A STUDY OF DRUGS THAT INHIBIT PROLACTIN SECRETION

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ABSTRACT

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PROLACTIN SECRETION

By

Marie Catherine Gelato

1. The effects of pentobarbital (PB) on the pituitary and on hypothalamic PIF activity were studied in female rats. An injection of PB completely depleted the hypothalamus of PIF activity by the end of 10 minutes, and 2 hours later PIF activity was still lower than in control rats injected with saline. Addition of PB to a pituitary incubation system resulted in inhibition of prolactin release, indicating that PB can directly inhibit prolactin release by the pituitary. The initial brief rise in serum prolactin observed after injecting PB is believed to be due to a rapid decrease in hypothalamic PIF activity, whereas the subsequent prolonged fall in prolactin is believed to be due to a direct inhibitory action on the pituitary.

The effects of another barbituric acid derivative, thalidomide, was determined on serum and pituitary prolactin levels on the afternoon of proestrus. All three doses

used, 5, 10, and 25 mg/100 gm body weight, completely blocked the proestrous afternoon surge. A dose-response effect of thalidomide was indicated, since the highest dose of thalidomide used produced the greatest blockage of the proestrous prolactin peak, whereas serum prolactin levels were progressively increased when lower doses of thalidomide were used. Pituitary prolactin concentrations were increased in all thalidomide-treated animals as compared to controls. Thalidomide, like PB, decreased serum prolactin levels and blocked the proestrous afternoon surge. It is possible that thalidomide exerts its inhibitory effect on prolactin release by directly blocking the pituitary.

2. A single intraperitoneal injection of iproniazid on the afternoon of proestrus, or cocaine on the morning of proestrus, in female rats caused a significant reduction in serum prolactin levels. Iproniazid completely blocked the afternoon proestrus rise of prolactin whereas cocaine caused a reduction in the pre-peak values of prolactin on the morning of proestrus. Several doses of cocaine were tested, and in some cases high doses of cocaine first inhibited and then stimulated prolactin release. All doses of cocaine used had no effect on pituitary prolactin concentration as compared to controls. The inhibitory effects of iproniazid and cocaine on prolactin release may be related to their demonstrated ability to increase catecholamine levels in the hypothalamus.

3. Daily injections of ergocornine, 400 μg and 800 μg per rat, a dose known to suppress both prolactin and LH levels, were given to female rats for one estrous cycle. Sixteen of the 32 rats also were given a single injection of 1 mg of prolactin on the afternoon of proestrus. As a result of the ergocornine treatment the size of the ovaries and the number of corpora lutea were significantly increased as compared to controls. The animals receiving both ergocornine and prolactin showed no change in ovarian weight or number of corpora lutea when compared to controls.

Similar experiments using iproniazid were carried out for one and three estrous cycles. The animals given iproniazid in both studies showed an increased number of corpora lutea and increased ovarian weight. Iproniazid-prolactin treated animals showed no change in ovarian size or number of corpora lutea as compared to controls. These experiments add further proof for the role of prolactin as a luteolytic agent during the estrous cycle.

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Marie Catherine Gelato

A THESIS

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Dedicated

to my

Mother and Father

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INTRODUCTION

Drugs have served as tools for studying the control and functions of prolactin and other pituitary hormones. Reviews by Meites (1962; 1963) and Coppola (1971) have shown the importance of drug studies in elucidating prolactin physiology. Drugs have been used to demonstrate the relationship of the hypothalamus and its hypophysiotropic hormones or factors to prolactin release and have led to a better understanding of the role of prolactin in lactation, mammary development, mammary tumorigenesis, and ovarian function.

Recently it was reported (Wuttke and Meites, 1970) that Na pentobarbital initially stimulated and then inhibited prolactin release. Therefore, it was of interest to determine the site of action of Na pentobarbital by studying its effects on the hypothalamus and pituitary. Since Na pentobarbital is a barbituric acid derivative, it also was of interest to know if another drug in this class, thalidomide, would have the same effect on prolactin release.

Brain catecholamines recently have been demonstrated to influence the release of prolactin and other pituitary hormones. An increase of catecholamine levels in the

hypothalamus is believed to inhibit prolactin release, while a decrease of catecholamine levels in the hypothalamus is believed to stimulate prolactin release. Certain drugs can be injected to alter catecholamine levels in the hypothalamus. Iproniazid blocks the enzyme monoamine oxidase and cocaine blocks the reuptake of catecholamines by nerve endings. Through these mechanisms both drugs increase catecholamine concentrations in the hypothalamus. Because of these actions of iproniazid and cocaine on brain catecholamine levels, it was of interest to study their effects on prolactin release.

Prolactin levels in the serum rise on the afternoon of proestrus together with LH and FSH, and remains high on the morning of estrus during each cycle. Suppression of this rise of prolactin by ergocornine resulted in a significant enlargement of the ovaries due to the accumulation of many old corpora lutea, whereas administration of prolactin prevented these effects of ergocornine (Wuttke and Meites, 1971). It was postulated from this work that the rise of prolactin during the estrous cycle causes the luteolysis of the old crop of corpora lutea from the previous cycle. It was of interest therefore to determine the effects of high doses of ergocornine known to suppress both prolactin and LH on luteal function, and also to observe whether another drug that depresses prolactin secretion, iproniazid, could produce the same effects as ergocornine.

LITERATURE REVIEW

I. Functions of Prolactin

Prolactin is one of six hormones secreted by the anterior pituitary. In mammals it has been closely associated with growth hormone (GH) and has growth promoting properties in some species (Bern and Nicoll, 1968). Both prolactin and growth hormone are synthesized and released by acidophils within the anterior pituitary (Meites, 1966). Prolactin is a large polypeptide, and only the structure of ovine prolactin has been determined thus far (Li *et al.*, 1969).

The role of prolactin in mammals has become defined more clearly in the last decade. It has been well established that prolactin plays a major part in normal and neoplastic mammary gland development, lactation, and luteal function in the rat, mouse, ferret and hamster (Meites and Nicoll, 1966). Since the literature on prolactin physiology is vast, I will attempt to present some of the most important and classical references. Other more detailed reviews include those by Cowie and Folley (1955), Meites (1962), Nicoll and Bern (1964), Meites and Nicoll (1966) and Bern and Nicoll (1968).

The ovaries and the anterior pituitary are the two most important organs in the control of growth and development of the mammary glands. The classic experiments by Lyons and co-workers (1958) used hypophysectomized, ovariectomized, and adrenalectomized rats as a model, and showed that injections of estrogen and GH produced duct growth whereas injections of estrogen, progesterone, GH and prolactin elicited lobulo-alveolar growth. These studies were confirmed by the in vitro experiments of Elias (1957) and Rivera (1964). Undeveloped mammary glands from mice and quinea pigs were cultured for 5-7 days with combinations of insulin, estrogen, progesterone, GH and prolactin, and these mammary glands showed lobulo-alveolar growth.

However, other evidence indicates that anterior pituitary hormones alone, prolactin and GH, can induce mammary lobulo-alveolar growth equivalent to that seen in pregnancy in the absence of ovarian hormones. Transplantation of a pituitary mammotropic tumor into adreno-orchidectomized rats of the inbred Fischer strain resulted in mammary lobulo-alveolar growth equivalent to that seen during pregnancy (Clifton and Furth, 1960). Related experiments by Talwalker and Meites (1961) showed that prolactin and GH injections into randomly bred Sprague-Dawley rats are enough to induce lobulo-alveolar growth in the mammary glands. They used rats which were adrenalectomized and ovariectomized or adrenalectomized, ovariectomized and hypophysectomized and

gave injections of GH and prolactin or prolactin alone, and observed considerable lobulo-alveolar growth by either treatment. In sexually immature female rats hypophysectomized and implanted in the right inguinal region with an anterior pituitary from a mature rat, mammary lobulo-alveolar growth occurred locally only near the implanted area. The transplanted pituitary has been shown to release both prolactin and GH (Meites and Kragt, 1964).

It seems evident that prolactin and GH are the predominant factors in mammary gland development. It has been demonstrated by Turner (1939), Lyons et al. (1958) and by Meites and Hopkins (1961), that ovarian hormones have no ability to stimulate mammary gland development in the absence of anterior pituitary hormones. It has been suggested by Meites and Nicoll (1966) that the gonadal steroids stimulate secretion of anterior pituitary hormones (prolactin and GH), and synergize with the pituitary hormones to sensitize the mammary tissue to these hormones.

In addition to its role in normal mammary development, prolactin plays an important part in the development of mammary tumors. Huggins (1965) reported that a single intravenous injection of 7,12-dimethylbenzanthracene (DMBA) into female Sprague-Dawley rats, 50-60 days old, was able to produce mammary tumors. This has become a useful model for studying mammary cancer. With this preparation it is possible to study the relationships between mammary tumors,

the hypothalamus, the anterior pituitary and the ovaries. Ovariectomy, performed before carcinogen treatment, prevents tumor induction (Huggins et al., 1961; Dao, 1962; Talwalker et al., 1964; Welsch et al., 1968). However, it lengthens mean latency period and reduces incidence of tumors when performed after carcinogen treatment but before tumor appearance (Huggins et al., 1959). After mammary tumors have developed, ovariectomy causes regression of these tumors (Huggins et al., 1959; Sterental et al., 1964). Hypophysectomy has a more drastic effect on mammary tumors in the rat (Huggins et al., 1959; Kim et al., 1960; Dao, 1964; Sterental et al., 1964). Both ovariectomy and hypophysectomy have been shown to depress prolactin secretion (Pearson et al., 1969; Chen et al., 1970). The current view is that ovariectomy and hypophysectomy depress or remove prolactin levels, resulting in suppression of growth of mammary tumors (Kim et al., 1960; Meites, in press). It also has been shown that injections of prolactin into rats enhances growth of existing DMBA induced tumors and development of new mammary tumors (Meites et al., in press).

