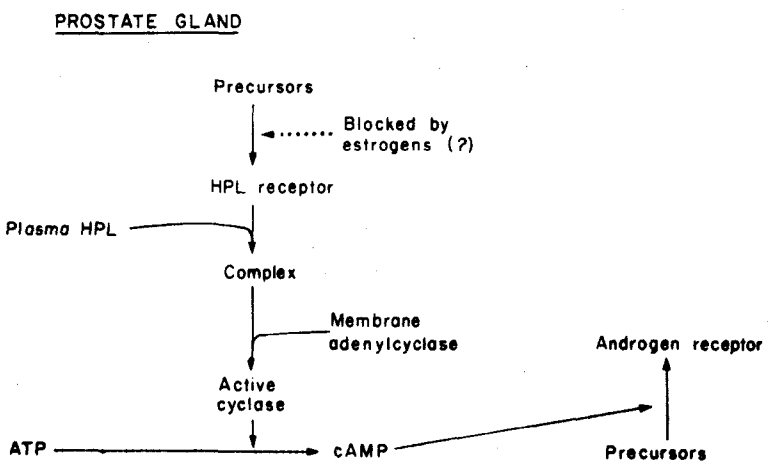


Fig. 2.

It is now well known that plasma testosterone is taken into prostatic cells, converted to 5α-dihydrotestosterone, which then binds to a specific receptor and effects biological changes by interaction with nuclear chromatin. Some evidence has now been published which suggests that androgen binding activity of the prostate is increased by prolactin. If this is true, a situation analogous to breast carcinoma may exist. Men with prostate carcinoma are often treated with estrogens. It is now clear that such men show high plasma HPL during this treatment. It would be tempting to suggest that estrogens inhibit the formation of prostate cell HPL receptors, so that no biological effect occurs, despite the high HPL concentration. Figure 2 suggests a simplified view of the process.

It has become clear in recent years that breast cancers differ significantly in the presence or absence of steroid hormone receptors. Some possess receptors for estradiol, others possess receptors for 5α-dihydrotestosterone, others possess both while others possess neither. Clinical response to endocrine therapy can be predicted relatively accurately in the advanced or recurrent disease from a knowledge of which receptors are present and which are absent. It is possible that a similar situation may exist for prostate carcinoma, and investigations of response to particular therapy in terms of receptors present in primary and secondary tumours are urgently needed. An assay for HPL receptors might greatly aid such studies.

Finally, clinical trials of various drug combinations in patients with carcinoma of the breast or prostate gland seem worth undertaking. Inhibitors of prolactin secretion (*L*-dopa or 2-bromo-α-ergocryptine) could be used in combination with estrogens to determine whether synergistic therapeutic effects can be developed. A study of estrogenic compounds for effects on prolactin secretion and inhibition of lactation could yield interesting new compounds with potential clinical usefulness. A substance which inhibited lactation without releasing prolactin, yet which yielded responses similar to estrogen in post-menopausal breast cancer, or metastatic prostate carcinoma, would be a major advance.

Michael Briggs

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LONG-ACTING INJECTABLE CONTRACEPTIVES

T. J. VECCHIO

Medical Research,
Upjohn International Inc.
Kalamazoo, Michigan, USA

I. GENERAL CONSIDERATIONS

The use of slow-release injectable preparations of progestogens for female contraception was first reported by Siegel (1963), who used hydroxyprogesterone caproate alone or in combination with estradiol valerate. Injectable contraception at present continues to be confined to the use of steroid hormones, either progestogens alone or progestogens and estrogens in combination. As such it is similar in effect to the oral contraceptives, with clinical differences dependent on differences in pharmacodynamics resulting from the parenteral route of administration, and the characteristic curve of release from an injection depot. The curve of activity of oral contraceptives, given for 21 days

and omitted for seven may be presented in schematic form as in Fig. 1, in comparison with that of three-monthly depot injections.

