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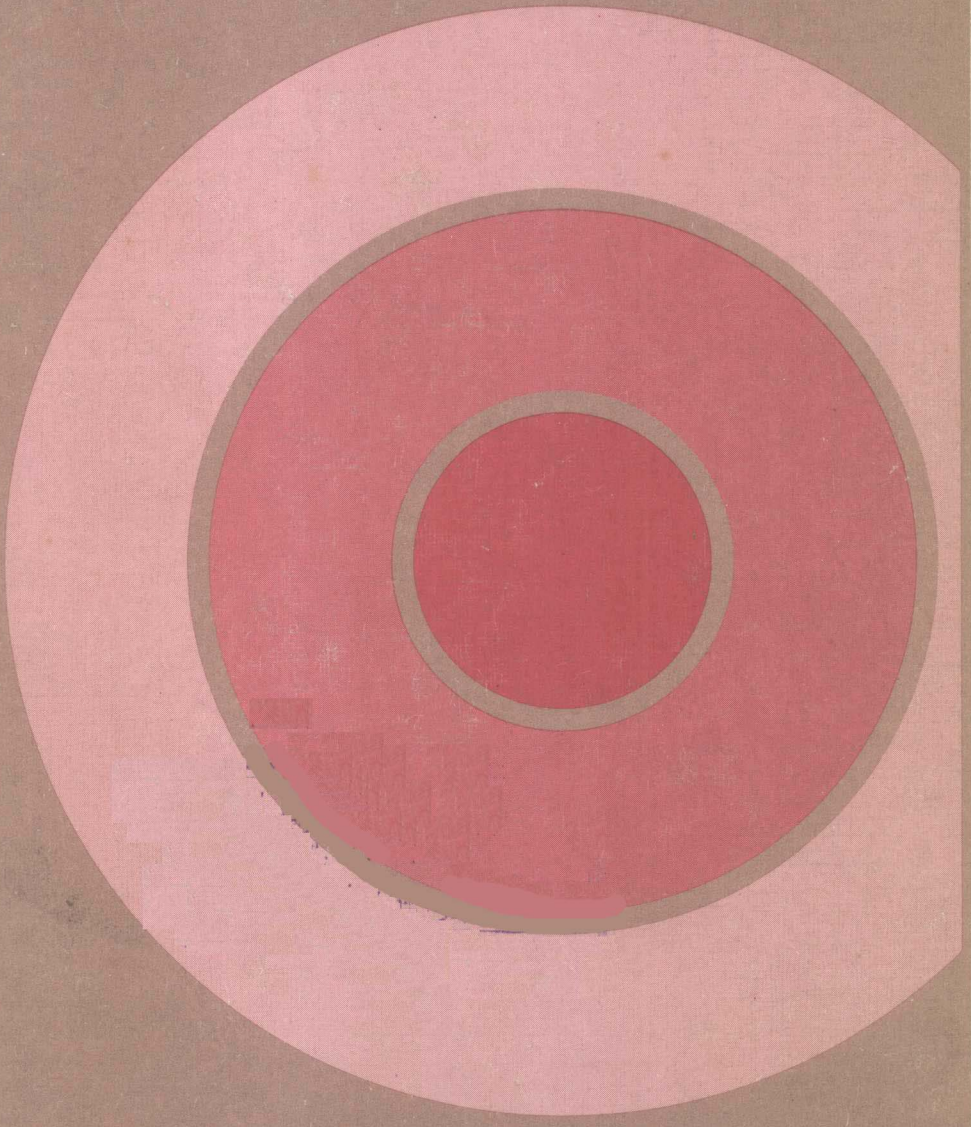

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V.H.T. James

St. Mary's Hospital Medical School
University of London

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V International Congress of Endocrinology

Hamburg, July 18-24, 1976

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Introduction

The scientific program of the V International Congress of Endocrinology was comprised of six plenary lectures; two hundred and twenty-two symposia lectures; nine hundred and thirty-one short scientific communications, of which a significant number were poster sessions, the latter format being a very popular one for the participants; six workshops dealing with new methods in Endocrinology; twenty-five noontime sessions devoted to recent developments in the diagnosis and treatment of important endocrine diseases; and five technical sessions dealing with advances in instrumentation applicable to research and patient care. Two thousand, eight hundred and fifty scientists and physicians from fifty-eight countries gathered to present and discuss results of their investigations and of their clinical experiences.