Initiation and maintenance of lactation is dependent on adequate amounts of prolactin and adrenal corticosteroids. Other hormones, including GH, thyroid stimulating hormone, parathormone, and insulin, also may be necessary to promote maximum milk production (Meites and Nicoll, 1966). In hypophysectomized guinea pigs, prolactin and adrenal cortical

hormones represented the minimal requirements for milk secretion (Folley and Young, I941; Nelson et al., 1943). However, in intact rats (Talwalker et al., 1961) and mice (Nandi and Bern, 1961) the corticosteroids rather than prolactin were the limiting factor for initiating lactation during pregnancy, since administration of adrenal corticoids alone was effective in initiating lactation whereas prolactin alone was ineffective. In mammary tissue cultures, insulin as well as prolactin and corticosteroids were found to be necessary to initiate and maintain mammary secretion (Rivera, 1964). A rise in prolactin and ACTH secretion has been reported to occur at about the time of parturition (Meites, 1959; Cowie and Folley, 1955) and both serve to initiate lactation.

Prolactin also functions as a luteotropin but it may act synergistically with LH. It maintains the corpus luteum of the ovary. Astwood (I941) demonstrated in hypophysectomized rats, that administration of prolactin produced functional corpora lutea. It has been shown that in rats the luteotropic effect of prolactin is necessary, during the first six days of pregnancy to maintain progesterone secretion by the corpora lutea (Clemens et al., 1969). It seems that increased levels of prolactin tend to maintain functional corpora lutea. Everett (1956) showed that removal of the anterior pituitary from its connection with the diencephalon and placing it under the kidney capsule causes an

increased secretion of prolactin and prolongs luteotropic activity in rats. Subsequent studies by Nikitovitch-Winer and Everett (1958) in which anterior pituitary grafts were placed under the kidney and in the anterior chamber of the eye, also demonstrated a prolonged luteotropic activity in rats. Chlorpromazine and reserpine (Barraclough and Sawyer, 1959) or perphenazine (Ben-David, 1968) administration to rats also prolongs luteotropic activity. However, as mentioned by Meites and Nicoll (1966) prolactin can not be considered the only hormone involved in luteal function since luteinizing hormone (LH) has been shown to be an important regulator of luteal function in several species, including the rat.

Prolactin has the ability to be luteolytic as well as luteotropic in rats (Malven and Sawyer, 1966). Hypophysectomized rats were treated with LH and FSH in order to induce ovulation and luteal formation. Prolactin injections were given several days later and structural luteolysis occurred whereas prolonged treatment with FSH and LH had no effect on luteolysis. Further work by Malven (1969) demonstrated both a luteotropic and luteolytic response to prolactin in hypophysectomized rats. However, the function of prolactin in a normal cycling rat was unknown until recently. Radioimmunoassay of prolactin in the blood serum has shown that it is highest on the afternoon of proestrus and remains elevated in early estrus; it is low during most of pregnancy

(Amenomori et al., 1970; Gay et al., 1970). It has been demonstrated very recently by Wuttke and Meites (1971) that this rise of prolactin during the normal cycle serves to induce luteolysis of the older crop of corpora lutea during each cycle. The normal rise of prolactin on the afternoon of proestrus and estrus was blocked by a drug, ergocornine. A dose of ergocornine was used that is known to markedly decrease prolactin levels but has relatively little effect on LH levels and does not interfere with regular cycling or ovulation (Wuttke et al., 1971). Luteolysis was blocked in those animals treated with ergocornine. When exogenous prolactin was administered on the afternoon of proestrus or on the days of proestrus and estrus, luteolysis occurred as determined by the number of corpora lutea and ovarian weight. Related experiments by Billeter and Fluckiger (1971) are in agreement with those presented above, although carried out over a longer time period. Further evidence for the luteolytic action of prolactin in the normal cycling rat will be presented in this thesis.

II. Hypothalamic Inhibition

The hypothalamus is intimately involved in regulation of secretion of all the hormones from the anterior pituitary. However, the regulation of prolactin secretion is unique in that it is the only anterior pituitary hormone that is chronically inhibited by the hypothalamus. The hypothalamus

produces a neurohumor, termed appropriately prolactin inhibiting factor (PIF), which traverses the hypothalamo-hypophyseal portal system and acts directly on the anterior pituitary to inhibit prolactin synthesis and release (Talwalker et al., 1963).

Any disturbance in the connection of the hypothalamus with the anterior pituitary can cause an increased secretion of prolactin. Various experimental techniques have been employed to accomplish the latter, such as pituitary stalk section and administration of various CNS-depressant drugs (Meites et al., 1963). Recently, it was reported that transplantation of a pituitary beneath the kidney capsule of hypophysectomized-ovariectomized rats or placement of electrolytic lesions in the median eminence of ovariectomized rats, resulted in increased serum prolactin levels (Chen et al., 1970).

Culture of anterior pituitary tissue in vitro has demonstrated that the anterior pituitary can synthesize and release prolactin when removed from the hypothalamus and other body influences (Meites, 1959). Other in vitro work with incubated pituitaries and hypothalamic and cerebral extracts, resulted in decreased release of prolactin only when the pituitary tissue was incubated with hypothalamic extract (Talwalker et al., 1963). Pasteels et al. (1963) did similar experiments with human pituitaries and observed the same results. This work has been confirmed in vivo by

Grosvenor et al. (1964) who injected hypothalamic extracts into postpartum lactating rats and reported that they inhibited the usual prolactin release following suckling.

There are several stimuli which are known to increase prolactin secretion and these have been employed to measure PIF activity. Ratner and Meites (1964) demonstrated that estradiol and suckling increased prolactin secretion by depleting the hypothalamus of PIF activity. In a similar series of experiments Sar and Meites (1967) showed that PIF activity during proestrus and estrus in rats was less than during the stage of diestrus. This corresponds to the serum prolactin levels during these phases of the cycle (Amenomori et al., 1970; Wuttke and Meites, 1970).

While the existence of PIF has been well documented in mammals, its presence has not been demonstrated in other species. Meites and Nicoll (1966) reported the presence of a prolactin releasing factor (PRF) in 6 species of birds. The chemical structures of these substances have not been determined. However, this is a very active area of neuroendocrine research and it is very likely that within the next few years the structures for most of the hypophysiotropic hormones of the hypothalamus will be determined. Very recently the structures for thyrotropic releasing factor (TRF or TRH) (Boler et al., 1969) and luteinizing hormone releasing factor (LRF) (Matsuo et al., 1971) have been reported; also that for melanocyte stimulating hormone inhibiting factor (MIF) (Nair et al., 1971).

III. Catecholamines

In the last few years much attention has been given to the role of the catecholamines on gonadotropic secretion. As mentioned earlier, the hypothalamus serves as a regulator of the function of the anterior pituitary and possibly the release of the hypothalamic hormones is mediated through these catecholamines. There is much controversy as to which of these biogenic amines, norepinephrine or dopamine, has the major role in release of pituitary hormones. In an excellent review by Fuxe and Hokfelt (1969), much of the current research on this question was presented. Evidence is mounting in favor of dopamine as the major neurotransmitter substance. It was postulated by Coppola et al. (1966) that a sympathetic tonus, originating in the hypothalamus, normally acted to stimulate the release of FSH and LH while restraining the secretion of prolactin. In the absence of this tonus, FSH and LH secretion were suppressed while prolactin release was stimulated. Evidence for this postulate came from pharmacological studies in which certain drugs which interferred with or enhanced catecholamine activity were used, and gonadotropin secretion was monitored.

An early study by Barraclough and Sawyer (1959) demonstrated that reserpine, a catecholamine depletor, and chlorpromazine, a potent adrenergic blocker, induced the sustained release of prolactin in rats, as judged by deciduoma formation, and also blocked ovulation, indicating

interference with LH release (Coppola *et al.*, 1966). In more recent studies, Coppola *et al.* (1965) also reported that reserpine induced pseudopregnancy and attributed this to depletion of brain norepinephrine stores. Kanematsu *et al.* (1963) reported the site of action of reserpine to be the basal tuberal hypothalamic area. This area of the hypothalamus is considered to be the most important in relation to control of the anterior pituitary function (Flerko, 1966). Ratner *et al.* (1965) demonstrated that reserpine can deplete rat hypothalamus of PIF activity when administered *in vivo* and can act directly on the hypothalamus *in vitro* to decrease PIF release. Van Maanen and Smelik (1968) provided direct evidence for localization of a monoaminergic prolactin inhibitory system in the tuberal part of the hypothalamus. They observed induction of pseudopregnancy in those rats implanted with reserpine and blockage of pseudopregnancy in those rats pretreated with iproniazid. It appears therefore, that sympathetic neurotransmitters may act on the nerve endings in the hypothalamus containing the releasing and inhibiting factors, to control their release into the hypophyseal portal vessels.

The above evidence has been further substantiated by the work of Lu *et al.* (1970) in which serum and pituitary prolactin levels were measured after administration of reserpine, chlorpromazine, α-methyl-meta-tyrosine and α-methyl-para-tyrosine. These are all catecholamine

depressants. All drugs produced increases in serum prolactin levels indicating that they decreased hypothalamic PIF activity. It is interesting to note that systemic injections of catecholamines such as epinephrine and dopamine produced no effect on prolactin release (Lu *et al.*, 1970). Koch *et al.* (1970) has shown by an *in vitro* study that the effects of catecholamines on prolactin release may be dose dependent. Kamberi *et al.* (1971) perfused the anterior pituitary with catecholamines and observed no effect on prolactin release. However, when these same catecholamines were injected into the third ventricle of the hypothalamus, prolactin release was decreased. This indicates that catecholamines act indirectly through the hypothalamic-hypophyseal system to influence prolactin secretion.

IV. Iproniazid

Monoamine oxidase (MAO) inhibitors block the enzyme, monoamine oxidase which catalyses the oxidative deamination of epinephrine, norepinephrine, dopamine, serotonin and others. The result is an accumulation of these amines in the brain. One such MAO inhibitor is iproniazid which is a "psychic energizer". Iproniazid was developed as a treatment for tuberculosis but because of its excessive stimulating effects on the CNS and its toxic effects, it was discarded as an anti-tuberculosis drug (Bernheim, 1965).

