SENESCENT ALTERATIONS OF LH AND PROLACTIN REGULATION IN THE FEMALE RAT

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ABSTRACT

SENESCENT ALTERATIONS OF LH AND PROLACTIN REGULATION IN THE FEMALE RAT

By

Barry Ernest Watkins

Effects of L-dopa and LRH on serum LH and prolactin were determined in young (4 mo) proestrous, estrous and second day diestrous, as well as in aged (23-33 mo) constant estrous and constant diestrous female Long-Evans rats. For all experiments a pretreatment control sub-orbital sinus blood sample was obtained about 10 minutes before drug treatment. In the L-dopa studies, intraperitoneal administration of 0.5 ml saline or a saline suspension of 3 or 30 mg L-dopa was made at 1:00 p.m. Serial bleedings were then performed at 15, 60 and 120 minutes after drug injection. In other experiments, 0.5 ml of saline or LRH doses of 5, 50 or 500 ng were intravenously injected at about 10:00 a.m., followed by bleedings at 15, 30 and 60 minutes. Further experiments were conducted to analyze effects of altered steroid feedback on prolactin and LH responses following L-dopa or LRH treatment. Two hour progesterone pretreatment (5 mg s.c.) was used for animals in all reproductive states of concern. A similar 2 hour priming regime using 20 µg estradiol benzoate was used for all groups except the young diestrous and aged constant diestrous rats, which received 24 hours of estrogen pretreatment. Serum prolactin and LH were then measured

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following acute administration of saline, 30 mg L-dopa or 50 mg LRH in the same schedules as previously described.

Basal serum prolactin was elevated during proestrus and estrus compared to diestrus. Aged rats had prolactin levels which were intermediate between those of young proestrous or estrous, and those of diestrous rats. The 30 mg dose of L-dopa maximally suppressed serum prolactin levels in both young and old rats by 15 minutes, with the suppression lasting for at least two hours. Conversely, 3 mg of L-dopa caused only transient depression of serum prolactin, which was of longer duration in young than in senescent rats. These results suggest that perhaps old rats are less capable of releasing prolactin release-inhibiting factor (PIF) or that pituitary responsiveness to PIF decreases with age. The possibility of age related changes in control of prolactin releasing factor secretion also exists.

Although neither estradiol benzoate nor progesterone priming affected the ability of 30 mg L-dopa to depress serum prolactin, the duration of prolactin suppression was generally more transient in both young and old rats than that occurring in those not receiving steroid priming. These changes induced by ovarian steroid pretreatment may be due to decreased L-dopa availability within the central nervous system (CNS) following impaired drug absorption or accelerated metabolism. These steroids may also suppress pituitary responsiveness to endogenously released PIF following L-dopa administration.

Basal serum LH was greatly elevated only on the afternoon of proestrus. Of the low levels representative of all other reproductive

states, LH values of old constant estrous rats were slightly greater than those of either estrus or diestrus, which in turn exceeded those found in aged constant diestrous animals. Young diestrous rats had a smaller LH increase following 30 mg L-dopa than did those in either proestrus or estrus. LH levels in old constant estrous rats increased more slowly than did those of younger ones, while aged constant diestrous rats were completely unresponsive to 30 mg of the drug. The delayed LH enhancement in old constant estrous rats and the lack of response in the aged constant diestrous group could indicate the senescent development of a variable degree of latency for either endogenous LRH release or of delayed pituitary response to LRH.

Fifty and 500 ng LRH elevated serum LH in all groups by 15 minutes in a dose related manner. The magnitude of LH response was greater in young proestrous and estrous rats than in the diestrous animals. Both groups of aged rats showed a smaller LH response to LRH induction than did any of the younger ones. The induced LH elevation was also delayed in both groups of aged rats. These results indicate that with senescence, the pituitary becomes less capable of secreting LH in response to a given LRH stimulus, and that the response generated occurs after a longer latency period.

Pretreatment with either estradiol benzoate or progesterone did not affect basal serum LH concentrations. Although progesterone pretreatment did not greatly alter the ability of L-dopa to increase serum LH levels, estradiol benzoate therapy abolished the L-dopa effect in all groups other than that of young proestrous rats. On the other

hand, estrogen pretreatment increased pituitary responsiveness to LRH in estrous, diestrous and old constant estrous animals. Progesterone pretreatment preferentially enhanced pituitary responsiveness in all young rats without significantly affecting that of senescent animals. These results show that while estradiol benzoate therapy can restore pituitary responsiveness of constant estrous rats to levels corresponding to young cycling rats, increasing the steroid's availability is incapable of restoring functional pituitary response capacity of aged constant diestrous rats. It was also shown that pituitaries of aged rats were refractory to the progesterone effect of enhancing pituitary responsiveness. Thus estradiol benzoate plays a dual role in providing feedback information for control of LH secretion by augmenting the ability of the pituitary to secrete LH while under LRH stimulation, while simultaneously blunting L-dopa's effect to elevate serum LH through presumed dopaminergic activation of hypothalamic LRH release.

SENESCENT ALTERATIONS OF LH AND PROLACTIN REGULATION IN THE FEMALE RAT

By

Barry Ernest Watkins

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INTRODUCTION

The field of experimental gerontology has been less extensively investigated than most other areas of bio-medical research concern. The current status of knowledge regarding the physiology of ageing has been aptly summarized by the Russian scientist Vladimir Frolkis (1968) who stated that "the essence of ageing is the sum total of unequal changes in regulatory processes at the molecular, cellular and systemic levels of the organism." In other words, debilitations of ageing appear to be related to impaired functioning of homeostatic control mechanisms. The endocrine and nervous systems are recognized to be major mediators of long and short term regulation of homeostasis, respectively. Maintenance of homeostasis through time is thus to a large part dependent on functional patency of endocrine control systems as they are influenced by neural and neuroendocrine regulation. Reproduction appears to be an ideal system to investigate senescent alteration of regulatory processes because mammals typically exhibit impaired reproductive capacity with ageing. The rat was chosen as an experimental model for this research because of its short life span. Also normal neuroendocrine control of gonadotropin and prolactin secretion, as well as ovulation, has already been rather fully characterized in this species. The research described here was primarily conducted to determine the influence of senescence on LH and prolactin control in the female rat.

LITERATURE REVIEW

Ageing

The rat has been extensively used as a convenient experimental model to study the impairment of reproductive performance that occurs during senescence. Cyclic patterns of vaginal cytology are altered with advancing age. Aged rats have irregular cycles of vaginal cytology which show unpredictable patterns, long periods of vaginal cornification, or persistent leucocytic smears characteristic of pseudopregnancy (Ingram, 1959; Clemens and Meites, 1971; Peng and Huang, 1972). Some aged rats can show continuous leucocytic smears accompanied with long term ovarian atrophy, suggesting that loss of gonadotropin secretion may be responsible for onset of anestrus (Peng and Huang, 1972). Sexual acceptance of the male was also shown to be less strongly associated with periods of vaginal "estrous" cornification in old than in young adult rats (Ingram, 1959). Mandl and Shelton (1959) further reported that reduced fertility precedes the exhausting of ovarian oocyte supply in the rat. This decreased reproductive capacity could be related to the generalized ovarian atrophy which was reported to occur in old constant estrous rats by Clemens and Meites in 1971. However it has been demonstrated that ovaries of old constant estrous animals do not appear overtly abnormal, typically having well developed follicles with no evidence of luteal tissue (Clemens and Meites, 1971). In addition,

it was shown by Aschheim in 1965 that intravenous administration of purified LH to aged constant estrous rats caused resumption of estrous cycling, presumably through the induction of ovulation. Further, transplantation of ovaries from young adult or immature female rats into old constant estrous or pseudopregnant recipients did not restore normal estrous cycles (Aschheim, 1964; Peng and Huang, 1972). On the other hand, young adult female rats receiving transplants of ovaries from aged donor rats can continue to show normal estrous cycling (Aschheim, 1964; Peng and Huang, 1972). These observations suggest that ovarian dysfunction is not the primary cause of reproductive decline in the rat.

Age related changes are known to develop at the hypophysial level in the rat. Pecile *et al.* (1966) reported that pituitaries of old rats have proportionally higher numbers of acidophils than do young counterparts, a finding which is compatible with the observation that old constant estrous rats have elevated pituitary prolactin content (Clemens and Meites, 1971). Clemens and Meites (1971) further indicated that such senescent rats have higher pituitary FSH and lower LH content than do those of young cycling rats on the day of estrus. Age related changes in the integrity of pitutary function was evaluated by Pecile *et al.* in 1966. Using young hypophysectomized female rats they found that gonadal function, as assessed by vaginal estrous cyclicity and by uterine and ovarian weight, could be restored by transplanting pituitary tissue from 30 day old donor rats into the evacuated pituitary capsule of the hypophysectomized assay animals. However, hypophysial

transplantation from 8 or 24 month old donors did not restore gonadotropic function. In a similar series of experiments Peng and Huang (1972) transplanted pituitaries from rats over 16 months old under the hypothalamic median eminence of hypophysectomized young adults. Following such surgical manipulation estrous cycling was restored in 10 of 30 rats treated, and fertility was demonstrated. They also found that 3 out of 4 young rats which received both pituitary and ovarian transplants from old donors showed continued estrous cycling. These pituitary transplant studies indicate that some aged rat pituitaries can release gonadotropins in cyclic patterns when transplanted to the median eminence of young recipients. Although functional alterations at the hypophysial level are implicated, they may not totally be responsible for senescent impairment of reproduction.