The plenary lectures were especially chosen to represent the most significant advances in the field of Endocrinology in the past four years. The Geoffrey Harris Memorial Lecture delivered by Dr. Roger Guillemin encapsulated the role of the brain as one of the most important endocrine organs of the body and related the discovery of new brain hormones and their actions on the brain itself and other endocrine systems. Dr. Friesen discussed new information dealing with the role of the pituitary and placental hormones in the normal and abnormal function of the breast, and Professor Gorski brought the highly complex subject of the molecular mechanism of action of sex hormones up to date. Dr. Grossman's lecture summarized recent discoveries which place the gastrointestinal tract as one of the major endocrine organs in the body and discussed current attempts to understand the role of the gastrointestinal hormones in digestion, metabolism and other bodily functions. Professor Orci's lecture addressed new insights into the manner in which hormones are secreted by endocrine cells, insights provided by the availability of powerful new technologies. Dr. Vermeulen's lecture considered the role of hormones in aging, a process which begins at birth, with a special reference to the patterns of sex hormone production throughout the human life span.

Each of the 53 symposia consisted of four or five lectures delivered by internationally recognized experts in the field. The symposia of the Congress were devoted to problems of hormones in behavior, hypertension, diabetes, obesity, cancer, male infertility, population control, growth and development, puberty and aging, nutrition and malnutrition and to newly-discovered endocrine diseases and other subjects of medical and biological importance.

One of the major functions of the International Congresses of Endocrinology is to provide a forum for the exchange of newly-generated scientific information by the Endocrinologists of the world. The noontime clinical 'Meet the Professor' sessions represented a new portion of the program at the International Congress. They were designed to provide opportunities for relatively intimate in-depth discussions between a distinguished clinical professor and small groups of practicing physicians devoted to recent developments in clinical medicine, and to the day-to-day problems in the treatment of patients with endocrine diseases.

Space and financial consideration do not permit the publication of all scientific activities at the Congress. Under the distinguished editorship of Professor Vivian James, a large number of the plenary lectures and symposia papers follow in this and its companion volume, thus permitting wider dissemination of the scientific contributions to interested scientists and students throughout the world.

J.C. Beck
Secretary General, International Society of Endocrinology

E. Knobil
Chairman, Programme Committee

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HUMAN PROLACTIN, PROLACTIN RECEPTORS AND PLACENTAL LACTOGENS

H.G. Friesen and R.P.C. Shiu

Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada

In this review we propose to examine three related topics. The first will be a consideration of some significant developments in our understanding of the role of human prolactin (hPRL) in states of health and disease. Secondly important advances in studies of PRL receptors will be highlighted. Finally the application of radioreceptor assays to the detection and measurement of placental lactogens will be illustrated.

HUMAN PROLACTIN

At the time of the IV International Endocrine Congress in 1972 the identity of human prolactin had just been established (1) and homologous sensitive and specific radioimmunoassay procedures were being employed for the first time to examine hPRL secretion in health and disease. In the intervening period a great deal of additional information has accumulated on the chemistry and circulating forms of hPRL, the physiological variables and pharmacological agents which influence serum hPRL concentrations. The physiological role of hPRL in a number of clinical settings has been established and the pathophysiology of hyperprolactinemia particularly as it affects gonadal function has been clarified to some extent. Several reviews concerned with hPRL have appeared (2-5). Because of limitations of space we will focus on the last mentioned topic to emphasize the important therapeutic advance that the introduction of bromocriptine represents in the treatment of hyperprolactinemia.

Continued research on the chemistry and biosynthesis of prolactin have led to several exciting developments. A prohormone of prolactin has been identified (6). Its molecular weight (MW) is approximately 28 000 compared to the principal component in pituitary extracts which has a (MW) of 22 000. Although the prohormone thus far has been identified only in nonprimate pituitary glands it is reasonable to suppose that similar biosynthetic mechanisms exist in human pituitary tissue. Both in the pituitary and in the circulation prolactin consists of several different molecular weight species (7). The major proportion (75-85%) in both pituitaries and in serum is found as "little" PRL, approximately 10-20% has a molecular weight twice that of the monomeric form and is referred to as "big" PRL and finally 1-2% may be of the "big big" form which has little biological activity as judged by radioreceptor assays.