Iproniazid has been used in order to study changes in gonadotropic secretion since it is known that drugs of this class alter catecholamine levels. Setnikar et al. (1960) showed that iproniazid retards sexual maturation, lengthens the estrous cycle, and causes an increase in gonadotropic activity of the rat hypophysis. Poulson et al. (1960) demonstrated that iproniazid interrupted pregnancy when administered before mid-pregnancy. In mice iproniazid prevented the formation of decidual tissue (Lindsay et al., 1963). This evidence suggested that iproniazid alters gonadotropic and possibly prolactin secretion. Mizuno et al. (1964) reported that iproniazid also may inhibit prolactin release. They observed that the mammary secretory response following electrical stimulation of the uterine cervix and also postpartum lactation in rats were suppressed by iproniazid treatment. Nagasawa and Meites (1970) demonstrated that iproniazid suppressed carcinogen-induced mammary tumor growth in rats. This suggests a decrease in prolactin secretion. Moreover, Lu and Meites (1971) demonstrated that a systemic injection of iproniazid reduces serum prolactin levels in female rats. It was of interest to determine if iproniazid could inhibit the late afternoon proestrous surge of prolactin.

V. Cocaine

Cocaine produces effects similar to stimulation of adrenergic nerves and is an alkaloid obtained from leaves of the coca tree. It paralyzes the termination of the sensory nerves which is the basis for its therapeutic use (Grollman, 1965).

In a review on drugs which affect norepinephrine uptake, a hypothesis was proposed to explain the sympathomimetic action of cocaine. It was stated that cocaine acts by inhibiting the normally rapid inactivation of catecholamines by nerve uptake. When the uptake system is blocked high concentrations of norepinephrine or other catecholamines are available in the vicinity of adrenergic receptors, this produces exaggerated and prolonged pharmacological responses (Iversen, 1967). Direct evidence for an inhibition of norepinephrine uptake by cocaine was provided by Whitby *et al.* (1960) and by Muscholl (1961). Cocaine was administered intravenously followed by an intravenous injection of tritum labelled norepinephrine. It was observed that cocaine markedly reduced the uptake of circulating norepinephrine into certain tissues as measured by increased plasma levels of norepinephrine (Whitby *et al.*, 1960). This work has been confirmed by various *in vitro* studies. Dengler *et al.* (1961) incubated cat cortex, spleen, and heart tissues for 45 minutes in a medium containing cocaine and labelled norepinephrine. Cocaine was observed to inhibit

norepinephrine uptake. Similar studies were performed by Thoenen et al. (1964) and Gillespie and Kirpekar (1965) in which they also demonstrated an inhibition of norepinephrine uptake by cocaine. Fuxe and Hokfelt (1969) suggested that cocaine could be used to study the relation of catecholamines to endocrine functions. Since no work had been done on the relation of cocaine to anterior pituitary hormone secretions until this time, it was of interest to determine its effects on prolactin secretion.

VI. Barbiturates

Another class of drugs unrelated to catecholamines that influence prolactin secretion are the barbiturates. The barbiturates are derivatives of barbituric acid which is a combination of urea and malonic acid. These drugs depress the central nervous system and induce sedative and hypnotic effects. The sedatives and hypnotics are used to allay nervousness, to induce sleep if pain is absent and to control convulsions (Grollman, 1965).

The barbiturates were first shown to influence gonadotropic secretion by Everett and Sawyer (1949). They injected Nembutal into rats and were able to block ovulation in spontaneously ovulating animals. Further work by the same authors showed that other barbiturates, such as phenobarbital and barbital, also blocked ovulation (1950). Recently, with the development of radioimmunoassays, work has been done in

which serum levels of hormones have been measured after administration of particular drugs. Wuttke and Meites (1970) reported that Na pentobarbital initially increases and then depresses serum prolactin levels. Wakabayashi and Arimura (1971) confirmed this report. In this thesis further work was done to elucidate the site of action of Na pentobarbital.

Thalidomide is structurally related to phenobarbital and possesses properties similar to the barbiturates (Grollman, 1965). It is a central nervous system depressant but in contrast to barbiturates it does not cause the loss of the righting reflex. It acts in conjunction with other drugs to potentiate their effects such as potentiating the depressant effect of chlorpromazine (Kuhn and Van Maanen, 1961). It has been used widely as a sedative and mild hypnotic drug until it was discovered that thalidomide has teratogenic effects (Grollman, 1965).

Since the discovery of its teratogenic properties, comprehensive studies have been done on thalidomide and much of the work has included tumor research. In 1966 it was reported that thalidomide influenced the rate of growth and disappearance of DMBA-induced tumors in Sprague-Dawley rats (Muckter, 1966). Subsequent work by the same researcher and co-workers showed that thalidomide derivatives, cyclic imides, had more effect than thalidomide on DMBA induced tumors in rats (Muckter et al., 1967). More recently they

have reported that 1-(Morphalinomethyl)-4-phthalimido-piperidendione-2,6, a thalidomide derivative, had a greater therapeutic effect than bilateral ovariectomy, estrogen or androgen on DMBA induced tumors (Muckter et al., 1969). They proposed that thalidomide and its derivatives exert their effects on DMBA-induced tumors through the endocrine system. It was of interest to know how thalidomide affects prolactin secretion since it has been well established that prolactin is influential in mammary tumorigenesis (Meites and Nicoll, 1966).

VII. Ergot Drugs

Ergot alkaloids are derivatives of lysergic acid and are extracted from a rye or wheat fungus, ergot. Therapeutically ergot alkaloids were used to alleviate headaches due to their vasoconstrictor activity. They also cause smooth muscle contraction and are adrenergic blocking agents (Grollman, 1965).

Shelesnyak (1954, 1955) showed that ergotoxine inhibited deciduoma formation and terminated pseudopregnancy and early pregnancy. This suggested that ergotoxine interrupted prolactin secretion. In subsequent work it was demonstrated that exogenous prolactin was able to overcome the effects of ergotoxine on interrupting gestation (Shelesnyak, 1958). Zeilmaker and Carlsen (1962) reported that ergocornine produced morphological changes in the corpora lutea which

prohibited decidual formation and inhibited milk production in lactating rats, and this was overcome by an injection of lactogenic hormone. Nagasawa and Meites (1970) reported that ergocornine reduced serum and pituitary prolactin levels and suppressed DMBA-induced mammary tumor growth when injected for 15 days. A systemic injection or an implant into the median eminence area of the hypothalamus of ergocornine significantly depressed cyclic fluctuations in serum prolactin levels (Wuttke et al., 1971). Wuttke et al. (1971) also reported that part of the inhibitory action of ergocornine on prolactin secretion was mediated through the hypothalamus by increasing PIF activity. In vitro studies by Lu et al. (1971) have shown that ergocornine also can act directly on the anterior pituitary to inhibit prolactin secretion. Further evidence will be presented in this thesis that ergocornine inhibits prolactin release.

VIII. Effects of Hormones

Prolactin is also influenced by other hormones as well as various drugs. It has been demonstrated that administration of gonadal, adrenal cortical, and thyroid hormones increased prolactin secretion by the in situ anterior pituitary of rats (Meites et al., 1963). Estrogen is the most potent stimulator of prolactin release. Nicoll and Meites (1962) added estrogens (0.5 μg per ml) to the medium of organ cultures of rat anterior pituitaries and found a significant

increase in prolactin production *in vitro*. Subsequent work by Nicoll and Meites (1964) showed that lower doses of estrogen are more effective in stimulating prolactin production *in vitro* than are higher doses of estrogen.

In an *in vivo* study, Ramirez and McCann (1964) implanted estrogen tipped needles into the anterior pituitary and median eminence of rats, and observed that the intrahypothalamic and intrahypophyseal implants promoted pseudopregnancy, mammary growth, and lactation. This confirmed the *in vitro* findings that estrogen acts directly on the anterior pituitary to stimulate prolactin secretion. Ratner and Meites (1962) demonstrated that the anterior pituitary in estrogen-injected rats released greater amounts of prolactin than the pituitary of untreated rats during a short time incubation. More recently Nagasawa *et al*. (1969) showed that an implant of estradiol benzoate into the median eminence of rats significantly increased serum and pituitary prolactin levels. Therefore estrogen has two modes of action in releasing prolactin, one by a direct action on the pituitary and the other indirectly through the hypothalamus.

Other steroids such as progesterone, androgens, and corticosteroids slightly stimulate prolactin secretion *in vitro* (Meites, 1959), but were observed to be without effect on prolactin secretion *in vitro* (Nicoll and Meites, 1966). The thyroid hormones also influence prolactin secretion. Injections of thyroid hormones in rats increased

prolactin secretion by the *in situ* pituitary (Meites, 1963). Nicoll and Meites (1963) further demonstrated that addition of either thyroxine or triiodothyronine to the medium of organ cultures of rat anterior pituitary resulted in a significant stimulation of prolactin release. Thus a direct action of thyroid hormones on the anterior pituitary was established.

MATERIALS AND METHODS

I. Animals

All rats were of the Sprague-Dawley strain obtained from two sources: Spartan Research Labs. (Haslett, Michigan) and Carworth Farms (Portage, Michigan). Female and male rats were used and housed 6 or 8 to a cage. After drug treatment was started the animals were separated into different groups. All animals were fed Wayne Lab Blox pellets (Allied Mills, Chicago, Illinois) and water ad libitum. The temperature of the animal room was maintained at 25±1°C and artificially illuminated with 14 hours of light (5:00 AM to 7:00 PM) and 10 hours of darkness daily.

II. Treatment of Pituitary and Blood Samples

All blood samples were taken by cardiac puncture under light ether anesthesia and pituitaries were removed at the end of each experiment. The pituitaries were frozen at -20°C until assayed, at which time pituitary homogenates were made. The pituitaries were sonified with a Sonifier cell disruptor (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) in a tube containing 0.01 M phosphate buffered saline

(pH 7.0). The blood samples were first stored for 24 hours at 2.5°C and the clots were then removed. The sera were frozen at -20°C until assayed.