There is evidence that central nervous system parameters, particularly hypothalamic structures involved with neuroendocrine feedback control systems, are functionally altered during the course of ageing. A recent series of experiments has assessed age related changes within the hypophysial-adrenocortical axis (Hess and Riegle, 1972; Riegle and Hess, 1972; Riegle, 1973). These studies have shown that experimental procedures entailing elevation of endogenous blood plasma corticosteroid levels following depo ACTH treatment, direct enhancement of glucocorticoid titers with dexamethasone therapy, or chronic stress-induced corticosteroid increases; all result in attenuation of acute stress-related plasma corticosteroid elevation to a greater degree in young than in old rats. These results indicate that

adrenocortical feedback control system sensitivity is depressed in the senescent rat. It has also been suggested that the threshold for hypothalamic feedback control of gonadotropin secretion elevates with age (Dilman, 1971), possibly due to impaired neurotransmitter synthesis (Frolkis, 1966). In support of this concept, Clemens and Meites (1971) reported elevated hypothalamic follicle stimulating hormone-releasing factor (FRF) activity in aged constant estrous rats compared to that of young ones on the day of estrus. Attempts have recently been made to experimentally restore reproductive capacity of senescent rats. It has been shown that intravenous LH administration (Aschheim, 1965) causes resumption of estrous cycling. Similarly, systemic progesterone treatment of several days duration to aged rats in constant estrus is capable of inducing ovulation in association with at least one apparently normal estrous cycle (Clemens *et al.*, 1969). Clemens *et al.* (1969) also demonstrated that direct electrical stimulation of the hypothalamic preoptic area of old constant estrous rats can cause ovulation. Their report further indicated that prolonged systemic epinepherine administration could induce ovulation in old rats; the effect possibly mediated through alteration of certain central nervous system neuroendocrine functions. Similarly, chronic administration of L-β-3-4-dihydroxyphenylalanine (L-dopa) or iproniazid, drugs assumed to increase hypothalamic catecholamine availability, restored regular cycling patterns in old constant estrous rats (Quadry *et al.*, 1973).

Prolactin Control

General Considerations

In vitro work by Meites *et al.* in 1961 suggested that pituitary prolactin production is twice as great in mature female rats as in prepuberal ones. By incubating pituitaries with ^{14}C leucine, Ieire *et al.* (1972) reported that *in vitro* prolactin synthesis rates in mature female rats is highest during proestrus and estrus, and that prolactin release is lowest on the day of diestrus. In the normal cycling rat, serum prolactin levels have been found to surge on the day of proestrus (Amenomori *et al.*, 1970; Wuttke and Meites, 1970; Neill *et al.*, 1971; Uchida *et al.*, 1972; Taya and Igarashi, 1973). The precise duration of serum prolactin elevation has been variously reported by different groups of investigators. Neill *et al.* (1971) indicated that serum prolactin returns to baseline levels by midnight on the day of proestrus. However other investigators have demonstrated elevated serum prolactin levels which lasted from the afternoon of proestrus through the day of estrus (Niswender *et al.*, 1969; Amenomori *et al.*, 1970; Taya and Igarashi, 1973). It was further shown by Uchida *et al.* in 1972 that about two-thirds of their rats exhibited prolactin surges which ended during the night of proestrus while the remaining animals had prolactin elevations which were still evident on the day of estrus. It has recently been demonstrated that serum prolactin levels can also show two separate elevations which occur on the afternoon of proestrus and on the day of estrus (Riegle, unpublished). The exact cause of these

nonuniform patterns of prolactin surging is not yet known. These findings could be a reflection of variability in normal secretion patterns between animals or between strains of rats. It is also possible that on the day of estrus some rats have exaggerated sensitivity to nonspecific stressors associated with experimental manipulation which are thought to increase serum prolactin concentrations (Neill, 1970; Valverde-R. *et al.*, 1973).

Hypothalamic Monoamines and Prolactin Control

Considerable investigative effort has recently been devoted to attempt to elucidate the role that the central nervous system (CNS) plays in the control of hypophysial prolactin secretion. It is generally accepted that the major central regulation of prolactin secretion is of suppressive nature mediated through a hypothalamic prolactin inhibiting factor (PIF) (Meites *et al.*, 1961; Talwalker *et al.*, 1963; Kamberi *et al.*, 1971c; Kanematsu and Sawyer, 1973). In addition, recent evidence suggests the presence of a hypothalamic prolactin releasing factor (PRF) which appears to be elaborated during conditions such as those associated with ether stress (Valverde-R. *et al.*, 1973). It has recently been shown that the synthetic tripeptide pGlu-His-ProNH_2, known as thyrotropin releasing hormone or TRH, can cause prolactin to be released from the pituitary (Bowers *et al.*, 1973). In addition the findings of Bowers *et al.* (1973) suggest that since TRH is equally capable of increasing secretion of prolactin as well as of thyroid stimulating hormone (TSH), TRH might be a physiological PRF. In support of this concept, it has been reported that intravenous TRH injection

results in elevation of serum prolactin concentrations (Mueller *et al.*, 1974), even in rats with median eminence lesions (Porteus and Malven, 1974).

As a consequence of the discovery of hypothalamic regulating factors, many investigations were carried out to evaluate possible involvement of various CNS neuro-transmitters in the control of PIF release. Coppola *et al.* (1965) found an inverse relationship between CNS catecholamine availability and the ease of pseudopregnancy induction in the rat. In these experiments, the use of reserpine, α-methyldopa or tetrabenazine to deplete brain catecholamine levels stimulated the onset of pseudopregnancy, presumably due to drug-evoked impairment of PIF release. They also found that the effect could be reversed following restoration of monoamine stores with systemic treatment of L-dopa, the metabolic precursor of dopamine, norepinepherine, and epinepherine. The assumption that these effects occurred within the brain were confirmed in 1968 by van Maanen and Smelik, who demonstrated the induction of pseudopregnancy associated with monoamine depletion in the hypothalamic median eminence following implantation of reserpine within the basal hypothalamus. They also showed that simultaneous enhancement of hypothalamic catecholamine levels with the monoamine oxidase inhibitor iproniazid could overcome the reserpine effect. With the advent of radioimmuno-assay techniques for assessment of rat prolactin (Niswender *et al.*, 1969), an attempt was made by Lu *et al.* (1970) to verify and expand these findings concerning the neuro-humoral control of prolactin secretion. Intraperitoneal administration of drugs known

to inhibit catecholamine activity (reserpine, chlorpromazine, α-methyl-para-tyrosine, or α-methyl-meta-tyrosine) markedly elevated serum prolactin levels in proestrous rats by 30 minutes after injection and generally decreased pituitary prolactin content when measured at 4 hours after drug treatment. However direct systemic treatment of suspected neuro-transmitters such as dopamine, epinepherine, norepinepherine or serotonin, did not affect serum prolactin, presumably due to their inability to diffuse across the blood-brain barrier (Lu *et al.*, 1970). It has further been shown by Kleinberg *et al.* (1971) that the ability of systemic chlorpromazine therapy to elevate serum prolactin concentrations is strikingly inhibited when coupled with L-dopa pretreatment. Intraperitoneal L-dopa administration has been reported to effectively suppress serum prolactin levels in both intact female rats and in hypophysectomized rats bearing anterior pituitary grafts (Lu and Meites, 1971; Lu and Meites, 1972). Using an *in vitro* assay protocol, Lu and Meites (1972) also showed that L-dopa treatment elevated PIF activity both in peripheral serum and within the hypothalamus. In addition, the drug was effective in elevating hypothalamic and blood serum PIF activity in hypophysectomized rats (Lu and Meites, 1972). These results present strong evidence that systemic administration of L-dopa can suppress serum prolactin concentration by acting within the CNS to increase dopamine availability which in turn enhances hypothalamic synthesis and/or secretion of PIF. Using *in vitro* incubation methods, Kamberi *et al.* (1970a) demonstrated that injection of dopamine directly into the third ventricle of the brain caused

enhancement of PIF activity in hypophysial stalk portal blood plasma of adult male rats. These results can be interpreted to mean that dopamine acted within the hypothalamus or associated CNS areas to stimulate secretion of hypothalamic PIF into the hypophysial portal system. Alternatively, it is possible that dopamine diffused into the pituitary stalk portal circulation where it directly acted on the pituitary as a prolactin inhibiting factor in a manner which had been earlier hypothesized by van Maanen and Smelik (1968). In an attempt to clarify the role of hypothalamic neuro-transmitters on prolactin secretion, Kamberi *et al.* (1971a) found that intraventricular administration of dopamine hydrochloride to male rats markedly decreased serum prolactin levels while similar injection of epinepherine or norepinepherine was without effect. However very high nonphysiological doses of epinepherine and norepinepherine did result in significant serum prolactin suppression. In addition, none of the monoamines altered prolactin concentration either when perfused to the anterior pituitary by way of a cannulated hypophysial portal vessel or when injected into infundibular or peduncular arteries (Kamberi *et al.*, 1971a). Kamberi *et al.* (1970c) also found that simultaneous intraventricular injection of dopamine and pronethalol (a β-adrenergic blocker) caused typical prolactin suppression, while dopamine in association with an α-adrenergic blocker such as phentolamine or phenoxybenzamine, prevented the prolactin response seen with dopamine alone. These observations indicate that dopamine, rather than epinepherine or norepinepherine, is the monoamine which exerts greatest inhibition of pituitary prolactin secretion. It

is also apparent that the dopamine effect is mediated through activation of certain hypothalamic α-adrenergic receptors to induce the release of a hypothalamic PIF since dopamine itself is not able to suppress pituitary prolactin when directly perfused into the pituitary or when delivered via general cephalic arteries.

However evidence is accumulating which suggests that catecholamines may directly inhibit prolactin release at the pituitary level. There have been reports that *in vitro* incubation of pituitaries with dopamine, epinepherine or norepinepherine causes decreased prolactin release accompanied by accumulation of the hormone within the adenohypophysis (MacLeod, 1969; Birge *et al.*, 1970; Schally *et al.*, 1974; Shaar and Clemens, 1974). In *in vitro* experiments Shaar and Clemens (1974) have also shown that the PIF activity of hypothalamic extracts can be abolished with pre-incubation with monoamine oxidase or with aluminum oxide to absorb available catecholamines. Other *in vivo* evidence presented by Donoso *et al.* (1973) indicates that L-dopa treatment can suppress serum prolactin in castrate rats with complete median eminence lesions, suggesting that high catecholamine levels may directly inhibit pituitary prolactin release. Perfusion of hypophysial portal vessels with glucose solutions containing dopamine or norepinepherine also has been shown to decrease serum prolactin, implying that these monoamines may be prolactin release-inhibiting factors (Takahara *et al.*, 1974).