The oral contraceptives provide a relatively steady hormonal support to the endometrium, with a daily fluctuation in level from peak values a few hours after administration to a nadir just before the administration of the following daily dose (Cornette *et al.*, 1971). At the end of the 20- or 21-day administration period there is a clear-cut drop in hormonal levels resulting in relatively predictable withdrawal bleeding and prompt return of fertility. The one-month depot injectables, combining progestin and estrogen, also produce regular cyclic withdrawal bleeding, but with less precision than the oral contraceptives—due to the gradual decline in hormonal levels. Return of fertility is slow after one to two years of treatment. The three-month depot injections, containing progestin

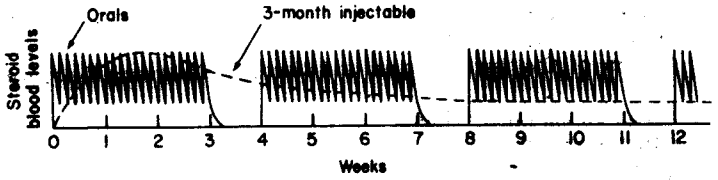

Fig. 1. Schematic representation of the progestogen blood levels achieved with combination oral contraceptives and three-month injectable contraceptives.

alone, do not produce a regular cycle at all, so that bleeding is irregular and usually sparse, and return of fertility is slow. The progestogen-only injectables also differ from the orals in not having an estrogen component, which produces certain differences in their metabolic effects. These distinct characteristics will be discussed in detail in the sections on the various injectable contraceptives. Much of this chapter will deal with medroxyprogesterone acetate, since it is the injectable in most widespread use, with by far the greatest amount of literature available.

II. MEDROXYPROGESTERONE ACETATE (MPA)

Chemically, MPA is 17α-hydroxy-6α-methylprogesterone, whose structure is shown in Fig. 2 along with that of natural progesterone, revealing the close structural similarity between them. Its molecular weight is 386.5, its melting point 205–209°C, and its solubility in water is less than 1 mg/ml. It is an active progestogen, with a subcutaneous potency in rabbits 20 times that of progesterone, both in preventing ovulation (Barnes *et al.*, 1959), and by the McPhail index (Jones *et al.*, 1966). Like progesterone it has a thermogenic effect, a single

injection of 50–100 mg resulting in a sharp rise in basal body temperature within 48 h, prolonged for as long as 50 days in some patients (Barfield and Greenblatt, 1961). It has been employed in oral contraceptives, which have contained 5 or 10 mg of MPA in combination with an estrogen.

MPA is available as a sterile aqueous suspension of the micronized steroid (Depo-Provera, Depo-Clinovir, Provera, Depo-Prodasone, Depo-Progevera)* in concentrations of 50, 100, 150 and 400 mg/ml. All concentrations have been employed for contraception, but most work has been done with 50 mg/ml preparation, given in a dose of 150 mg (3 ml). Subsequently, the 150 mg/ml

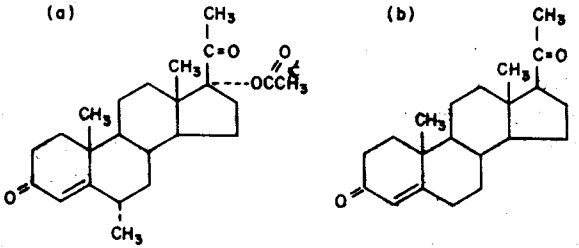

Fig. 2. The structural formulas for (a) medroxyprogesterone acetate and (b) natural progesterone.

preparation was developed so that the entire dose could be given in 1 ml. The 100 mg/ml concentration has been used experimentally in a dose of 3 ml (300 mg) every 6 months, and the 150 mg/ml has been employed in doses of 2 ml (300 mg) and 3 ml (450 mg) every 6 months. The 400 mg/ml preparation, currently in use in the USA in the palliative treatment of endometrial carcinoma where high doses (400–1,000 mg/week) are required, was dropped from study as a contraceptive in a dose of 1 ml (400 mg) every 6 months because of a high failure rate. This is perhaps due to a delayed absorption from the site of injection and is currently under study.

A. ABSORPTION, FATE AND DISTRIBUTION

Within 24 h after injection of a sterile aqueous suspension of MPA in the dog the suspending fluid disappears from the site, leaving the steroid in the form of a whitish amorphous pellet. Residual drug was found in two dogs 127 and 78 days after the intramuscular injection of 100 mg, 40.9 mg and 5.3 mg, respectively,

* Trademarks of the Upjohn Company. Also referred to in some publications as depot, or incorrectly, as 'depo-'medroxyprogesterone acetate or DMPA. In some European publications the designation MAP is used.