With homologous radioimmunoassays mean serum prolactin concentrations have been reported to range from a low of 3 ng/ml to a high of 20-30 ng/ml. It appears that over several years there has been a gradual decrease in mean levels in normal subjects and perhaps representative figures available now would be in the neighbourhood of 5-7 ng/ml. The upper limit of the normal range varies somewhat among laboratories and may depend on how carefully standardization of time and circumstances of sampling have been controlled. In our laboratory concentrations which are repeatedly above 20 ng/ml in resting, fasting individuals are regarded as abnormal. On the other hand single random samples in otherwise normal individuals may occasionally reach values as high as 30 ng/ml.

Serum prolactin concentrations are affected by a number of physiological variables including age, sex, pregnancy, lactation and stress. In addition to these factors which cause major differences in serum prolactin concentrations, diurnal variations of serum prolactin occur with the highest levels observed at night (8). The nocturnal increases in fact are sleep related and normally account for levels which are perhaps 150-170% of the 24-hour mean. Frequent sampling (every 20 minutes) has revealed that

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serum prolactin concentrations also vary episodically in a manner similar to that observed for serum LH. The amplitude of these oscillations increase during the night and possibly at various stages in the menstrual cycle.

PROLACTIN EFFECTS ON OVARY AND TESTIS

One of the important clinical advances in studies of human prolactin has been the recognition of the role played by prolactin in regulating gonadal function. The possibility that prolactin is involved in ovarian function was dismissed by some after initial studies failed to reveal significant differences in serum levels throughout the menstrual cycle. Studies by McNatty and his colleagues (9,10) however, indicated that although serum prolactin concentrations did not vary greatly throughout the menstrual cycle, in ovarian follicular fluid major differences in prolactin concentrations occurred during the cycle. Moreover, changes in prolactin concentration in tissue culture media had a profound effect on progesterone secretion by human granulosa cells in vitro. High concentrations of prolactin (100 ng/ml) inhibited progesterone secretion whereas prolactin concentrations in the neighbourhood of 10-20 ng/ml maintained progesterone secretion. Finally the complete elimination of prolactin with the use of antiserum markedly reduced progesterone secretion. This compelling evidence leaves no doubt that prolactin by a direct action on human ovarian cells influences progesterone secretion. A second direct action of prolactin on the ovary has been identified; namely, the regulation of LH receptors in the ovary (11). The latter in turn mediate the action of LH and presumably an excess and more certainly a deficiency of LH receptors would be expected respectively to enhance or diminish the steroidogenic response of the ovary to LH.

Abundant clinical evidence also is available that hyperprolactinemia has a deleterious influence on gonadal function in human subjects. It has been suggested that the more prolonged periods of amenorrhea and anovulatory cycles in women who breast feed result from increased serum prolactin levels found in this group compared with women who do not (12). Other experiments also suggest that the suckling stimulus may be less important in this circumstance than the prolactin surges (13). Increases in serum prolactin produced by pharmacological agents however have been successful in some circumstances in preventing ovulation by blocking the midcycle LH release. The most clear clinical demonstration that hyperprolactinemia causes amenorrhea, however, is observed in patients with PRL secreting microadenomas of the pituitary gland. Upon successful selective extirpation of the tumour, serum PRL levels fall to the normal range in a matter of hours and menses resume in a matter of weeks (14). Similar effects are seen when patients with hyperprolactinemia are treated with the ergot alkaloid bromocryptine (CB-154). This dopaminergic agent is a remarkably effective inhibitor of PRL secretion regardless of whether a tumour or a functional disorder is present. Hyperprolactinemia is observed in some 5-20% of women with amenorrhea (15) so that it now becomes important to make the diagnosis as therapy of these patients is possible whereas previously therapeutic regimens using gonadotropins or clomiphene often met with failure.

The mechanism by which hyperprolactinemia causes amenorrhea has been examined, and it appears that elevated PRL levels act at three anatomical sites to impair gonadal function. PRL has a direct antigonadal effect inhibiting the response of the ovary to exogenous gonadotropins (16). In addition, PRL at low levels enhances and at high levels inhibits progesterone secretion by ovarian cells (9,10). At the hypothalamic level two abnormal responses have been noted in hyperprolactinemic subjects. The first is the failure of estrogen to elicit a positive feedback on LH secretion (17) and the second is a loss of the LH episodic secretory pattern at least in women with longstanding hyperprolactinemia (18). Finally at the level of the pituitary a diminished LH response has been noted in the postpartum period in women who breast feed compared with those who do not (19). One explanation which has been offered for the difference in response is that the increased PRL levels in women who breast feed may have altered the pituitary sensitivity to LH-RH, but other possibilities such as decreased LH stores must be considered as well. In women therefore PRL unquestionably influences gonadal function.