Techniques employing histochemical fluorescence of hypothalamic catecholamines have allowed qualitative assessment of catecholamine

content within the predominately dopaminergic tubero-infundibular neurons of the median eminence. Such procedures, accompanied by treatment with an inhibitor of catecholamine synthesis (α-methyltyrosine-methylester), makes possible general determination of catecholamine turnover as reflected by the relative depletion of histochemical fluorescence (Ahrén *et al.*, 1971; Hökfelt and Fuxe, 1972; Fuxe *et al.*, 1973). Absolute levels of hypothalamic dopamine content as gauged by fluorescent intensity in tubero-infundibular neurons are not altered by hypophysectomy, castration, or treatment with exogeneous pituitary hormones (Hökfelt and Fuxe, 1972). However tubero-infundibular catecholamine turnover varies as a function of the estrous cycle, being lowest during proestrus and early estrus (Fuxe *et al.*, 1969; Ahrén *et al.*, 1971). Intravenous prolactin pretreatment in normal and hypophysectomized rats of both sexes generally enhanced hypothalamic dopamine turnover rate to that characteristic of cycling female rats during diestrus (Hökfelt and Fuxe, 1972). These findings provide further evidence linking deopminergic activity within the hypothalamus with PIF release since an inverse relationship is demonstrated between previously reported prolactin levels during the estrous cycle (Amenomori *et al.*, 1970; Taya and Igarashi, 1973), and corresponding alterations in tubero-infundibular dopamine turnover rates. These results support the concept that prolactin treatment acts to inhibit the endogenous secretion of prolactin by increasing hypothalamic dopamine turnover, which presumably causes enhancement of PIF release (Hökfelt and Fuxe, 1972).

Investigations have also been carried out to evaluate possible involvement of other suspected CNS neuro-transmitters on prolactin control. Intraperitoneal administration of serotonin and its immediate metabolic precursor, 5-hydroxy-L-tryptophan, had no effect on serum prolactin concentrations in rats (Lu *et al.*, 1970; Smythe and Lazarus, 1973). However a recent report by Lu and Meites (1973) indicated that high intravenous doses of 5-hydroxy-L-tryptophan markedly increased serum prolactin by 30 minutes after injection, while serotonin itself did not alter peripheral prolactin levels. They theorized that the reason serotonin was without effect on serum prolactin was likely due to its inability to pass the blood-brain barrier. Using intraventricular administration of serotonin and melatonin, Kamberi *et al.* (1971b) showed that these indolamines could elevate serum prolactin. They also discounted the possibility that serotonin or melatonin directly promotes hypophysial prolactin release when they found that perfusion of the pituitary by way of a cannulated hypophysial stalk portal vessel did not affect serum prolactin levels. These observations suggest the additional existence of a serotonergic system in the hypothalamus which may act in antagonism to the dopaminergic inhibitory system by facilitating pituitary prolactin secretion. In addition, cholinergic pathways have recently been implicated in the control of pituitary prolactin release. The blocking of cholinergic synapses with atropine has been shown to depress serum prolactin in rats on the day of estrus (Gala *et al.*, 1972) as well as to block the proestrous preovulatory prolactin surge (Libertun and McCann, 1973). Similarly systemic treatment with

nicotine, known to be a cholinergic pre-ganglionic blocking agent, has been reported to delay or totally block the proestrous prolactin elevation in the rat (Blake *et al.*, 1973; Blake, 1974). Therefore involvement of certain CNS cholinergic neuron systems may also promote preovulatory prolactin surging in the rat.

Electrophysiologic studies have also been performed to try to anatomically define hypothalamic structures involved in the control of prolactin release. Although acute electrochemical stimulation of the ventral medial hypothalamus and medial preoptic area of the hypothalamus did not alter serum prolactin (Wuttke and Meites, 1972; Gala *et al.*, 1973), stimulation of the hypothalamic medial preoptic area with chronically implanted electrodes was shown to evoke marked elevations of serum prolactin (Wuttke and Meites, 1972).

It is well established that estrogen therapy to ovariectomized rats elevates serum and pituitary prolactin levels (Amenomori *et al.*, 1970; Lu and Meites, 1971; Bishop *et al.*, 1972; Kalra *et al.*, 1973). *In vitro* experiments have demonstrated that this effect is at least partially mediated by a direct stimulatory action of the hormone on pituitary prolactin release (Nicoll and Meites, 1964; Lu *et al.*, 1971). In 1972 Bishop *et al.* reported that suprachiasmic lesions cannot affect estrogen's ability to elevate serum prolactin, while lesions in the median eminence prevent the estrogen effect. The authors concluded both that estrogen's stimulatory effect on prolactin release is mediated at the median eminence, and that the direct enhancement of estrogen on pituitary prolactin secretion may not be physiologically important since

the steroid was ineffective in ovariectomized rats with medium eminence lesions. By injecting normally cycling rats with an antiserum to estradiol on the second day of diestrus, Neill *et al.* (1971) were able to block the expected proestrous surge of prolactin. In 1972 Neill also demonstrated that estrogen treatment of ovariectomized adult rats resulted in initiation of periodic prolactin surges which could be overcome by disrupting anterior neural input to the hypothalamus. These results indicate that estrogen is essential for induction of the pre-ovulatory prolactin surge, and that it likely acts within CNS centers rostral of the hypothalamus to promote prolactin secretion. In intact female rats, systemic estradiol benzoate given on the morning of estrus resulted in significant plasma prolactin elevation by the afternoon of the following day, while if the steroid was aministered on the first day of diestrus, serum prolactin remained unchanged when measured on the second day of diestrus (Kalra *et al.*, 1973). However Ying and Greep (1972) showed that estradiol benzoate given on day one of diestrus invariably induced the onset of pseudopregnancy, presumably associated with elevated serum prolactin levels. These data suggest that the sensitivity of feedback mechanisms by which estradiol benzoate affects prolactin secretion alters during various stages of the estrous cycle.

In vitro experiments by Nicoll and Meites (1964) indicated that progesterone does not directly affect hypophysial prolactin release. On the other hand, systemic injection of high doses of progesterone elevated serum prolactin in castrate female rats (Kalra *et al.*, 1973). In intact rats, progesterone administration is capable of both increasing

and decreasing estrous cycle length, depending on time of injection (Paup, 1973). It has also been reported that progesterone therapy on the morning of proestrus in intact rats can advance the onset time of the preovulatory prolactin surge by about 3 hours, as well as enhance the magnitude of response (Uchida *et al.*, 1972; Kalra *et al.*, 1973).

LH Control

General Considerations

Using the ovarian ascorbic acid depletion technique, Ramirez and McCann (1964) determined normal LH levels during the estrous cycle of the rat. They found that plasma LH began to rise on the morning of proestrus, peaked that afternoon, and had not yet returned to basal levels by the morning of estrus. With the development of a sensitive radioimmuno-assay specific for rat LH (Monroe *et al.*, 1968), it became possible to more precisely evaluate normal patterns of LH secretion as reflected by peripheral hormone concentrations. Subsequent investigations have shown that the preovulatory LH surge occurs in its entirety during the afternoon or evening of proestrus, with measurable serum LH elevation between 3 and 9 p.m. in most rats (Goldman *et al.*, 1969; Monroe *et al.*, 1969; Wuttke and Meites, 1970; Neill *et al.*, 1971; Taya and Igarashi, 1973). However in any given rat, the duration of the LH surge is thought to last for only 1 to 3 hours (Monroe *et al.*, 1969).

It is believed that control of gonadotropin secretion in cycling female rats involves tonic CNS negative feedback regulation by ovarian steroids and by LH itself. Both *in vitro* and *in vivo* experiments have

shown that estrogen can act within the hypothalamus to presumably decrease LRH-induced pituitary LH secretion, thereby depressing serum LH levels (Kalra *et al.*, 1973; Legan *et al.*, 1973; Saksena *et al.*, 1973). In addition, the existence of short loop negative feedback of LH on gonadotropin control was shown by the report that implantation of LH into the medial basal hypothalamus can inhibit ovarian function (Ojeda and Ramirez, 1969).

Activation of hypothalamic preoptic mechanisms which induce LH release is periodically superimposed on this tonic inhibition of gonadotropin secretion. It has been reported that lesions within dorsal hypothalamic areas have no effect on elevated serum luteinizing hormone-releasing factor (LRF) activity in chronically hypophysectomized rats, while median eminence lesions depress LRF activity (Naller and McCann, 1965). These results can be interpreted to mean either that the median eminence is itself the site of LRF synthesis or that the median eminence acts as an area for storage of LRF which had been synthetized in more anterior hypothalamic regions. The combined findings that suprachiasmatic lesions decreased hypothalamic LRF activity and that marked LRH activity was present in the supra-optic area of the hypothalamus indicate that this region may constitute a major site of LRH synthesis (Crighton and Schneider, 1969).

The normal cyclic changes in serum LH levels of mature female rats can be altered by appropriately timed administration of gonadal steroids or of certain centrally acting pharmacologic agents, notably barbiturates (Wuttke and Meites, 1970; Krey and Everett, 1971; Ying and

Greep, 1972; Beattie and Schwartz, 1973). Pentobarbital is capable of blocking both the expected preovulatory LH surge and subsequent ovulation when given between 1:30 p.m. of the last day of diestrus and 1:30 p.m. of proestrus (Redmond, 1968; Wuttke and Meites, 1970; Beattie and Schwartz, 1973). The report by Beattie and Schwartz (1973) demonstrating pentobarbital's ability to inhibit LH surging 24 hours after administration, suggests that the short acting drug may not have direct adenohypophysial action but rather that it interferes with a neural clock mechanism involved with preovulatory gonadotropin elaboration. In accord with this hypothesis, it has been found that administration of pentobarbital in the early afternoon on any day in four day cyclic rats could delay the next ovulation by 24 hours (Domingues and Smith, 1971). Wuttke and Meites (1972) reported that the pentobarbital induced suppression of preovulatory gonadotropin release could be overcome by electrochemical stimulation of electrodes which were chronically implanted in the hypothalamic medial preoptic area or within the arcuate nucleus region. It was also concluded from these studies that the neural triggering mechanisms causing LH release are at least partially located within the medial preoptic area. Estrogen can also influence the onset time of LH surging. Injections of estradiol benzoate given on the morning of the first day of diestrus in 4 day cycling rats advanced ovulation time by 24 hours (Ying and Greep, 1972; Krey *et al.*, 1973). Ying and Greep (1972) also showed that this estrogen effect can be overcome by pentobarbital treatment during the critical period before the expected precocious gonadotropin

surge. Recent work has suggested that the stimulatory feedback effects of exogenous estrogen on gonadotropin release depended on progesterone synergism (Krey *et al.*, 1973; Mann and Barraclough, 1973). Further evidence suggests that subcutaneous progesterone injections can partially overcome the inhibitory influence of minimally effective pentobarbital doses on ovulation (Kabayashi *et al.*, 1973). In addition, administration of progesterone on the second day of diestrus or during proestrus increased serum LH within 6 hours after therapy (Naller *et al.*, 1966). It was also shown by Redmond (1968) that progesterone administration on the morning of proestrus could induce premature gonadotropin release as indicated by the time of ovulation. More recently, Uchida *et al.* (1972) extended the work of Redmond with the demonstration that progesterone given during the morning of proestrus advanced the preovulatory gonadotropin surge by about 3 hours in intact cycling rats. Although the mechanism of action for these progesterone effects is unknown, it is quite possible that the steroid acts within the CNS both to counteract pentobarbital induced suppression of LH release and to alter normal clock mechanisms that control the timing of preovulatory gonadotropin secretion.