III. Radioimmunoassay

Prolactin in individual serum samples and pituitaries were measured by radioimmunoassay (Niswender et al., 1969). Each serum sample was assayed at 2 dilutions and pituitary samples at 3 and 4 dilutions in 1% bovine serum albumin and 1% egg white. These values were averaged and expressed in terms of a standard rat prolactin preparation (NIAMD-RP-1) obtained from National Institutes of Arthritis and Metabolic Diseases of National Institutes of Health, Bethesda, Maryland. The hormone (H-10-10-B) used for iodination in these assays was provided by Dr. S. Ellis, NASA, Ames Research Center, Moffet Field, California. Part of the radioimmunoassays done utilized iodine 131 (Cambridge Nuclear, Billerica, Mass.) for iodination of the hormone and in the last two experiments (cocaine and thalidomide) the isotope, iodine 125 (New England Nuclear, Boston, Mass.) was used for labelling of the hormone.

IV. Histological Treatment

Ovarian tissue was fixed in Bouin's fluid, sectioned and stained with hematoxylin and eosin and examined under the microscope.

V. Statistical Treatment

Sample means and standard error of means were calculated within each experimental group and significance of differences between groups was determined by Student's t test and Analysis of Variance (Sokal and Rohlf, 1969).

EXPERIMENTAL

I. Effects of Barbiturates on Prolactin Secretion

A. Effect of Injecting Na Pentobarbital (PB) on Hypothalamic PIF Activity and Effect of Na Pentobarbital on Pituitary Prolactin Release In Vitro in the Rat

1. Objective

Recently it was reported that an injection of PB during the early afternoon of proestrus produced an initial increase in serum prolactin for about 30 minutes duration, but completely prevented the late afternoon rise in serum prolactin (Wuttke and Meites, 1970). The purpose of this experiment was to determine whether PB acted on the hypothalamus, on the anterior pituitary or on both to influence prolactin release.

2. Procedure

Adult female (160-200 g) and male (180-220 g) rats were used. Thirty female rats were divided into 3 groups and treated at 1 PM on the day of proestrus as follows:

Ten rats were injected intraperitoneally (i.p.) with 31.5 mg NaPB base in .9% saline/kg BW and guillotined 10 minutes later. Ten other animals were injected i.p. with

31.5 mg PB/kg and guillotined 2 hours later. Ten control animals were injected i.p. with .9% saline and guillotined 2 hours later.

Immediately after each rat was killed, the hypothalamus and an equivalent amount of cerebral cortex were removed. The hypothalamic and cerebral cortical tissues from each group were placed in ice cold .1 N HCl, homogenized at 12,000 g for 40 minutes at 4°C. The supernatant was kept frozen, thawed just prior to use, adjusted to pH 7.4 with 1 N NaOH and assayed for PIF activity by the in vitro method of Kragt and Meites (1965). Donor anterior pituitaries were removed from mature male rats, hemisected, and each half was incubated separately in 1 ml medium 199 with .1 ml of hypothalamic or cerebral cortical extract (equivalent to approximately 1 hypothalamus). Incubation time was 4 hours.

In order to test the direct effect of PB on the anterior pituitary, 10 anterior pituitary halves from male rats were each incubated separately in medium 199 without brain extracts, and the corresponding 10 halves were each incubated in medium 199 to which 3 μg PB was added. Incubation time was 4 hours.

3. Results

Pituitary halves incubated with hypothalamic extract from saline-injected control rats (Group A1) released

significantly less prolactin into the medium than the corresponding halves incubated with cerebral cortical extract from the same rats (Group A2), indicating the presence of PIF activity in the hypothalamus (Table 1). Ten minutes after PB injection, no significant PIF activity was found in the hypothalamus, as indicated by release of similar amounts of prolactin from AP halves incubated with either hypothalamic or cerebral cortical extract. Two hours after PB injection, hypothalamic PIF activity was partially restored, as indicated by lower prolactin release from pituitary halves incubated with hypothalamic extract than by the corresponding halves incubated with cerebral cortical extract. This shows that PB decreases hypothalamic PIF activity, and is believed to account for the initial rise in serum prolactin after injection of PB.

Pituitary halves removed from untreated male rats and incubated without brain extracts (Group B1) released significantly more prolactin than the corresponding halves incubated with 3 μg PB (Group B2). This indicates that PB directly inhibits AP prolactin release, and is believed to account for the inhibition of prolactin release by PB.

Table 1.A. Effect of Injecting Pentobarbital (PB) on Hypothalamic PIF Activity

B. Effect of PB on Pituitary Prolactin Release *in vitro*

	Group and Treatment	PIF Activity (NG Prolactin Released/MG AP)			Direct Effect of PB (NG Prol. released/MG AP)
		2 hrs. after Saline inj.	10 min. after PB inj.	2 hrs. after PB inj.	
A.	1. Hypothal. Extract + 1/2 AP	383.1± 56.3	964.1± 46.9[a]	679.5±45.7[a]	
	2. Cortical Extract + 1/2 AP	1164.5±100.0	974.7±177.7	1120.1±50.0	
B.	1. Medium + 1/2 AP				1100.1±59.3
	2. Medium + 1/2 AP + 3 ug PB				712.2±40.0[b]

[a]PB vs. Saline inj. controls, $P < 0.001$

[b]PB vs. Controls, $P < 0.001$

B. Effects of Thalidomide on Serum and Pituitary Prolactin Levels in the Rat

1. Objective

The purpose of this study was to determine the effects of thalidomide on serum and pituitary prolactin levels and relate these findings to the effects of other barbiturates on prolactin release.

2. Procedure

Adult female rats weighing between 210 and 245 grams were used. A pre-treatment blood sample was taken at 12 AM on the day of proestrus from each rat. A dose of 5, 10, or 25 mg thalidomide/100 gm body weight was injected i.p. at 12:30 AM and rats were bled at 1, 2, 3, 4, and 5 PM. Control rats were injected with saline, the drug vehicle. The animals were guillotined after the 5 PM bleeding and their pituitaries were removed. Both pituitary and serum samples were assayed for prolactin by radioimmunoassay.

3. Results

Table 2 shows that a single i.p. injection of any of the 3 doses of thalidomide significantly reduced serum prolactin. The afternoon proestrous surge was completely blocked. A dose-response effect of thalidomide is indicated, since the highest dose of thalidomide used (25 mg/100 g body weight) produced the greatest blockage of the proestrous prolactin peak, whereas serum prolactin levels were

Table 2. Effects of Thalidomide on Serum and Pituitary Prolactin Levels

Treatment and no. of rats	Pre-treatment (12 AM)	Post-treatment 30 min. (1 PM)	1 1/2 hrs (2 PM)	2 1/2 hrs (3 PM)	4 1/2 hrs (5 PM)	Pituitary ug/mg
Controls (17)	339.7±40.3‡	399.9±40.1	737.9±101.8	989.4±75.3	1300.0±81.8	3.87±0.16(14)
Thalidomide (8) 5 mg/100 gm BW	277.5±44.5	328.4±51.5	357.1± 58.6[a]	391.3±76.1[b]	560.4±91.0[b]	5.36±0.18[b](7)
Thalidomide (13) 10 mg/100 gm BW	256.4±48.2	252.3±37.5[a]	250.9± 54.5[b]	441.8±53.2[b]	473.5±84.9[b]	5.04±0.19[b](12)
Thalidomide (12) 25 mg/100 gm BW	236.6±39.1	170.2±33.4[b]	173.0± 31.8[b]	193.6±42.8[b]	422.0±71.5[b]	5.59±0.16[b](10)

Each group was compared to controls

‡Prolactin values = ng/ml (NIAMD-RP-1 STD)

[a] $P < .05$
[b] $P < .001$

increasingly higher when lower doses of thalidomide (5 and 10 mg/100 g body weight) were used. The serum prolactin levels appeared to be reflected by the pituitary prolactin concentrations. Thus pituitary concentrations were significantly higher in all animals treated with thalidomide. This might be expected since the release of prolactin from the pituitary was prevented by the drug.

C. Conclusions

Na pentobarbital produced a disappearance of hypothalamic PIF activity by 10 minutes after injection and depressed PIF activity after 2 hours. Cerebral cortical extract from the same animals had no effect on pituitary prolactin release *in vitro*. Incorporation of 3 μg PB directly into the incubation medium significantly inhibited prolactin release by a direct action on the anterior pituitary. These results suggest that the ability of injected PB to initially increase prolactin release for a period of about 30 minutes and then to inhibit prolactin release for a more prolonged period (Wuttke and Meites, 1970) is due to a rapid reduction in hypothalamic PIF activity followed by a prolonged inhibitory action directly on the pituitary.

Thalidomide, like PB, decreased serum prolactin levels and blocked the afternoon proestrous rise. Thalidomide also increased pituitary prolactin concentration as compared to that in control rats. However, thalidomide unlike PB,

did not cause an initial increase in serum prolactin. This may be due to the fact that the doses of thalidomide used did not completely anesthetize the animals as did PB. Whether thalidomide, like PB, directly blocks pituitary release of prolactin remains to be determined.

II. Effects of Drugs that Alter Hypothalamic Catecholamines on Prolactin Release

A. Effects of a Single Injection of Iproniazid on Pituitary Prolactin Release

1. Objective

Iproniazid has been shown to inhibit prolactin release on the morning of proestrus in female rats (Lu and Meites, 1971). It was of interest to determine if iproniazid would block the proestrous afternoon rise in prolactin as well.

2. Procedure

Adult female rats weighing between 205 and 245 grams were used. A pre-treatment blood sample was taken at 1 PM on the day of proestrus. The animals were injected with a dose of 40 mg/rat of iproniazid at 1:15 PM and bled at 2, 3, and 4 PM. The animals were killed by guillotine. The control animals were injected with saline, the drug vehicle. Serum samples were assayed for prolactin by radioimmunoassay.