Clinically the major problem in the evaluation and management of patients with hyperprolactinemia arises from the difficulty of distinguishing patients with a "functional" disorder from patients with a pituitary lesion - microadenoma. No dynamic tests are particularly helpful and the overlap in basal serum PRL values in the two groups is very great indeed. In large lesions, of course, serum PRL values increase and as

values exceed 200 ng/ml the diagnosis of pituitary tumour becomes more certain. The only objective clue to differentiating a functional lesion from a pituitary tumour depends on the recognition of asymmetry of the floor or "blistering" of the sella turcica often visible only in tomograms of the region (20).

Treatment of patients with amenorrhea and/or galactorrhea and hyperprolactinemia depends on the cause. All secondary causes of hyperprolactinemia first must be excluded (21). If radiographic abnormalities are present, transsphenoidal hypophysectomy is recommended. However, occasionally even the most skillful neurosurgeon may find no tumour and we know of this circumstance in several cases (22). However, when a tumour can be identified and totally removed at operation the results are most gratifying (14). Medical therapy with bromocryptine is now a practical alternative and in almost all cases is effective in decreasing prolactin to the normal range. In a Canadian study of 80 patients menses resumed in 80% and of women who desired pregnancy 75% achieved their goal within six months of the onset of treatment (23). Unfortunately remission of the hyperprolactinemia is not permanent in the majority. One added benefit of bromocryptine therapy which was observed in one patient with a PRL secreting pituitary tumour who refused surgery was that visual field defects which were present initially disappeared during the six month period of therapy suggesting that bromocryptine was exerting an antitumourigenic effect (Ezrin, C. and Corenblum, B. unpublished observation). From experimental studies in rats such an effect might have been anticipated.

In the male basal prolactin levels influence testosterone secretion as well (24) and again abnormal elevations of serum PRL cause a decrease in testosterone secretion accompanied by loss of libido (25). The syndrome is reversible when the hyperprolactinemia is corrected. The mechanism of the decreased testosterone secretion is not clear. In patients with hyperprolactinemia the testes are capable of responding to exogenous hCG with an increase in testosterone secretion and Tolis et. al. (26) have suggested the major defect in this syndrome is due to a decrease in LH secretion, presumably via a central mechanism. In the testes as in the ovary PRL also modulates the LH receptor - a decrease of PRL results in a decrease of LH receptors (27). It has been generally assumed that the profound loss of libido observed in this syndrome is the consequence of diminished testosterone secretion. However, in at least one patient the exogenous administration of testosterone for several months failed to improve libido whereas two weeks after bromocryptine therapy was initiated, prolactin levels fell to the normal range and libido and potency returned (G.E. Wilkins unpublished observations). These findings strongly suggest that PRL may be exerting a direct central inhibitory effect on libidinous drive. An alternate possibility to be considered is that PRL may be inhibiting the conversion in the brain of testosterone to dihydrotestosterone and perhaps the latter exerts a more potent behavioral effect on sex drive.

PROLACTIN RECEPTORS

The biological effect of a hormone is dependent on at least two factors, serum concentrations of a hormone and target tissue responsiveness. Although in endocrinology it is recognized that both parameters may vary independently less emphasis has been placed on changes in tissue responsiveness as a major variable which influences the biological effectiveness of a hormone. In the past few years, however, increasing attention has been focused on changes in hormone receptors as one determinant of tissue responsiveness (28,29). From this research the concept has emerged that receptors are discrete and dynamic entities with defined tissue and intracellular distribution. Hormone receptors like hormone secretions are under the control of complex regulatory mechanisms. In the case of prolactin the subcellular distribution of receptors has been examined and plasma membranes (30) as well as Golgi (31) contain PRL receptors. The tissue distribution of PRL receptors in each species is unique and in each species there are major sex differences in tissue content of PRL receptors (32,33). Tissue content of receptors also varies with age and hormonal milieu and each tissue receptor responds in a specific manner to the same stimulus. Thus for example estrogens stimulate PRL receptors in the rat liver while in the kidney or prostate there is a decrease in PRL specific binding sites and in other tissues no changes are noted.