Surgical ovariectomy is another approach which has been employed as an experimental tool to determine requirements for initiation of pulsatile gonadotropin surging. Ovariectomy has often been shown to remove negative feedback of ovarian steroids on gonadotropin secretion, thereby resulting in elevated serum gonadotropin levels (Ramirez and McCann, 1963 and 1965; Blake *et al.*, 1972; Kawakami *et al.*, 1973) as

well as enhanced serum and hypophysial portal vessel LRF activity (Schneider and McCann, 1970a; Ajika *et al.*, 1972; Ben-Jonathan *et al.*, 1973). Ajika *et al.* (1972) also demonstrated that 24 or 48 hours of estradiol benzoate therapy decreased both pituitary LH content and hypothalamic LRF activity. Likewise, replacement therapy with estradiol benzoate and progesterone in long term ovariectomized rats can suppress the elevated serum LH concentrations (Naller *et al.*, 1966; Blake *et al.*, 1972; Kalra *et al.*, 1973). There is also evidence that estrogen alone is able to markedly decrease serum LH levels in ovariectomized rats (Saksena *et al.*, 1972; Kalra *et al.*, 1973). Estrogen availability may be the only requirement to induce pulsatile gonadotropin surging. Treatment of ovariectomized rats solely with estrogen can result in resumption of LH surging on the second day after injection (Neill, 1972; Legan *et al.*, 1973). Further, estrogen is capable of inducing daily afternoon LH surges in long term ovariectomized rats which are of comparable duration to normal proestrus gonadotropin surging in intact cycling rats (Legan and Midgely, personal communication). However lesions in the median eminence of ovariectomized rats prevented this estrogen effect (Bishop *et al.*, 1972b). Collectively, these observations suggest that positive feedback may act as a stimulus to implement the triggering of neural clock mechanisms responsible for pulsatile LH release. Support for this hypothesis was found in intact rats, where treatment with antiserum to estradiol at 1 p.m. of the second day of diestrus abolished the expected preovulatory gonadotropin surge (Neill *et al.*, 1971).

Progesterone may interact with estrogen to control gonadotropin secretion. Increased serum LH concentrations were induced within 4 hours after progesterone administration in chronically ovariectomized rats pretreated with testosterone propionate to depress serum LH levels (Jackson, 1973). Using ovariectomized rats, Kalra and co-workers (1972) indicated that systemic progesterone given 48 hours after a priming dose of estradiol benzoate caused elevation of serum LH evidenced at 12 hours after injection. Activation of α-adrenergic norepinepherine pathways within the CNS was thought to be responsible for this effect since selective inhibition of hypothalamic norepinepherine content with diethyldithiocarbamate, or treatment with the α-adrenergic blocking agent phenoxybenzamine inhibited the gonadotropin elevation. Thus use of the ovariectomized rat as a model for assessment of the influence of gonadal steroids on CNS triggering mechanisms for pulsatile LH release shows the existence of complex relationships between steroid availability and subsequent timing of neuroendocrine events.

Precise anatomical location of the sites of steroid action on control of gonadotropin secretion is not yet well understood. In addition to proposed hypothalamic areas within the arcuate nucleus and median eminence area for negative steroid feedback interaction (Schneider and McCann, 1970; Bishop *et al.*, 1972; Piva *et al.*, 1973), there is evidence that the limbic system may affect control of gonadotropin secretion (Ellendorf *et al.*, 1972; Kawakami *et al.*, 1973; Piva *et al.*, 1973). Ellendorf *et al.* (1972) reported that micro-electrode stimulation of electrodes implanted in the amygdala during the afternoon of proestrus blocked ovulation. They also demonstrated that amygdalar stimulation

in chronically ovariectomized rats caused transient decrease in serum LH concentration. On the other hand, Kawakami *et al.* (1973) found that stimulation of either medial amygdaloid regions or of the dorsal hippocampus increased serum gonadotropin levels. There is evidence that tritiated progestins are concentrated in both the hypothalamus and hippocampus in the mouse (Luttge *et al.*, 1973). It has also recently been shown that implantation of progesterone into the amygdala causes decreased hypothalamic LRH content, and presumably depressed levels of serum LH (Piva *et al.*, 1973). Although these results are somewhat conflicting, they nonetheless provide strong evidence for involvement of the limbic system in gonadotropin control.

Hypothalamic Monoamines and LH Control

Much experimentation has been done in an attempt to clarify that part played by the CNS in control of ovulation. In 1969 Kordon and Glowinski investigated the effect of altered CNS catecholamine availability on gonadotropin induced ovulation in prepuberal female rats. They found that inhibition of catecholamine synthesis with α-methyl tyrosine administration on the afternoon of the day preceding expected ovulation blocked its occurrence. They also made the observation that L-dopa treatment to restore catecholamines in these blocked rats partially restored ovulation, while L-threodihydroxyphenlyserine therapy to selectively restore brain norepinepherine levels was ineffective. These results suggest that functional patency of CNS dopaminergic synapses was necessary for successful ovulation induction. In a

similar series of experiments, Rubinstein and Sawyer (1970) examined the effects of depressed CNS catecholamine availability on ovulation in cycling rats. They found that depletion of brain catecholamines with reserpine treatment in the morning of proestrus inhibited ovulation and decreased the ability of electrochemical stimulation of the hypothalamic medial preoptic area to induce ovulation. Intraventricular injection of epinepherine was better able to overcome the pentobarbital block to ovulation than was norepinepherine, dopamine or serotonin, suggesting that hypothalamic epinepherine releasing synapses could be important in bringing about ovulation (Rubinstein and Sawyer, 1970). Kamberi *et al.* (1969) utilized an *in vitro* assay technique in an attempt to link brain catecholamine availability to associated LRH activity and LH release. They found that co-incubation of norepinepherine or dopamine with rat pituitaries generally did not affect the rate of LH release as measured by the ovarian ascorbic acid depletion assay. When stalk median eminence fragments were included in the incubations, dopamine rather than norepinepherine or serotonin caused enhanced pituitary LH release. These results indicated that dopamine stimulated pituitary LH release by promoting LRF secretion from hypothalamic stalk median eminence tissue. The development of a sensitive radioimmuno-assay for rat LH (Monroe *et al.*, 1968) made possible a more critical examination of CNS catecholamine involvement in the control of gonadotropin secretion and of subsequent ovulation. Using radioimmuno-assay for LH determination, further *in vitro* studies were performed showing that co-incubation of pituitaries with dopamine, norepinepherine or serotonin did not alter

basal LH release, while epinepherine caused slight elevation of LH release (Schneider and McCann, 1969). It was also demonstrated by Schneider and McCann (1969) that with stalk median eminence added to incubations, only dopamine was able to facilitate pituitary LH release. Further experiments employing α- and β-adrenergic blocking agents demonstrated that dopamine's action to enhance LRH secretion was mediated through α-adrenergic mechanisms (Kamberi *et al.*, 1969; Schneider and McCann, 1969; Schneider and McCann, 1970b). Additional evidence that the dopamine effect to enhance pituitary LH elaboration is mediated through promotion of hypothalamic LRH release comes from the report that LRF activity is increased in hypophysial portal stalk plasma of rats treated with intraventricular dopamine (Kamberi *et al.*, 1969). In *in vivo* experiments it has been shown that while intraventricular dopamine therapy increased LH levels, norepinepherine or serotonin was without effect (Schneider and McCann, 1970b; Kamberi *et al.*, 1970b). The observation that dopamine was ineffective in promoting pituitary LH release when perfused directly into the pituitary through portal vessels or when injected into the basilar artery (Kamberi *et al.*, 1970b) also indicates that the monoamine induces its effect through mediation of hypothalamic LRH secretion. Schneider and McCann (1970b) reported that dopamine is more effective to induce serum LH elevation on the second day of diestrus and on proestrus than during estrus or day one of diestrus. They also indicated that the catecholamine could elevate serum LH in ovariectomized rats which had been primed with estrogen and progesterone pretreatment.

Studies have also been conducted to assess possible involvement of other CNS neuro-transmitters on the control of gonadotropin release. Intraventricular administration of the indolamines serotonin and melatonin are known to decrease serum LH levels in ovariectomized female rats (Schneider and McCann, 1970b) and in intact male rats (Kamberi *et al.*, 1970b). These findings indicate that, in addition to catecholamine mechanisms promoting LRH release, there is an opposing serotonergic pathway in the hypothalamus that acts in some manner to inhibit LRH secretion. Evidence has recently been published that implicates the presence of CNS cholinergic pathways which facilitate gonadotropin secretion. Both systemic and intraventricular treatment with atropine to block cholinergic receptors were able to block the preovulatory proestrous gonadotropin surge and to suppress serum LH levels in ovariectomized female rats (Libertun and McCann, 1973). Since exogenous LRH still increased serum LH in rats which had been treated with atropine, the major site of the drug's action was assumed to be not at the hypophysial level, but rather at higher CNS centers.