3. Results

It is evident from the data shown (Table 3) that iproniazid blocked the proestrous afternoon peak. The action of iproniazid was seen approximately 45 minutes after injection (1 hr group). At this time there was no increase in prolactin release as compared to the control rats. When the control animals reached their peak level (1200±100.4 ng/ml) of prolactin at 4 PM, the iproniazid group showed no significant rise in serum prolactin. Since iproniazid is a monoamine oxidase inhibitor, and thus inhibits the metabolism of catecholamines, it acts to increase catecholamine activity in the brain (Grollman, 1965). An increase in catecholamine activity has been shown to evoke an increased release of PIF by the hypothalamus (Lu and Meites, 1971) and depress prolactin release.

B. Effects of Cocaine on Pituitary and Serum Prolactin Levels

1. Objective

Cocaine is a weak monoamine oxidase inhibitor. More importantly it potentiates the action of catecholamines. Since it has been shown that catecholamines inhibit prolactin release, it was of interest to determine the effects of cocaine on serum prolactin values.

2. Procedure

Adult female rats weighing between 210 and 260 grams were used. A pre-treatment blood sample was taken at 9 AM

Table 3. Effects of Iproniazid on the Proestrous Rise of Prolactin

Treatment and no. of rats	Serum Prolactin (ng/ml) 0 1 PM	1 hr 2 PM	2 hr 3 PM	3 hr 4 PM
Controls (6) saline	153.0±15.8##	368.7±46.9	891.7±123.6	1200.0±100.4
Iproniazid (8) 40 mg/rat	229.0±27.2	233.3±29.0[a]	299.1± 39.6[b]	247.0± 37.3[a]

All groups compared to controls

##Prolactin - ng/ml - NIAMD-RP-1 STDS

[a] $P < .05$
[b] $P < .001$

on the morning of proestrus and the last sample was taken before the proestrous afternoon rise of prolactin. A dose of 1, 5, 10, or 20 mg/kg was injected i.p. at 9:15 AM and rats were bled 30 minutes, one hour, two hours, and four hours after injection. Control rats were injected with saline, the drug vehicle. The animals were guillotined at the end of the experiment and their pituitaries were removed and weighed. Both pituitary and serum samples were assayed for prolactin by radioimmunoassay.

3. Results

The data (Table 4) shows that cocaine elicited a decrease in serum prolactin levels. The lower doses (1 and 5 mg/kg) lowered serum prolactin levels below the pre-injection values. However, the action of the higher doses (10 and 20 mg/kg) appeared to be less consistent. The 10 mg dose evoked a significant reduction in serum prolactin 0.5 hour after injection, followed by an increase at 1 and 2 hours after injection and then a fall at 4 hours below pre-treatment values. The dose of 20 mg/kg appeared to be more effective in reducing serum prolactin levels, but after 4 hours there was a significant increase in serum prolactin. None of the doses used had any effect on pituitary prolactin concentration by the end of 4 hours.

Table 4. Effects of Cocaine on Serum and Pituitary Prolactin Levels

Treatment and no. of rats	Serum Prolactin (ng/ml) 0 (9 AM)	1/2 hr	1 hr	2 hr	4 hr	Pituitary ug/mg
Controls (12) saline	188.3±10.8‡	294.0±38.8	270.9±28.9	267.1±51.4	198.6±25.0	14.8±1.8
Cocaine (8) 1 mg/kg	207.9±44.5	154.7±36.4[b]	136.8±15.4[c]	139.3± 8.4[a]	152.1±19.4	12.4±2.3
Cocaine (9) 5 mg/kg	170.3±19.7	148.4±21.0[c]	142.1±22.5[c]	169.2±26.0	115.2±20.3[b]	13.3±1.1
Cocaine (7) 10 mg/kg	221.4±30.6	136.0±21.3[c]	262.0±46.1	398.1±47.4	167.8±40.7	10.9±0.81
Cocaine (10) 20 mg/kg	169.6±23.0	82.5± 9.9[d]	116.8±23.6[d]	123.0±11.9[b]	368.6±74.0[a]	11.4±1.1

All groups compared to controls

‡Prolactin values - ng/ml - NIAMD-RP-1 STDS

[a] $P < .05$
[b] $P < .02$
[c] $P < .01$
[d] $P < .001$

C. Conclusions

Both iproniazid and cocaine have been reported to increase catecholamines in the brain and other tissues (Iversen, 1967), although they do not accomplish it in the same way. As mentioned earlier iproniazid blocks the enzyme, monoamine oxidase which catalyzes the oxidative deamination of epinephrine, norepinephrine, dopamine, and others, whereas cocaine blocks the reuptake of catecholamines by the nerve endings. Both processes result in increased levels of catecholamines in specific tissues. Increases in hypothalamic catecholamines have been shown to be associated with increased hypothalamic PIF activity and decreased serum prolactin values (Lu and Meites, 1971). Cocaine appeared to produce a biphasic effect on prolactin release. The higher doses (10 and 20 mg/kg) caused both an inhibition and stimulation of prolactin release. It is possible that the biphasic effect on prolactin release is due to other actions of cocaine besides its ability to potentiate the actions of catecholamines. It is known that high doses of cocaine cause a heightened excitability in animals (Grollman, 1965) and this may stimulate prolactin release. There are many indications that stresses increase prolactin release (Meites et al., 1963). It remains to be determined whether these drugs act only via the hypothalamus or also on the pituitary.

III. Luteolytic Role of Prolactin During the Estrous Cycle in Female Rats

A. Effects of Ergocornine (EC) Treatment

1. Objective

Recently it was reported that injections of ergocornine, at a dose known to markedly reduce serum prolactin during the estrous cycle prevented luteolysis of old corpora lutea (Wuttke and Meites, 1971). Luteolysis of corpora lutea during the estrous cycle of the rat has generally been ascribed to LH (Rothchild, 1965). It was of interest to repeat some of the work previously reported (Wuttke and Meites, 1971), using higher doses of ergocornine known to reduce serum LH as well as prolactin levels to determine the effects of reducing the levels of both hormones on luteal maintenance.

2. Procedure

Adult female rats weighing 205 to 245 grams were used. The animals were injected at 9 AM and 6 PM daily with ergocornine methanesulfonate (EC), 400 μg and 800 μg/rat i.p., dissolved in 70% ethanol and then in .9% saline to give a solution of 1% ethanol - 99% saline. The treatment was started on the last afternoon of diestrus prior to the expected day of proestrus and continued through the cycle until the following first day of diestrus. Control animals were injected with the ethanol-saline solution. At 2 PM on

the day of proestrus, 8 of the 16 rats given each dose of ergocornine, received a subcutaneous injection of 1 mg prolactin (ovine, NIH-P-S8, 28 IU/mg) dissolved in .9% saline. At the end of treatment, during diestrus, the rats were killed and the ovaries, adrenals, and uteri were removed, cleaned and weighed. The ovaries were prepared for histological examination. Corpora lutea were counted only from a mid-sagittal cross section and not from the entire ovary.

3. Results

Injections of both doses of ergocornine (400 μg and 800 μg/rat) resulted in significant increases in both ovarian and adrenal weight, and no effect on uterine weight as compared to controls (Table 5). The number of corpora lutea per ovary in the EC-treated rats was significantly greater than the corpora lutea per ovary in the controls or rats given both EC and prolactin. It was noted during microscopic examination that many of the ovaries of the EC-treated animals had large numbers of follicles. Body weight was significantly although not greatly reduced in the EC-injected rats as compared to body weight in the control rats. Thus the large doses of EC prevented luteolysis of the corpora lutea and prolactin counteracted the effect of the EC on the ovaries.

Table 5. Effects of Ergocornine Injections During One Estrous Cycle on Weight of Endocrine Organs and Average Number of Corpora Lutea

Treatment and no. of rats	Av. body weight (g)	Av. ovarian weight (mg)	Av. uterine weight (mg)	Av. adrenal weight (mg)	Av. no. corpora lutea+
Saline (8)	235.0±2.5	71.9±2.6	330.1±13.1	60.7±1.9	6.04±0.29
Ergocornine (8) 400 ug, PEDD*	214.0±4.9	93.5±2.7[b]	338.5±28.1	71.7±2.7[a]	8.18±0.32[b]
Ergocornine (8) 400 ug, PEDD + prolactin 1 mg, P	223.0±2.3[a]	71.2±1.4	307.1±23.5	69.3±2.1[a]	5.86±0.38
Ergocornine (8) 800 ug,PEDD	217.4±2.9[b]	99.4±1.2[b]	327.6±26.4	74.8±1.6[b]	8.07±0.61[a]
Ergocornine (8) 800 ug, PEDD + prolactin 1 mg, P	221.5±2.3[a]	72.3±3.1	329.7±15.1	72.8±1.7[b]	6.44±0.41

All groups compared to saline controls
+Counted from mid-sagittal cross section
*P - Proestrus
E - Estrus
D - Diestrus
[a]P < .01
[b]P < .001

B. Effects of Iproniazid Treatment

1. Objective

It has been reported in this thesis that iproniazid decreases serum prolactin levels and also blocks the proestrous afternoon surge of prolactin. It was of interest therefore to see whether iproniazid could be used to provide evidence for a luteolytic role of prolactin during the estrous cycle, since it should decrease prolactin release.

2. Procedure

Adult female rats weighing 200 to 240 grams were used. The animals were divided into four groups as follows: (1) saline controls, treated with the drug vehicle, (2) animals given 10 mg of iproniazid daily, and 45 mg on the day of proestrus, (3) iproniazid treated animals given 5 mg of iproniazid daily and 25 mg on the day of proestrus, and (4) iproniazid as in group 2, and at 2 PM on the afternoon of proestrus 1 mg of prolactin (ovine, NIH-P-S8, 28 IU/mg) dissolved in saline. The treatments were started on the last PM of diestrus prior to the expected day of proestrus and continuing throughout one cycle, terminating on the next first day of diestrus. In another group of rats, this same schedule and grouping of animals was carried out for three cycles as well. However, in the three cycle study, only one group was treated with iproniazid alone (same doses as group 3 in the one cycle study). At the end of the treatment, during diestrus, the rats were killed and the ovaries

and pituitaries were removed. At the end of the three cycle experiment, the adrenals and uteri were also removed. The ovaries were cross sectioned and prepared for microscopic examination. Corpora lutea were counted only from the cross section and not from the whole ovary.