We have used the rat liver as a model for studies of the control of PRL receptors. It is apparent from these data that many factors influence PRL binding sites in the liver. These include estrogen, testosterone, growth hormone, ACTH through an adrenal mechanism and PRL itself (34,35,36). Of particular interest is the autoregulatory influence of PRL on specific binding sites in the liver (37) and more recently similar results

have been observed in the rat mammary gland in the early postpartum period (38). This positive interaction by PRL on its own receptor is in sharp contrast to the situation observed with insulin or catecholamines where there appears to be a down regulation by the hormone of its own receptors. It would seem that the positive effect of PRL on its own receptors provides a powerful amplification mechanism for the action of PRL. Parenthetically it might be worth mentioning that at least three effects of PRL on the liver have been reported in order to dispel the notion that PRL binding to the liver occurs principally because the latter degrades or inactivates the hormone. PRL increases the number of estrogen receptors (39), ornithine decarboxylase activity (40) and somatomedin release (41) in the liver.

Studies of hormone receptors also suggest that synergism between hormones may be mediated at the receptor level. For example in rats made deficient in PRL in both ovary and testes the LH receptor decreases. The steroidogenic response of the ovary to LH after PRL treatment is often greater than to LH alone and possibly the former is acting by maintaining LH receptors and thus enhancing the response to LH. However, the precise relationship between receptor level and tissue responsiveness is complicated. Unless the receptor is rate limiting, significant changes in receptor levels may occur without important differences in hormone response. Indeed the whole subject of occupancy, negative cooperativity, spare receptors, etc. as it influences hormone responsiveness is a topic which is under active investigation in a number of laboratories. There can be no doubt, however, that when receptors are reduced to a very low level, either by natural antibodies in the case of insulin (42) or by exogenous antibodies in the case of PRL (43), that biological effectiveness of the hormone is either greatly reduced or abolished.

Studies with antibodies to PRL receptors began when PRL receptors from rabbit mammary glands were solubilized and purified by affinity chromatography (44). Guinea pigs were immunized with partially purified receptor preparations and the antiserum obtained was used for both in vitro and in vivo studies. The antiserum (or gamma-globulin fraction) inhibited the binding of PRL to rabbit mammary gland explants, as well as to crude or purified membrane fractions from this tissue without affecting the binding of insulin. In addition, the antiserum inhibited PRL binding to membranes prepared from other rabbit tissues as well as membranes from tissues obtained from other species(45). Finally and most importantly the antiserum blocked the action of PRL in stimulating casein synthesis and aminoisobutyric acid transport, but was without effect on insulin stimulated glucose transport (43). When the antiserum was administered to rats during an estrus cycle the most notable finding was an increase in the number of old corpora lutea and an increase in serum PRL concentrations (46). These in vivo results suggested that the antiserum had blocked the luteolytic effect of PRL on the ovary and that a deficiency of PRL was sensed, activating compensatory mechanisms to increase PRL secretion in an attempt to overcome the deficient state. These experiments provide conclusive evidence that PRL binding sites are involved in mediating the action of the hormone on the mammary gland and the ovary.

RADIORECEPTOR ASSAYS AND PLACENTAL LACTOGENS

In our studies of the radioreceptor assay for PRL and lactogens we employed the rabbit mammary gland membrane fraction as the source of the receptor. The specificity and sensitivity of the receptor assay has been reported elsewhere (47). In striking contrast to radioimmunoassays, radioreceptor assays are not species specific, but rather "class" specific. Thus in both a RRA for prolactin or growth hormone, pituitary hormones from a member of species can be detected. Indeed the RRA specificity in general will be very similar to the bioassay data obtained using a particular tissue. However, if there are major differences in peripheral degradation or clearance rates among hormone preparations, the RRA estimates of potency may deviate considerably from estimates based on in vivo studies. In the case of the rabbit mammary gland, human growth hormone (hGH) and human placental lactogen (hPL) were previously shown using in vivo bioassays to exhibit PRL-like activity equal to that observed with ovine PRL standards. Similar results were obtained with the RRA. Using a variety of classical bioassays placental lactogens have been identified in a number of species (48) but had been measured only in the human and monkey during pregnancy. As the radioimmunoassays for hPL and mPL were species specific they could not be used for measurement of PL in any other species. On the other hand with RRA we have been able to identify and measure PL in nine species (49). We and others have used the RRA to purify and characterize ovine (50,51), bovine (52), caprine (Currie and Friesen unpublished observations) and rat (53) PL. Pituitary PRL from these and other species could also be detected using