Feedback characteristics of estrogen on neuroendocrine control of LRH secretion have likewise been examined. Schneider and McCann (1970c) showed that co-incubation of estradiol with pituitaries directly increased basal LH release, while the response to the addition of stalk median eminence fragments or purified LRF was not affected. They also demonstrated that inclusion of estrogen with incubates containing pituitaries, stalk median eminence fragments and dopamine inhibited the catecholamine's ability to enhance LH release through LRH mediation.

It was assumed that estrogen's suppressive effect on dopamine induced LRH release was brought about through synthesis of an inhibitory peptide since inhibition of protein synthesis with puromycin or cycloheximide abolished the effect. By using steroid blocked ovariectomized rats as assay animals for LRH activity, Schneider and McCann (1970a) extended the study of estrogen effects on dopaminergic control of LRH secretion. They reported that intraventricular dopamine injections increased LRF activity in serum of hypophysectomized rats. However pretreatment with intraventricular estrogen at 2 hours before dopamine administration prevented the monoamine's ability to increase serum LRF. These results indicate that although estrogen acts directly at the adenohypophysis to enhance LH release, it interferes with hypothalamic dopaminergic mechanisms which promote LRH secretion. The recognized ability of estrogen to exert negative feedback on gonadotropin control may therefore be implemented by inhibiting dopaminergic LRH release. However estrogen's action to facilitate pulsatile gonadotropin surging appears to involve different neural mechanisms.

Fluorescent histochemical examination of catecholamine content within infundibular dopaminergic neurons has been conducted in order to relate hypothalamic dopamine turnover rates with conditions known to alter endogenous LH secretion. After treating rats with α-methyltyrosine-methylester to inhibit catecholamine synthesis, a qualitative assessment of monoamine turnover is possible by measuring the relative degree of fluorescence attenuation through time. Fuxe *et al.* (1969 and 1972) reported that the low dopamine turnover rates characteristic of

ovariectomized or immature female rats is elevated after treatment with low doses of exogenous estrogen or pregnant mare gonadotropin. Similarly, in intact female rats, it was shown that dopamine turnover was lowest during proestrus and early estrus, times of depleted pituitary LH content and presumed elevation of serum LH (Ahrén *et al.*, 1971). Finally, intravenous LH therapy did not alter the low dopamine turnover of hypophysectomized rats. These results provide evidence that dopaminergic neural systems within the tubero-infundibular area of the hypothalamus may act to suppress LRH secretion. The conclusions from histochemical experiments concerning neuroendocrine involvement in control of LRH and gonadotropin secretion are in complete opposition to those concepts of gonadotropin control generated from direct alteration of hypothalamic neuro-transmitter availability. Future studies involving simultaneous use of both of these experimental techniques will be required to fully reconcile these currently contradictory hypotheses.

Effects of LRH on Pituitary LH Secretion

In 1966, Antunes-Rodrigues and co-workers attempted to gauge pituitary responsiveness to purified LH-releasing factor throughout the estrous cycle of normally cycling rats. Using bioassay techniques for serum LH determination, they found that the releasing factor elevated serum LH of rats in every stage of the estrous cycle. Although there were no significant changes in pituitary responsiveness during the estrous cycle, their results were suggestive of possible enhancement on the morning of proestrus. More precise assessment of pituitary responsiveness for LH release awaited the report of Matsuo *et al.* (1971)

describing successful synthesis of the decapeptide (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (LRH), which was shown to have potency comparable to that of the natural porcine LH-releasing factor (Arimuri *et al.*, 1972). Synthetic LRH was demonstrated to have a circulating half life of about seven minutes, and was shown to be selectively taken up by the pineal and the pituitary as well as by kidney and liver (Redding and Schally, 1972). Intravenous injection of LRH was reported to be effective in inducing ovulation only on the day of proestrus in both normally cycling and in pentobarbital blocked proestrous rats (Rippel *et al.*, 1973). Similarly, injection of synthetic LRH into cycling female rats preferentially enhanced pituitary sensitivity to the releasing hormone on the afternoon of proestrus (Cooper *et al.*, 1972; Martin *et al.*, 1974). The infusion of small doses of synthetic LRH directly into the anterior pituitary by way of a hypophysial portal vessel was also shown to effectively elevate serum LH levels (Ondo *et al.*, 1973). In a pair of papers, Debeljuk *et al.* (1972a and 1972b) demonstrated that such LRH administration was more effective in inducing pituitary LH secretion in immature rats of both sexes than in corresponding adult animals. These data suggest that pituitary responsiveness differs in animals of different physiological conditions.

Direct effects of ovarian steroids on pituitary sensitivity to exogenous LRH have recently been measured. In 1971 Arimura and Schally reported that estradiol benzoate given on day one of diestrus, 24 hours before LRH treatment, caused increased pituitary sensitivity to LRH

without altering basal LH levels. Similarly, when estradiol benzoate was given on the morning of estrus, pituitary response to LRH therapy was increased 48 hours later (Debeljuk *et al.*, 1972c). Using a different experimental approach, Clemens *et al.* (1972) provided indirect evidence that estradiol may increase pituitary responsiveness to LRH with the demonstration that estradiol benzoate augmented pituitary LH release in response to electrochemical stimulation in steroid blocked ovariectomized rats. On the other hand, Debeljuk *et al.* (1972c) demonstrated that treatment with progesterone or estradiol benzoate and progesterone on the day of estrus resulted in suppression of pituitary responsiveness to LRH treatment 48 hours later. Likewise it has been shown that when progesterone is given to cycling rats on the day of proestrus, basal LH concentrations are not different from those of rats receiving control oil treatment, while pituitary reactivity to LRH is suppressed when measured 48 hours after steroid therapy (Arimura and Schally, 1970). Using *in vitro* techniques, Schally *et al.* (1973) also indicated that steroids can alter adenohypophysial responsiveness to LRH. Their work suggests that low levels of estradiol enhanced basal LH release without affecting the response to LRH. On the other hand, higher estradiol doses acted to suppress both resting LH release and the response to LRH co-incubation. They also reported that progesterone decreased the response to LRH without changing basal LH release rates. In evaluating the effects of steroids at the hypophysial level these studies generally indicate that in *in vivo* conditions estrogen enhances pituitary responsiveness to LRH stimulation while progesterone has a

suppressive effect. However when examined *in vitro*, high levels of both steroids act to lessen the ability of LRH to induce pituitary LH secretion. In female rats the ability of ovarian steroids to enhance pituitary responsiveness to LRH is altered after ovariectomy, where in addition to estradiol benzoate's effect to suppress the already elevated basal serum LH levels, three days of treatment with the hormone results in decreasing pituitary capability to respond to LRH when compared to intact control animals (Negro-Vilar *et al.*, 1973). Libertun *et al.* (1974) presented both *in vivo* and *in vitro* evidence suggesting that ovariectomy decreased pituitary responsiveness to LRH, and that this suppression could be overcome following 3 days of pretreatment with subcutaneously administered estradiol benzoate. Rats also show a sex related difference in steroid feedback characteristics at the pituitary level. Debeljuk *et al.* (1972d) reported that 48 hour pretreatment with estradiol benzoate or testosterone depressed pituitary response to LRH in intact male rats. However after castration estradiol benzoate acted to facilitate the ability of LRH to elevate serum LH levels (Debeljuk *et al.*, 1973).

METHODS

Experimental Animals

Long-Evans rats were maintained in a temperature controlled colony having a twelve hour light/dark schedule, with lights on from 6 a.m. to 6 p.m. They received Wayne Lab-Blox diet for rats and mice, and water *ad libitum*. All experimental animals were of similar genetic constitution, having been bred and maintained within the Endocrine Research Unit's rat colony. Daily smears of vaginal cytology were taken to determine patterns of each rat's reproductive status within the estrous cycle. Endocrine studies were performed using 4 to 6 month old proestrous, estrous and second day diestrous, as well as 23 to 30 month old constant estrous and constant diestrous female rats. Old rats were considered to be in constant estrus or constant diestrus if they showed at least 10 consecutive days of cornified or leucocytic vaginal smears, respectively. Following experimental manipulation of any rat, a recovery period of at least 3 weeks was allowed to assure adequate hematocrit and blood volume recovery before the same animal was considered suitable for further experimentation.

Hormone and Drug Treatments

Rats in each of the previously defined reproductive states were treated with various doses of L-dopa (Hoffman-La Roche Inc.; Nutley, N.J.) or synthetic LRH (Eli Lily Inc.; Indianapolis, Ind.). In other experiments administration of L-dopa or LRH was coupled with subcutaneous pretreatment with estradiol benzoate (Upjohn Co.; Kalamazoo, Mich.) or progesterone (Mann Research Laboratories; N.Y., N.Y.). For experiments involving solely L-dopa therapy, intraperitoneal (i.p.) injection of 0.5 ml of saline or an equivalent volume saline suspension containing 3 or 30 mg L-dopa was made between 1:30 and 2:00 p.m. Intravenous (i.v.) treatment with LRH alone using 0.5 ml saline solutions of 0, 5, 50, and 500 ng LRH was performed during light ether anesthesia between 10:30 an 11:00 a.m. Similar experimental regimes were then performed coupling subcutaneous pretreatment of 5 mg progesterone dissolved in 0.2 ml corn oil 2 hours before i.p. administration of L-dopa at 30 mg per 0.5 ml, i.v. LRH at 50 ng per 0.5 ml, or suitable saline control. In addition, 24 hour pretreatment with subcutaneous estradiol benzoate at a dose of 20 μg in 0.2 ml corn oil was followed by L-dopa or LRH administration to young diestrous and old constant diestrous groups in the manner described for the progesterone treatment regime. The duration of estradiol benzoate pretreatment was 2 hours for rats in all other reproductive states of concern. All experiments using steroid priming were conducted in a manner to allow initiation of L-dopa and LRH therapy between 10:30 and 11:00 a.m.