3. Results

The results obtained with iproniazid in the one cycle study were very similar to those reported for ergocornine (Wuttke and Meites, 1971). Injections of iproniazid (the two dose groups) resulted in a significant increase in ovarian weight as compared to the controls (Table 6). The ovaries of rats injected with iproniazid and prolactin weighed about the same as in control rats. The pituitaries of one iproniazid treated group (Group 2) and iproniazid and prolactin treated animals (Group 4) were significantly decreased as compared to the controls. The number of corpora lutea per ovarian cross section in the iproniazid treated rats was significantly greater than the corpora lutea per ovary in the controls or rats given both iproniazid and prolactin.

The effects of iproniazid treatment during three estrous cycles (Table 7) resembled those during one cycle. The ovaries on the iproniazid treated animals were significantly increased as compared to the controls or rats given both iproniazid and prolactin. The number of corpora lutea per cross section of ovary were increased in weight in the

Table 6. Effects of Iproniazid Injections During One Estrous Cycle on Ovaries

Treatment and no. of rats	Av. body weight (g)	Av. ovarian weight (mg)	Av. pituitary weight (mg)	Av. no. corpora lutea+
1) Saline (6)	219.8±5.1	64.5±2.8	11.1±0.6	6.16±0.34
2) Iproniazid (6) 10 mg, EDD; 45 mg, P*	201.5±6.2	80.4±4.1[c]	9.2±0.4[a]	8.66±0.67[c]
3) Iproniazid (6) 5 mg, EDD; 25 mg, P	229.5±2.9	82.3±3.5[c]	10.5±0.45	8.50±0.51[d]
4) Iproniazid (6) 10 mg, EDD; 45 mg, P + prolactin 1 mg, P	206.0±3.2	61.0±4.2	8.9±0.5[b]	6.00±0.51

All groups compared to controls (saline)
+Counted from mid-sagittal cross section
*E - Estrus
D - Diestrus
P - Proestrus
[a]P < .05
[b]P < .02
[c]P < .01
[d]P < .001

Table 7. Effects of Iproniazid Injections During Three Estrous Cycles on Ovaries

Treatment and no. of rats	Av. body weight (g)	Av. ovarian weight (mg)	Av. no. corpora lutea[+]
Saline (6)	238.3±6.5	69.0±2.7	6.9±0.43
Iproniazid (6) 5 mg, EDD; 25 mg, P[*]	229.0±2.7	83.1±3.1[b]	8.1±0.38[a]
Iproniazid (6) 5 mg, EDD; 25 mg, P + Prolactin 1 mg, P	231.0±3.6	62.8±4.8	5.18±0.65

All groups compared to controls (saline)

[+]Counted from mid-sagittal cross section
[*]E - Estrus
D - Diestrus
P - Proestrus

[a]$P < .05$
[b]$P < .01$

rats given iproniazid alone, and returned to normal control numbers in the rats given iproniazid together with prolactin. There was no significant differences in uterine or pituitary weights in the iproniazid-treated rats as compared to controls in the three cycle study (Table 8). However, the average weight of the adrenals was significantly decreased in both the iproniazid and iproniazid prolactin treated animals as compared to the controls. In both the one cycle and three cycle studies iproniazid caused a slight decrease in body weight but this was not significant.

It should be noted that in the three cycle experiment the dosage of iproniazid was reduced (5 mg daily and 25 mg on day of proestrus to 5 mg daily and 10 on day of proestrus) in the middle of the experiment because of the overexcitability of the animals. Many large follicles were observed in the ovaries.

C. Conclusions

The data presented here provide further evidence that the function of prolactin during the estrous cycle is to induce luteolysis of the previous crop of corpora lutea. Both iproniazid and ergocornine significantly increased the weights of the ovaries and number of corpora lutea as compared to the controls. Prolactin administration prevented these actions of the 2 drugs. Although neither serum prolactin nor LH were measured in these rats, both drugs

Table 8. Effects of Iproniazid Injections During Three Estrous Cycles on Endocrine Organs

Treatment and no. of rats	Av. uterine weight (mg)	Av. adrenal weight (mg)	Av. pituitary weight (mg)
Saline (6)	353.2±8.7	67.8±2.9	10.1±0.85
Iproniazid (6) 5 mg, EDD; 25 mg, P*	382.4±30.8	49.5±1.8[a]	9.4±0.86
Iproniazid (6) 5 mg, EDD; 25 mg, P + Prolactin 1 mg, P	397.0±44.7	50.4±1.39[a]	9.4±0.57

All groups compared to controls (saline)

*E - Estrus
D - Diestrus
P - Proestrus

[a] $P < .001$

have been shown to significantly decrease serum prolactin levels, and the high doses of ergocornine used were also shown to decrease serum LH values (Wuttke et al., 1971). It does not appear therefore, that LH has any role in luteolysis of the old corpora lutea and that prolactin is solely responsible for this action during the estrous cycle.

DISCUSSION

It was the purpose of this thesis to use drugs to learn more about the control of prolactin secretion and the function of prolactin in the cycling animal. The drugs used in these experiments all depress serum prolactin levels. However, pentobarbital (Wuttke and Meites, 1970) has the ability to both stimulate and inhibit prolactin release, and cocaine may have the same properties. Two sites of action that these drugs may utilize to produce their effects on prolactin release are the hypothalamus and the pituitary.

As reported here, pentobarbital has a dual action. It depletes the hypothalamus of PIF activity and acts directly on the pituitary to inhibit prolactin release. This is believed to explain its ability to first stimulate prolactin release by depressing hypothalamic PIF activity, and then to inhibit prolactin release by a direct inhibitory effect on the pituitary. Ergocornine has also been shown to act on both the hypothalamus and the pituitary to inhibit prolactin release (Wuttke et al., 1971; Lu et al., 1971). Ergocornine increases PIF activity in the hypothalamus whereas pentobarbital decreases PIF activity in the hypothalamus, but both inhibit the pituitary directly.

The site of action of thalidomide has not yet been determined. Since it is a barbituric acid derivative, it may act similarly to pentobarbital. Apparently, it does not produce an initial increase in serum prolactin levels like pentobarbital, perhaps because it has less anesthetic effect. Its action may be solely on the pituitary. Thalidomide has been shown experimentally to act differently from the barbiturates in many respects and therefore a direct comparison can not be made between the 2 drugs (Somers, 1960). Unlike the barbiturate drugs, thalidomide does not cause an initial excitation in mice, incoordination, respiratory depression or narcosis, and it is virtually non-toxic.

Iproniazid and cocaine influence catecholamine levels in the hypothalamus. Iproniazid, a monoamine oxidase inhibitor depresses the metabolism of catecholamines and thereby increases catecholamine concentration in the hypothalamus (Grollman, 1965). Cocaine, on the other hand, blocks the reuptake of catecholamines by nerve endings so that the concentration of these amines is also increased in the hypothalamus (Iverson, 1967). The data presented reaffirms the hypothesis by Coppola et al. (1966) and others that an increased level of catecholamines inhibits prolactin and stimulates LH and FSH release. Kamberi et al. (1971) demonstrated that dopamine causes an increase in the release of PIF by diencephalic elements into the hypothalamo-pituitary portal vessels and thereby inhibits prolactin

release. It would prove interesting to measure catecholamine concentrations or turnover rates of catecholamines in the hypothalamus after administration of pentobarbital, ergocornine, and thalidomide to determine if there are any correlations with prolactin release.

The function of prolactin during the estrous cycle was not known until recently. Some of the data presented in this thesis provide further evidence for the action of prolactin as a luteolytic agent during the estrous cycle. As reported by Wuttke and Meites (1971), prolactin causes regression of the old crop of corpora lutea from the previous cycle. If the normal action of prolactin during proestrus and estrus is inhibited, by either ergocornine or iproniazid, ovarian weight increases due to the accumulation of corpora lutea. Injections of prolactin into ergocornine or iproniazid treated animals once during each cycle induced atropy of the old corpora lutea. Billeter and Fluckiger (1971) demonstrated a luteotropic as well as a luteolytic action of prolactin in the cycling rat. They used another ergot derivative, ergokryptine, to inhibit prolactin. However, instead of injecting prolactin only on the days of proestrus and estrus (Wuttke and Meites, 1971), they injected prolactin every day for 21 days. Some of their rats given ergokryptine and prolactin failed to ovulate and maintained the corpora lutea. Their failure to separate the luteotropic from the luteolytic effects of prolactin makes their study less valid

than that reported by Wuttke and Meites (1971) and the experiments presented here.

It is evident that prolactin can act as either a luteolytic or luteotropic agent. According to Malven (1969) the action of prolactin depends on the time of its administration after corpus luteum formation. An intriguing question which arises from these studies is--what is the mechanism which causes prolactin to function as either a luteotropic or luteolytic agent? The answer appears to lie in the ovary itself, since it has been shown that prolactin acts on fresh corpora lutea to elicit progesterone secretion but on old corpora lutea to induce luteolysis (Rothchild, 1965; Malven and Sawyer, 1966). It is well established that during pregnancy in rats prolactin is necessary to maintain the corpora lutea (Clemens et al., 1969). It has been reported that prolactin inhibits the enzyme, 20 α-hydroxysteroid dehydrogenase, which converts progesterone to 20 α-dehydroprogesterone, a less active compound (Wiest et al., 1968). This possibility has been discussed by Malven (1969). Apparently prolactin does not have this action on old corpora lutea, and induces luteolysis. The precise biochemical mechanism(s) involved in luteolysis remains to be determined.

LIST OF REFERENCES

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Amenomori, Y., C. L. Chen, and J. Meites. 1970. Serum prolactin levels in rats during different reproductive states. Endocrinology 86: 506-510.

Astwood, E. B. 1941. The regulation of corpus luteum function by hypophysial luteotrophin. Endocrinology 28: 309-320.

Barraclough, C. A., and C. H. Sawyer. 1959. Induction of pseudopregnancy in the rat by reserpine and chlorpromazine. Endocrinology 65: 563-579.