Blood Collection

Immediately after initial cage disturbance, pretreatment blood samples of about 1.5 ml were obtained during light ether anesthesia by suborbital sinus puncture using heparinized capillary tubes. This bleeding technique was previously shown to cause minimal stress effects on serum hormone concentrations (Riegle, unpublished). In all experimental designs, acute treatment with various doses of L-dopa or LRH was made about 10 minutes after the pretreatment bleeding. All animals receiving L-dopa therapy were serially bled at 15, 60 and 120 minutes after drug administration. Similarly, rats were serially bled at 15, 30 and 60 minutes after LRH treatment.

The resulting whole blood samples were then allowed to clot at room temperature for between 30 and 120 minutes. The samples were refrigerated overnight and then centrifuged to expedite serum collection. Serum samples were then frozen at -15°C for subsequent analysis of radioimmuno-assayable LH and prolactin.

Radioimmuno-Assay for LH and Prolactin

The respective radioimmuno-assay procedures for LH and prolactin determination are those described and validated by Monroe *et al.* in 1968 and Niswender *et al.* in 1969, and routinely used in the laboratory of Dr. J. Meites at Michigan State University. Purified rat prolactin (H-10-10-B Prolactin) and LH (LER 1056 LH) were radio-iodinated with ^{125}I at Dr. Meites' facilities, and subsequently eluted through a 1 x 15 cm Bio-Gel P60 column. The labeled hormone elutant was then

diluted with 0.1% gelatin phosphate buffered saline (PBS) solution to a concentration of about 30,000 counts per minute (CPM) per 100 µl as counted in an automatic gamma well counter (Nuclear-Chicago, model 1085 L; Des Plaines, Ill.). Anti-rat LH antiserum had been prepared by immunization of rabbits with the purified hormone, and was then diluted to a working concentration of 1:28,000. The anti-rat prolactin antiserum, which had been produced by immunization of rabbits with purified prolactin, was used at a working dilution of 1:5,000. Precipitation of the antigen-antibody complexes of either LH or prolactin was performed using a sheep antiserum resulting from specific immunization against rabbit gamma globulin. The ovine anti-rabbit gamma globulin serum was routinely used at a 1:60 dilution.

The radioimmuno-assay procedure is virtually identical for determination of LH and prolactin except for the use of different purified and labeled hormone species, and the administration of specific rabbit antisera for combination with each hormone. Duplicate aliquots of unknown serum samples were generally run at two dilution volumes for hormone determination in both prolactin and LH assays. In all cases, the selected volume of serum from samples to be tested was placed in 12 x 75 mm diSPo culture tubes (Scientific Products; McGaw Park, Ill.). All samples were diluted to a volume of 0.5 ml with 0.1% gelatin PBS. A volume of 0.2 ml of the working rabbit antiserum specific for rat LH or prolactin was then added to all culture tubes. Tubes were briefly vortexed and placed in a refrigerator at 4°C for 24 hours to allow equilibration of the complexing reaction between available hormone

antigen and the exogenously administered antibody. At the end of the incubation time 100 µl of radio-iodinated hormone with a total activity of about 30,000 CPM was pipeted into each tube, briefly vortexed, and re-incubated at 4°C for an additional 24 hour period. During this time the radio-labeled hormone competes with the unknown amount of native hormone within the original serum sample for available binding antibody in an equilibrium manner. Following the incubation 200 µl of the precipitating ovine anti-rat gamma globulin antibody was added to each tube, followed by short duration vortexing. A 72 hour incubation at 4°C was then carried out to allow near maximal antigen-antibody complexing and precipitation. At the end of this incubation period all tubes received an additional 3 ml of cold PBS, and were then centrifuged at 2200 revolutions per minute for 30 minutes in a refrigerated centrifuge (National Equipment Co., model K). After discarding the supernatant and drying the tube walls with tissue paper, the tubes were placed into plastic holding jackets and counted in the automatic gamma well counter.

In addition to tubes with unknown amounts of serum hormone to be assayed, sets of culture tubes containing known amounts of purified hormone were included in the assay procedures and counted to serve as reference standards. Standards for the prolactin assays consisted of triplicate samples containing 16 different quantities of purified NIH rat prolactin RP-1 ranging from 0.4 to 40 ng. Similarly, LH reference standards consisted of culture tubes containing triplicate samples of 16 different doses of purified NIH LH RP-1 ranging from 0.8 to 40 ng.

Determinations of general binding characteristics of the assays were accomplished through inclusion of total count tubes, normal rabbit serum (NRS) tubes, and total antibody binding tubes into the assay procedure. Total count tubes received only the radio-iodinated hormone, and were a reflection of total efficiency of count recovery. The NRS tubes contained 200 µl of diluted rabbit serum in 0.1% gelatin PBS rather than the hormone specific antibody, thus corresponding to non-specific binding activity. Total antibody binding tubes were equivalent to "zero hormone" standards. The counting time for all tubes in a given assay was calculated to equal 10,000 counts in the effective "zero hormone" standards (counts in total antibody binding tubes less those occurring in the NRS tubes). The non-specific activity represented by NRS tube counts was subtracted from all sample tubes counted by proper adjustment of the gamma counter's background setting.

Standard curves were drawn on 3 cycle semi-logarithmic paper correlating CPM with the log of reference standard hormone doses. The standard curves thus generated exhibited 50% cold hormone binding at 3.3 ± 0.1 ng for prolactin determinations and 15.6 ± 0.8 ng in LH assays. Tabular representations were then derived from the standard curve to facilitate translation of CPM data into corresponding hormone content for all unknown serum samples tested. Quantitative serum hormone data were then transformed to ng/ml concentration units.

Statistical Analysis

Daily vaginal smear data were recorded for all surviving rats in each of the experimental protocols used. Effects of the various treatments on the resultant vaginal cytology patterns were assessed after determination of the percentage of rats in each group whose patterns remained unchanged. Tests for differences of post-treatment cycling characteristics among animal groups were made using the non-parametric Kruskal-Wallis test for differences of location in ranked data (Sokal and Rolf, 1969), critical to a 5% significance level.

Analysis of serum hormone data for dose response effects of acute treatment of L-dopa or LRH, and for steroid pretreatment effects was performed using analysis of variance. Use was made of a multivariate analysis of variance program which incorporated a transformation matrix to accommodate a blocking effect of hormone measurements through time on individual rats. The statistical program had been modified for use on Michigan State University's CDC 6500 computer by the Office of Research Consultation, School for Advanced Studies, College of Education, Michigan State University. A critical alpha probability value of 0.05 was selected for these analyses. In those studies where significant response variability for specific experimental factors was demonstrated, Duncan's Multiple Range Test was used to test for differences among means.

RESULTS

Vaginal Cytology Data

The data in the first table summarize the various treatment effects on patterns of vaginal cytology. When no steroid pretreatment was given, neither acute L-dopa nor LRH therapy affected estrous cyclicity in 87 ± 2% of the young rats independent of their reproductive status at the time of experimentation. However 500 ng of LRH given either on the morning of proestrus or day two diestrus usually delayed onset of the succeeding vaginal estrus by 2 or 3 days. Those young rats experiencing altered vaginal cycling patterns following any of the experimental regimes typically became pseudopregnant. Likewise, cyclicity was generally not affected either by reproductive status or acute drug therapy in young rats which had been primed with 5 mg progesterone two hours before an experiment. The only difference was that progesterone pretreatment to young rats on the second day of diestrus usually either delayed the next estrus by 1 to 4 days or caused the next day's proestrous smears to be followed by cytologic profiles typical of diestrus rather than the expected cornified patterns characteristic of estrus. On the other hand, pretreatment of young rats with 20 μg estradiol benzoate invariably caused appearance of 2 to 3 days of cornification, followed by onset of pseudopregnancy which lasted from 9 to 15 days regardless of acute L-dopa or LRH administration.

Acute treatment with either L-dopa or LRH did not alter vaginal cytology patterns of old female rats when compared to the corresponding control groups (Table 1). While estradiol benzoate priming consistently precipitated development of pseudopregnancy in all young rats, the treatment was incapable of causing similar responses in aged rats. Pretreatment with estradiol benzoate was also unable to significantly increase the frequency of cyclicity induction in old rats. Although estradiol benzoate therapy appeared to partially restore estrous cycling in the aged constant diestrous rats, this change was not significant. Progesterone pretreatment likewise had no effect on vaginal cytology patterns in senescent rats when compared to animals which received no pretreatment. However simple experimental manipulation significantly restored vaginal cyclicity of old rats regardless of steroid pretreatment or acute drug therapy. Further, nonspecific stressors were more effective to cause resumption of estrous cycles in constant estrous than in constant diestrous rats, where 76 ± 6% of old constant estrous rats showed some recovery of cyclicity compared to 43 ± 7% of old rats originally in the constant diestrous state. However, although old cycling rats typically failed to show characteristic proestrous smears, normal cycle lengths of between 4 and 6 days were observed in 59 of 97 constant estrous rats and 31 of 40 rats that were normally in constant diestrus.

Table 1. Effects of acute L-dopa or LRH therapy, and of pretreatment with 20 μg estradiol benzoate or 5 mg progesterone on patterns of vaginal cytology

Hormone and drug therapy	Patterns of vaginal cytology in various reproductive states						
	Unchanging patterns					Onset of cyclicity	
	Proestrus	Estrus	Diestrus	Constant estrus	Constant diestrus	Constant estrus	Constant diestrus
No Pretreatment:							
Control	29[b]/33[a] (.88)[c]	34/36 (.94)	39/39 (.92)	1/27 (.04)	18/24 (.75)	25[d]/27 (.93)[e]	5/24 (.21)
3 mg L-dopa	13/14 (.93)	14/16 (.82)	14/18 (.78)	0/10 (.00)	6/9 (.67)	8/10 (.80)	3/9 (.33)
30 mg L-dopa	9/10 (.90)	11/13 (.85)	10/10 (1.00)	1/10 (.10)	5/9 (.56)	8/10 (.80)	4/9 (.44)
5 ng LRH	6/9 (.67)	8/10 (.80)	9/10 (.90)	0/8 (.00)	4/8 (.50)	5/9 (.56)	4/8 (.50)
50 ng LRH	10/11 (.91)	8/10 (.80)	7/10 (.70)	1/9 (.11)	7/11 (.64)	3/7 (.43)	4/11 (.36)
500 ng LRH	6/7 (.86)	8/8 (1.00)	9/9 (1.00)	0/7 (.00)	8/10 (.80)	5/10 (.50)	2/10 (.20)
Estradiol Benzoate Pretreatment:							
Control	0/11 (.00)	0/8 (.00)	0/9 (.00)	2/8 (.25)	2/9 (.22)	5/8 (.62)	7/9 (.78)
30 mg L-dopa	0/10 (.00)	0/9 (.00)	0/10 (.00)	4/9 (.44)	5/10 (.50)	5/9 (.56)	4/10 (.40)
50 ng LRH	0/9 (.00)	0/10 (.00)	0/10 (.00)	1/8 (.12)	2/9 (.22)	7/8 (.88)	7/9 (.78)
Progesterone Pretreatment:							
Control	9/10 (.90)	7/10 (.70)	7/10 (.70)	0/10 (.00)	6/10 (.60)	8/10 (.80)	4/10 (.40)
30 mg L-dopa	7/8 (.88)	8/8 (1.00)	9/10 (.90)	0/10 (.00)	5/7 (.71)	9/10 (.90)	2/7 (.29)
50 ng LRH	7/8 (.88)	5/9 (.55)	5/10 (.50)	0/10 (.00)	2/9 (.22)	9/10 (.90)	2/9 (.22)

[a]Total number of rats in the given reproductive state which received designated treatment.

[b]Number of rats which showed no change in vaginal patterns after experimental treatment (subset of "a").

[c]Proportion of rats exhibiting unchanged vaginal patterns.

[d]Number of old rats showing resumed vaginal cyclicity following experimental treatment.

[e]Proportion of old rats showing restored vaginal cycling.

Effects of L-Dopa on Serum Prolactin

The data in Table 2 and Figures 3 and 4 indicate that basal serum prolactin concentrations were different among the reproductive states studied. Prolactin levels were highest during proestrus and estrus, intermediate in both groups of aged rats, and lowest during the second day of diestrus. Injection of the saline vehicle did not affect serum prolactin in any of the groups other than that of young rats on the day of estrus (Table 2). Saline treated control estrous rats showed prolactin reduction 15 minutes after injection which was maintained for at least 2 hours. However statistical analysis indicated that there was no significant change in serum prolactin levels through time when evaluated across all reproductive states.

As is shown in Table 2, intraperitoneal administration of both 3 and 30 mg L-dopa generally caused marked suppression of circulating prolactin levels by 15 minutes in rats of all reproductive states (Table 2; Figures 3 and 4). Although the high dose of L-dopa invariably caused maximal lowering of serum prolactin for at least 2 hours, the 3 mg drug dose was less effective, as indicated by significant interaction between drug dose effect and time after administration (Figure 1). Analysis of the data indicates that, across all reproductive conditions, the low L-dopa dose was only effective in reducing prolactin at 15 minutes after injection. Further, not all reproductive states were equally susceptible to L-dopa induced inhibition of prolactin secretion as indexed by serum hormone levels. Figure 2, illustrating the interaction between drug dose and reproductive status, shows that 3 mg L-dopa was

more effective in decreasing prolactin in young proestrous and estrous rats than in those on the second day of diestrus or in either group of aged rats when assessed across all of the post-injection blood sampling times. The apparent diminutive effect of the low L-dopa dose on the diestrous group was likely due to the presence of already low basal prolactin levels in this status. Figure 3 shows the existence of a three way interaction among drug dose, reproductive status and time after L-dopa injection in young rats. The data depicted in this figure suggest that the 30 mg L-dopa dose was optimally effective in reducing serum prolactin for the entire 2 hour period after injection in all groups tested. Similarly, the low L-dopa dose caused depression of serum prolactin to comparable levels in all young rats throughout the ensuing 2 hours. Conversely, the ability of L-dopa to lower serum prolactin was more transient in both groups of aged rats, as is shown in Figure 4. Serum prolactin levels were restored to approximately those of saline injected control values by 60 and 120 minutes after 3 mg L-dopa administration. By 120 minutes after injection, mean serum hormone concentrations tended to rebound above corresponding saline controls, though this didfference was not significant.

Data in Tables 3 and 4 depict the effects of subcutaneous pretreatment with 20 μg estradiol benzoate and 5 mg progesterone, respectively. As is shown in these tables and in Figure 5, pretreatment with either of these ovarian steroids was equally able to depress resting prolactin levels in rats on the days of proestrus and estrus, but did not change those hormone concentrations characteristic of the other

reproductive conditions which were studied. Neither of the steroid pretreatment regimes generally altered the ability of L-dopa to inhibit prolactin secretion in any of the reproductive conditions tested. However the 30 mg L-dopa dose used in this study was found to be less effective across time in young estrous rats than in any of the other reproductive states studied. This decreased responsiveness was mainly due to response refractoriness of estrous animals which had been primed with estradiol benzoate (Table 3). The presence of a significant interaction between drug treatment and time after injection (Figure 6) also indicates that the suppressive effect of 30 mg L-dopa had generally begun to wane by 120 minutes after treatment in the steroid primed rats, a response which was not apparent in rats that received no steroid priming (Table 2; Figure 1).

Table 2. Effects of L-dopa treatment on serum prolactin levels of young and senescent female rats

Reproductive state	n	Serum prolactin (ng/ml) Time (minutes) -15	15	60	120
Proestrus:					
Control	10	304.0 ± 69.2[a]	308.2 ± 50.7	239.3 ± 37.3	283.2 ± 39.0
3 mg L-dopa	10		31.2 ± 13.2	81.8 ± 16.9	104.0 ± 18.2
30 mg L-dopa	10		55.4 ± 13.2	29.0 ± 12.6	40.4 ± 18.6
Estrus:					
Control	10	398.9 ± 63.2	180.1 ± 35.6	68.7 ± 22.3	126.6 ± 46.7
3 mg L-dopa	10		58.6 ± 11.8	59.1 ± 11.1	87.1 ± 16.7
30 mg L-dopa	10		22.9 ± 3.1	14.3 ± 6.1	12.0 ± 3.6
Diestrus:					
Control	10	62.4 ± 14.7	109.8 ± 38.3	79.7 ± 23.3	75.2 ± 19.6
3 mg L-dopa	10		42.0 ± 16.6	54.8 ± 17.1	59.8 ± 11.8
30 mg L-dopa	10		12.8 ± 5.2	9.7 ± 0.5	11.3 ± 1.6
Old Constant Estrus:					
Control	22	163.2 ± 18.2	211.9 ± 29.4	209.7 ± 32.0	189.4 ± 26.5
3 mg L-dopa	21		51.9 ± 11.9	260.6 ± 58.9	274.7 ± 52.8
30 mg L-dopa	10		21.7 ± 3.5	19.7 ± 3.5	21.1 ± 5.7
Old Constant Diestrus:					
Control	11	153.0 ± 28.5	179.0 ± 40.6	176.2 ± 39.5	199.0 ± 45.4
3 mg L-dopa	10		89.5 ± 42.9	134.0 ± 43.3	216.0 ± 66.7
30 mg L-dopa	10		35.7 ± 17.4	24.3 ± 9.8	66.4 ± 36.1

Hormone concentrations are shown as mean ± S.E.M.

[a]Pretreatment control values are pooled for all treatments in a given reproductive state.

Table 3. Effects of L-dopa therapy on serum prolactin levels of young and senescent female rats receiving 20 µg estradiol benzoate pretreatment

Reproductive state	n	Serum prolactin (ng/ml) Time (minutes) -15	15	60	120
Proestrus:[b]					
Control	11	86.8 ± 18.8[a]	195.0 ± 36.9	180.0 ± 41.9	189.0 ± 31.5
30 mg L-dopa	10		12.3 ± 0.3	18.4 ± 2.3	60.2 ± 14.6
Estrus:[b]					
Control	9	187.0 ± 47.9	225.0 ± 30.6	234.0 ± 43.1	144.0 ± 41.1
30 mg L-dopa	9		106.0 ± 48.9	95.6 ± 37.8	106.0 ± 39.5
Diestrus:[c]					
Control	10	100.7 ± 28.9	128.0 ± 20.3	78.6 ± 19.6	124.0 ± 35.7
30 mg L-dopa	10		10.6 ± 1.3	11.5 ± 2.4	14.6 ± 3.8
Old Constant Estrus:[b]					
Control	10	76.6 ± 18.6	189.0 ± 41.0	174.0 ± 29.5	235.0 ± 33.0
30 mg L-dopa	9		12.6 ± 0.4	22.8 ± 7.5	52.9 ± 15.0
Old Constant Diestrus:[c]					
Control	9	129.0 ± 25.0	121.0 ± 27.4	131.0 ± 28.3	118.0 ± 25.8
30 mg L-dopa	10		36.4 ± 5.1	51.6 ± 23.8	51.4 ± 27.6

Hormone concentrations are shown as mean ± S.E.M.

[a]Pretreatment control values are pooled for all treatments in a given reproduction state.

[b]Two hour estradiol benzoate pretreatment.

[c]Twenty-four hour estradiol benzoate pretreatment.

Table 4. Effects of L-dopa therapy on serum prolactin levels of young and senescent female rats receiving two hour pretreatment with 5 mg progesterone

Reproductive state	n	Serum prolactin (ng/ml) Time (minutes) -15	15	60	120
Proestrus:					
Control	10	77.8 ± 23.8[a]	121.0 ± 37.0	154.0 ± 34.9	160.0 ± 52.0
30 mg L-dopa	10		17.9 ± 5.6	23.5 ± 3.0	58.0 ± 21.5
Estrus:					
Control	10	196.0 ± 78.1	179.0 ± 36.0	129.0 ± 32.2	203.0 ± 68.5
30 mg L-dopa	10		15.2 ± 2.2	45.2 ± 8.4	68.6 ± 14.3
Diestrus:					
Control	10	67.0 ± 12.2	119.0 ± 29.6	111.0 ± 31.9	69.4 ± 15.8
30 mg L-dopa	11		35.4 ± 11.0	54.4 ± 29.1	83.5 ± 34.5
Old Constant Estrus:					
Control	10	171.5 ± 35.8	212.0 ± 57.7	342.0 ± 96.7	282.0 ± 74.1
30 mg L-dopa	10		81.3 ± 30.9	83.1 ± 37.6	85.3 ± 25.7
Old Constant Diestrus:					
Control	10	103.8 ± 20.6	180.0 ± 51.4	166.0 ± 39.3	206.0 ± 36.6
30 mg L-dopa	10		22.1 ± 5.8	30.5 ± 9.0	25.3 ± 5.8

Hormone concentrations are shown as mean ± S.E.M.