Ben-David, M. 1968. The role of the ovaries in perphenazine-induced lactation. J. Endocrinol. 41: 377-385.

Bern, H. A., and C. S. Nicoll. 1968. The comparative endocrinology of prolactin. Recent Progr. in Horm. Res. 24: 681-720.

Bernheim, F. 1965. Chemotherapy of bacterial infections: Drugs used in treatment of tuberculosis. In: Drill's Pharmacology in Medicine. J. R. DiPalma (Ed.). McGraw-Hill, New York. 1341-1342.

Billeter, E., and E. Fluckiger. 1971. Evidence for a luteolytic function of prolactin in the intact cyclic rat using 2-Br-α-ergokryptine (CB154). Experientia 27: 464-465.

Boler, J., F. Enzmann, K. Folkers, C. T. Bowers, and A. V. Schally. 1969. The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidyl-proline-amide. Biochem. Biophys. Res. Comm. 37: 705-710.

Chen, C. L., Y. Amenomori, K. H. Lu, J. L. Voogt, and J. Meites. 1970. Serum prolactin levels in rats with pituitary transplants or hypothalamic lesions. Neuroendrocrinology 6: 220-227.

Clemens, J. A., M. Sar and J. Meites. 1969. Termination of pregnancy by prolactin implants in the median eminence of the rat. Proc. Soc. Exp. Biol. Med. 130: 628-630.

Clifton, K. H., and J. Furth. 1960. Ducto-alveolar growth in mammary glands of adreno-gonadectomized rats bearing mammotropic pituitary tumors. Endocrinology 66: 893-897.

Coppola, J. A., R. G. Leonardi, W. Lippman, J. W. Perrine, and I. Ringler. 1965. Induction of pseudopregnancy in rats by depletors of endogenous catecholamines. Endocrinology 77: 485-490.

Coppola, J. A., R. G. Leonardi, and W. Lippman. 1966. Ovulatory failure in rats after treatment with brain norepinephrine depletors. Endocrinology 78: 225-228.

Coppola, J. A. 1971. Brain catecholamines and gonadotropin secretion. In: Frontiers in Neuroendocrinology. L. Martini and W. F. Ganong (eds.). Oxford University Press, New York. 129-143.

Cowie, A. T., and S. J. Folley. 1955. Physiology of the gonadotropins and the lactogenic hormone. In: The Hormones, Vol. IV. G. Pincus and K. V. Themann (eds.), Academic Press, New York. 309-387.

Dao, T. L. 1962. The role of ovarian hormones in initiating the induction of mammary cancer in rats by polynuclear hydrocarbons. Cancer Res. 22: 973-981.

Dengler, H. J., H. E. Spiegel, and E. O. Titus. 1961. Effects of drugs on uptake of isotopic norepinephrine. Nature 191: 816-817.

Elias, J. J. 1957. Cultivation of adult mouse mammary glands in hormone-enriched synthetic medium. Science 126: 842-844.

Everett, J. W., and C. H. Sawyer. 1959. The blocking effect of Nembutal on the ovulatory discharge of gonadotrophin in the cyclic rat. Proc. Soc. Exp. Biol. Med. 71: 696-698.

Everett, J. W., and C. H. Sawyer. 1950. A 24-hour periodicity in the "LH-release apparatus" of female rats disclosed by barbiturate sedation. Endocrinology 47: 198-217.

Everett, J. W., 1956. Functional corpora lutea maintained for months by autografts of rat hypophysis. Endocrinology 58: 786-796.

Flerko, Bela. 1966. Control of gonadotropin secretion in the female. In: Neuroendocrinology, Vol. I. L. Martini and W. F. Ganong (eds.). Academic Press, New York. 613-668.

Folley, S. J., and F. G. Young. 1941. Prolactin as a specific lactogenic hormone. Lancet 240: 380-381.

Fuxe, K., and T. Hokfelt. 1969. Catecholamines in the hypothalamus and the pituitary gland. In: Frontiers in Neuroendocrinology. W. F. Ganong and L. Martini (eds.). Oxford University Press, New York. 47-96.

Gay, V. L., A. R. Midgley, and G. D. Niswender. 1970. Patterns of gonadotropin secretion associated with ovulation. Fed. Proc. 29: 1880.

Gillespie, J. A., and S. M. Kirpekar. 1965. The inactivation of infused noradrenaline by the cat spleen. J. Physiol. 176: 205-227.

Grollman, A. 1965. Pharmacology and Therapeutics. Sixth Ed. Lea and Febiger, Philadelphia, Pa. 464-468; 335-343; 202-203.

Grosvenor, C. E., S. M. McCann, and M. D. Nallor. 1964. Inhibition of suckling-induced release of prolactin in rats injected with acid extract of bovine hypothalamus. Program 46th Meeting Endocrine Soc., San Francisco, p. 46.

Huggins, C., G. Briziarelli, and H. Sutton. 1959. Rapid induction of mammary carcinoma in the rat and the influence of hormones on the tumors. J. Exp. Med. 109: 25-42.

Huggins, C., L. C. Grand, and F. P. Bullantes. 1961. Mammary cancer induced by a single feeding of polynuclear hydrocarbons and its suppression. Nature 189: 204-207.

Huggins, C. 1965. Two principles in endocrine therapy of cancer: hormone deprival and hormone interference. Cancer Res. 25: 1163-1167.

Iversen, L. L. 1967. The uptake and storage of noradrenaline in sympathetic nerves. Cambridge University Press, London. 151-155.

Kamberi, I. A., R. S. Mical, and J. C. Porter. 1971. Effect of anterior pituitary perfusion and intraventricular injection of catecholamines on prolactin release. Endocrinology 88: 1012-1020.

Kanematsu, S., J. Hilliard, and C. H. Sawyer. 1963. Effect of reserpine on pituitary prolactin content and its hypothalamic site of action in the rabbit. Acta Endocrinologica 44: 467-474.

Kim, U., J. Furth, and K. H. Clifton. 1960. Relation of mammary tumors to mammotropes. I. Induction of mammary tumors in rats. II. Hormone responsiveness of 3-methylcholanthrene-induced mammary carcinomas. III. Hormone responsiveness of transplanted mammary tumors. Proc. Soc. Exp. Biol. Med. 103: 640-650.

Koch, Y., K. H. Lu, and J. Meites. 1970. Biphasic effects of catecholamines on pituitary prolactin release in vitro. Endocrinology 87: 673-675.

Kragt, C. L., and J. Meites. 1965. Stimulation of pigeon pituitary prolactin release by pigeon hypothalamic extract in vivo. Endocrinology 76: 1169-1176.

Kuhn, W. L., and E. F. Van Maanen. 1961. Central nervous system effects of thalidomide. J. Pharmacol. Exp. Therap. 134: 60-68.

Li, C. H., J. S. Dixon, K. D. Schmidt, Y. A. Pankov, and T. B. Lo. 1969. Amino and carboxy-terminal sequences of ovine lactogenic hormone. Nature 222: 1268-1269.

Lindsay, D., E. Poulson, and J. M. Robson. 1963. The effects of 5-hydroxytryptamine on pregnancy. J. Endocrinol. 26: 85-96.

Lu, K. H., Y. Amenomori, C. L. Chen, and J. Meites. 1970. Effects of central acting drugs on serum and pituitary prolactin levels in rats. Endocrinology 87: 667-672.

Lu, K. H., Y. Koch and J. Meites. 1971. Direct inhibition by ergocornine of pituitary prolactin release. Endocrinology 89: 229-233.

Lu, K. H., and J. Meites. 1971. Inhibition by l-dopa and monoamine oxidase inhibitors of pituitary prolactin release; stimulation by methyl-dopa and d-amphetamine. Proc. Soc. Exp. Biol. Med. 137: 480-483.

Lyons, W. R., C. H. Li, and R. E. Johnson. 1958. The hormonal control of mammary growth and lactation. Recent Progr. Horm. Res. 14: 219-254.

Malven, P. V. 1969. Luteotrophic and luteolytic responses to prolactin in hypophysectomized rats. Endocrinology 84: 1224-1229.

Malven, P. V., and C. H. Sawyer. 1966. A luteolytic action of prolactin in hypophysectomized rats. Endocrinology 79: 268-274.

Matsuo, H., Y. Baba, R. M. G. Nair, A. Arimura, and A. V. Schally. 1971. Structure of the procine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. Biochem. Biophys. Res. Comm. 43: 1334-1339.

Meites, J. 1959. Mammary growth and lactation. In: Reproduction in Domestic Animals, Vol. I. H. H. Cole and P. T. Cupps (eds.). Academic Press, New York. 539-593.

Meites, J. 1962. Pharmacological control of prolactin secretion and lactation. In: Pharmacological Control of Release of Hormones Including Antidiabetic Drugs, Vol. I. R. Guillemin (ed.). Pergamon Press, London. 151-181.

Meites, J. 1963. Neurohumoral and pharmacological control of prolactin secretion. In: Scientific Group on the Physiology of Lactation. World Health Organization, Geneva.

Meites, J. 1966. Control of mammary growth and lactation. In: Neuroendocrinology, Vol. I. L. Martini and W. F. Ganong (eds.). Academic Press, New York. 669-707.

Meites, J. (in press). The relation of estrogen and prolactin to mammary tumorigenesis in the rat. In: Breast Cancer Workshop: Estrogen, Target Tissue and Neoplasia. T. Dao (ed.). University of Chicago Press, Chicago.

Meites, J., and T. F. Hopkins. 1961. Mechanisms of oxytocin action in retarding mammary involution: study in hypophysectomized rats. J. Endocrinol. 22: 207-213.

Meites, J., C. S. Nicoll, and P. K. Talwalker. 1963. The central nervous system and the secretion and release of prolactin. In: Advances in Neuroendocrinology. A. V. Nalbandov (ed.). University of Illinois Press, Urbana. 238-277.

Meites, J., and C. L. Kragt. 1964. Effects of a pituitary homotransplant and thyroxine on body and mammary growth in immature hypophysectomized rats. Endocrinology 75: 575-570.

Meites, J., and C. S. Nicoll. 1966. Adenohypophysis: Prolactin. Ann. Rev. Physiol. 28: 57-88.

Meites, J., E. Cassell, and J. Clark (in press). Estrogen inhibition of mammary tumor growth in rats; counteraction by prolactin.

Mizuno, H., P. K. Talwalker, and J. Meites. 1964. Inhibition of mammary secretion in rats by iproniazid. Proc. Soc. Exp. Biol. Med. 115: 604-607.

Muckter, H. 1966. Thalidomide and Tumor. In: Antimicrobial Agents and Chemotherapy. Gladys L. Hobby (ed.). Ann Arbor, Michigan: American Society of Microbiology.

Muckter, H., E. Frankus, E. More, W. E. Kollmer, and M. Stoemmler. 1967. Experimentelle Untersuchungen mit cyclischen imiden bei Dimethylbenzanthracen-tumoren der Sprague-Dawley ratte. Zeitshrift fur Krebsforschung 69: 60-69.

Muckter, H., E. Frankus, and E. More. 1969. Experimental therapeutic investigations with 1-(morpholinomethyl)-4-phthalimido-piperidindione-2,6 on Dimethylbenzanthracene-induced tumors of Sprague-Dawley rats. Cancer Res. 29: 1212-1217.

Muscholl, E. 1961. Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. Br. J. Pharmac. Chemother. 16: 352-359.

Nagasawa, H., C. L. Chen, and J. Meites. 1969. Effects of estrogen implant in median eminence on serum and pituitary prolactin levels in the rat. Proc. Soc. Exp. Biol. Med. 132: 859-861.

Nagasawa, H., and J. Meites. 1970. Suppression by ergocornine and iproniazid of carcinogen-induced mammary tumors in rats; Effects on serum and pituitary prolactin levels. Proc. Soc. Exp. Biol. Med. 135: 469-472.

Nair, R. M. G., A. J. Kastin, and A. V. Schally. 1971. Isolation and structure of hypothalamic MSH Release-Inhibiting Hormone. Biochem. Biophys. Res. Comm. 43: 1376-1381.

Nandi, S., and H. A. Bern. 1961. The hormones responsible for lactogenesis in BALB/c Crgl mice. Gen. Comp. Endocrinol. 1: 195-210.

Nelson, W. O., R. Gaunt, and M. Schweizer. 1943. Effects of adrenal cortical compounds on lactation. Endocrinology 33: 325-332.

Nicoll, C. S., and J. Meites. 1962. Estrogen stimulation of prolactin production by rat adenohypophysis in vitro. Endocrinology 70: 272-277.

Nicoll, C. S., and J. Meites. 1963. Prolactin secretion in vitro: effects of thyroid hormones and insulin. Endocrinology 72: 544-551.

Nicoll, C. S., and H. A. Bern. 1964. Prolactin and the pituitary glands of fishes. Gen. Comp. Endocrinol. 4: 457-471.

Nicoll, C. S., and J. Meites. 1964. Prolactin secretion in vitro: effects of gonadal and adrenal cortical steroids. Proc. Soc. Exp. Biol. Med. 117: 579-583.

Nikitovitch-Winer, M. B., and J. W. Everett. 1958. Comparative study of luteotropin secretion by hypophysial autotransplants in the rat. Effects of site and stages of the estrous cycle. Endocrinology 62: 522-532.

Niswender, G. D., C. L. Chen, A. R. Midgley, Jr., J. Meites, and S. Ellis. 1969. Radioimmunoassay for rat prolactin. Proc. Soc. Exp. Biol. Med. 130: 793-797.

Pasteels, J. L., H. Brauman, and J. Brauman. 1963. Etude comparie de la secretion d'hormone somatotrope par l'hypophyse humaine in vitro, et de son activite lactogenique. Compt. Rend. Soc. Biol. 256: 2031-2033.

Pearson, O. H., O. Llerena, L. Llerena, A. Malina and T. Butler. 1969. Prolactin-dependent rat mammary cancer: A model for man? Trans. Ass. Amer. Physicians 82: 225-238.

Poulson, E., M. Botros, and J. M. Robson. 1960. The effects of 5-HT and iproniazid on pregnancy. J. Endocrinol. 20: Xl.

Ramirez, V. D., and S. M. McCann. 1964. Induction of prolactin secretion by implants of estrogen into the hypothalamo-hypophysial region of female rats. Endocrinology 75: 206-214.

Ratner, A., and J. Meites. 1962. Effect of estrogen administration in vivo on prolactin release in vitro. Proc. Soc. Exp. Biol. Med. 112:12-15.

Ratner, A., and J. Meites. 1964. Depletion of prolactin inhibiting activity of rat hypothalamus by estradiol or suckling stimulus. Endocrinology 75: 377-382.

Ratner, A., P. K. Talwalker, and J. Meites. 1965. Effect of reserpine on prolactin-inhibiting activity of rat hypothalamus. Endocrinology 77: 315-319.

Rivera, E. M. 1964. Interchangeability of adrenocortical hormones in initiating mammary secretion in vitro. Proc. Soc. Exp. Biol. Med. 116: 568-572.

Rothchild, I. 1965. Interrelations between progesterone and the ovary, pituitary, and central nervous system in the control of ovulation and the regulation of progesterone secretion. In: Vitamins and Hormones, Vol. 23. R. S. Harris, I. G. Wool, and J. A. Loraine (eds.). Academic Press, New York. 209-327.

Sar, M., and J. Meites. 1967. Changes in pituitary prolactin release and hypothalamic PIF content during the estrous cycle of rats. Proc. Soc. Exp. Biol. Med. 125: 1018-1021.

Setnikar, I., W. Murmann, and M. J. Magistretti. 1960. The retardation of sexual development in female rats due to iproniazid treatment. Endocrinology 67: 511-520.

Shelesnyak, M. C. 1954. Ergotoxine inhibition of deciduoma formation and its reversal by progesterone. Amer. J. Physiol. 179: 301-304.

Shelesnyak, M. C. 1955. Disturbance of hormone balance in the female rat by a single injection of ergotoxine ethanesulphonate. Amer. J. Physiol. 180: 47-49.

Shelesnyak, M. C. 1958. Maintenance of gestation in ergotoxine-treated pregnant rats by exogenous prolactin. Acta Endocrinol. 27: 99-109.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco. 218-230.

Somers, G. F 1960. Pharmacological properties of thalidomide (α-phthalimido glutarimide), a new sedative hypnotic drug. Br. J. Pharmac. Chemother. 15: 111-116.

Sterental, A., J. M. Dominquez, C. Weissmann, and O. H. Pearson. 1964. Pituitary role in the estrogen dependency of experimental mammary cancer. Cancer Research 23: 481-485.

Talwalker, P. K., and J. Meites. 1961. Mammary lubuloalveolar growth induced by anterior pituitary hormones in adreno-ovariectomized and adreno-ovariectomized-hypophysectomized rats. Proc. Soc. Exp. Biol. Med. 107: 880-883.

Talwalker, P. K., C. S. Nicoll, and J. Meites. 1961. Induction of Mammary secretion in pregnant rats and rabbits by hydrocortisone acetate. Endocrinology 69: 802-808.

Talwalker, P. K., A. Ratner, and J. Meites. 1963. In vitro inhibition of pituitary prolactin synthesis and release of hypothalamic extract. Amer. J. Physiol. 205: 213-218.

Talwalker, P. K., J. Meites, and H. Mizuno. 1964. Mammary tumor induction by estrogen or anterior pituitary hormones in ovariectomized rats given 7,12-dimethyl-1,2-benzanthracene. Proc. Soc. Exp. Biol. Med. 116: 531-534.

Thoenen, H., A. Hurlimann, and W. Haefely. 1964. The effect of sympathetic nerve stimulation on volume, vascular resistance and norepinephrine output in the isolated perfused spleen of the cat and its modification by cocaine. J. Pharmac. Exp. Ther. 143: 57-63.

Turner, C. W. 1939. The mammary glands. In: Sex and Internal Secretions, 2nd ed. E. Allen (ed.). Williams and Wilkins, Baltimore, Maryland. 740.

Van Maanen, J. H., and P. G. Smelik. 1968. Induction of pseudopregnancy in rats following local depletion of monoamines in the median eminence of the hypothalamus. Neuroendocrinology 3: 177-186.

Wakabayashi, I., and A. Arimura. 1971. Influence of stress and nembutal on serum prolactin levels in the rat. Fed. Proc. 30: 254.

Welsch, C. W., J. A. Clemens, and J. Meites. 1968. Effects of multiple pituitary homografts or progesterone on 7,12-dimethylbenzanthracene-induced mammary tumors in rats. J. Nat. Cancer. Inst. 41: 465-471.

Whitby, L. G., G. Hertting, and J. Axelrod. 1960. Effect of cocaine on the disposition of noradrenaline labelled with tritium. Nature 187: 604-605.

Wiest, W. G., W. R. Kidwell, and K. Balogh, Jr. 1968. Progesterone catabolism in the rat ovary: a regulatory mechanism for progestational potency during pregnancy. Endocrinology 82: 844-859.

Wuttke, W., and J. Meites. 1970. Effects of ether and pentobarbital on serum prolactin and LH levels in proestrous rats. Proc. Soc. Exp. Biol. Med. 135: 648-652.

Wuttke, W., and J. Meites. 1971. Luteolytic role of prolactin during the estrous cycle of the rat. Proc. Soc. Exp. Biol. Med. 137: 988-991.

Wuttke, W., E. Cassell, and J. Meites. 1971. Effects of ergocornine on serum prolactin and LH, and on hypothalamic content of PIF and LRF. Endocrinology 88: 737-741.

Wuttke, W., M. Gelato, and J. Meites (in press). Effects of pentobarbital on prolactin release.

Zeilmaker, G. H., and R. A. Carlsen. 1962. Experimental studies on the effect of ergocornine methane-sulphonate on the luteotrophic function of the rat pituitary gland. Acta Endocrinol. 44: 321-335.