[a]Pretreatment control values are pooled for all treatments in a given reproductive state.

Figure 1. Serum prolactin levels in female rats after administration of various doses of L-dopa, without regard to reproductive status.

The height of each bar represents mean serum prolactin concentration expressed as ng/ml, with brackets corresponding to the standard error of the mean. Each cluster of graphs indicates measured prolactin concentration at 15, 60 or 120 minutes after drug injection. Open bars depict control responses while striped and shaded bars correspond to 3 and 30 mg L-dopa treatment, respectively.

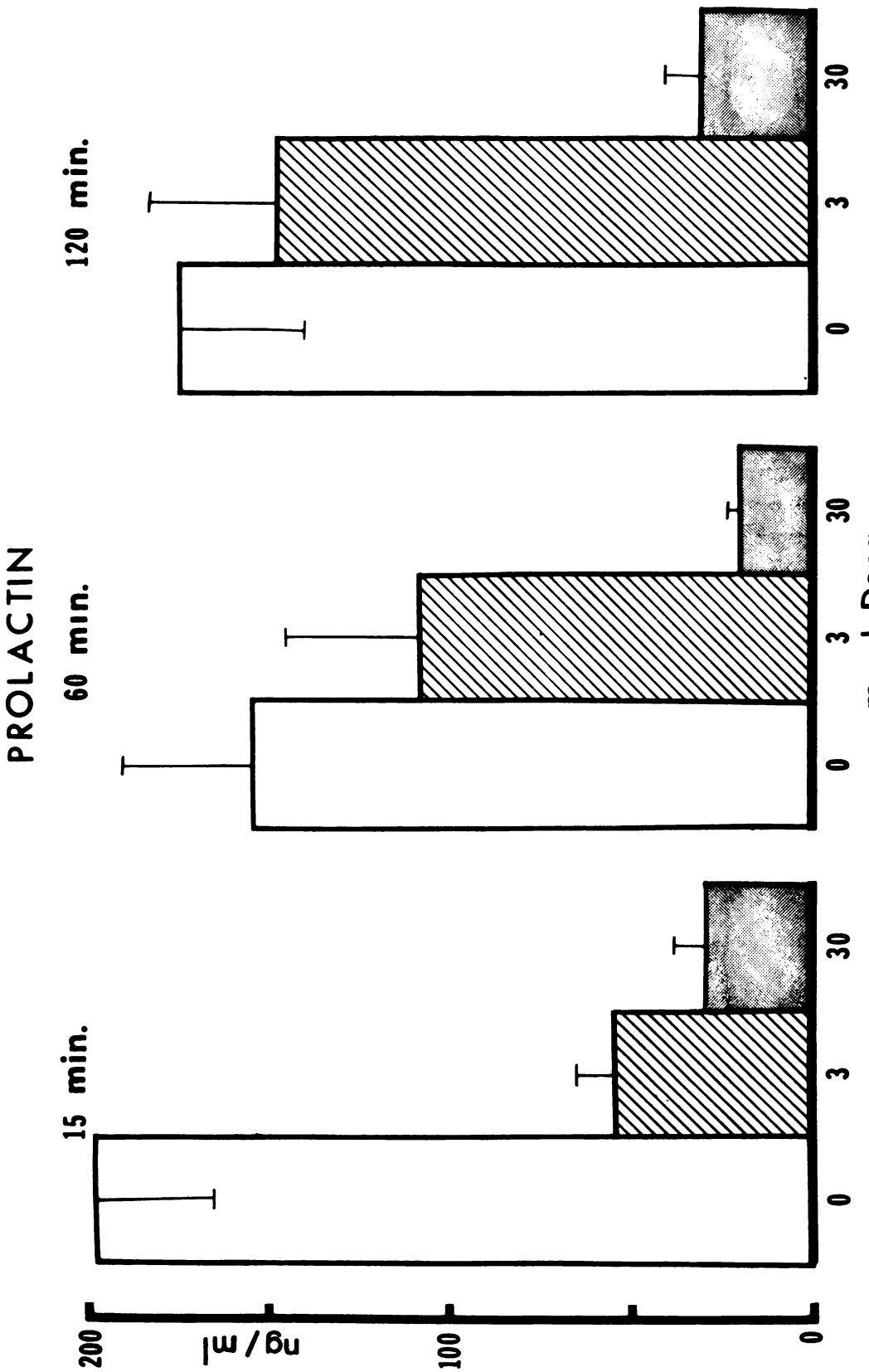

Figure 1

Figure 2. Serum prolactin concentrations in young and aged female rats after administration of various doses of L-dopa.

The height of each bar represents mean serum prolactin concentration expressed as ng/ml, with brackets corresponding to the standard error of the mean. Open bars signify responses to saline control while those which are striped or shaded correspond to prolactin levels measured after 3 or 30 mg L-dopa, respectively. The effects of the various L-dopa doses on serum prolactin levels are shown for young rats on the days of proestrus, estrus and the second day of diestrus, as well as in aged constant estrous and constant diestrous rats. Data illustrated in the figure represent pooled prolactin values from 15, 60 and 120 minutes after L-dopa therapy for the appropriate reproductive conditions.

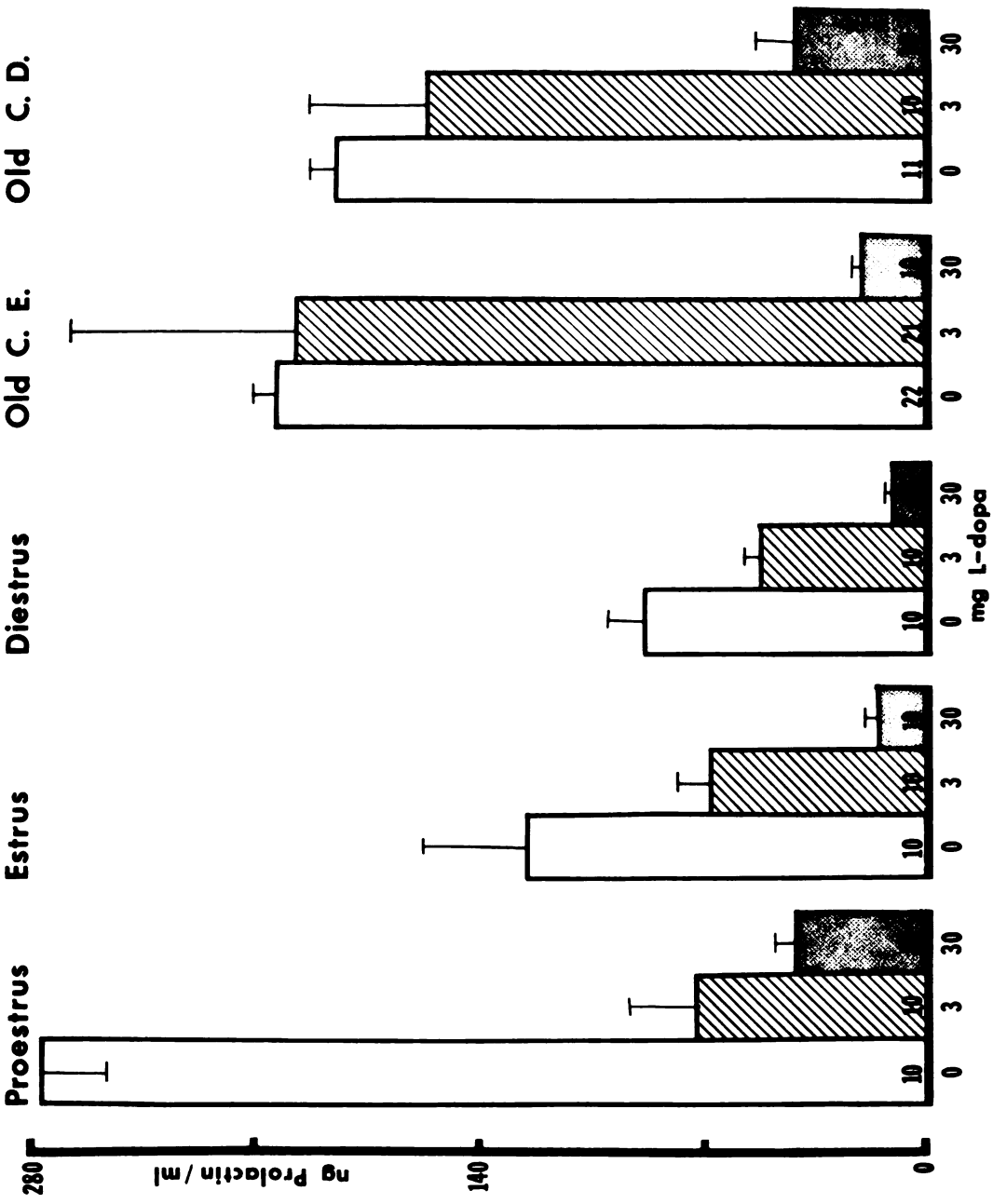
Proestrus
Estrus
Diestrus
Old C. E.
Old C. D.
ng Prolactin / ml
280
140
0
0
3
30
mg L-dopa
10
22
11

Figure 2

Figure 3. Effects of L-dopa therapy on serum prolactin concentrations in young cycling female rats.

These data illustrate the effects of L-dopa treatment on serum prolactin levels in young proestrous, estrous and second day diestrous rats. Mean serum prolactin levels expressed in ng/ml and their standard errors appear on the ordinate as a function of time relative to injection of various L-dopa doses. L-dopa administration is depicted by a caret on the abscissa, with blood samples taken 15 minutes before drug treatment, and at 15, 60 and 120 minutes afterwards. Solid circles represent control values while open circles and open squares signify 3 and 30 mg L-dopa treatment, respectively.

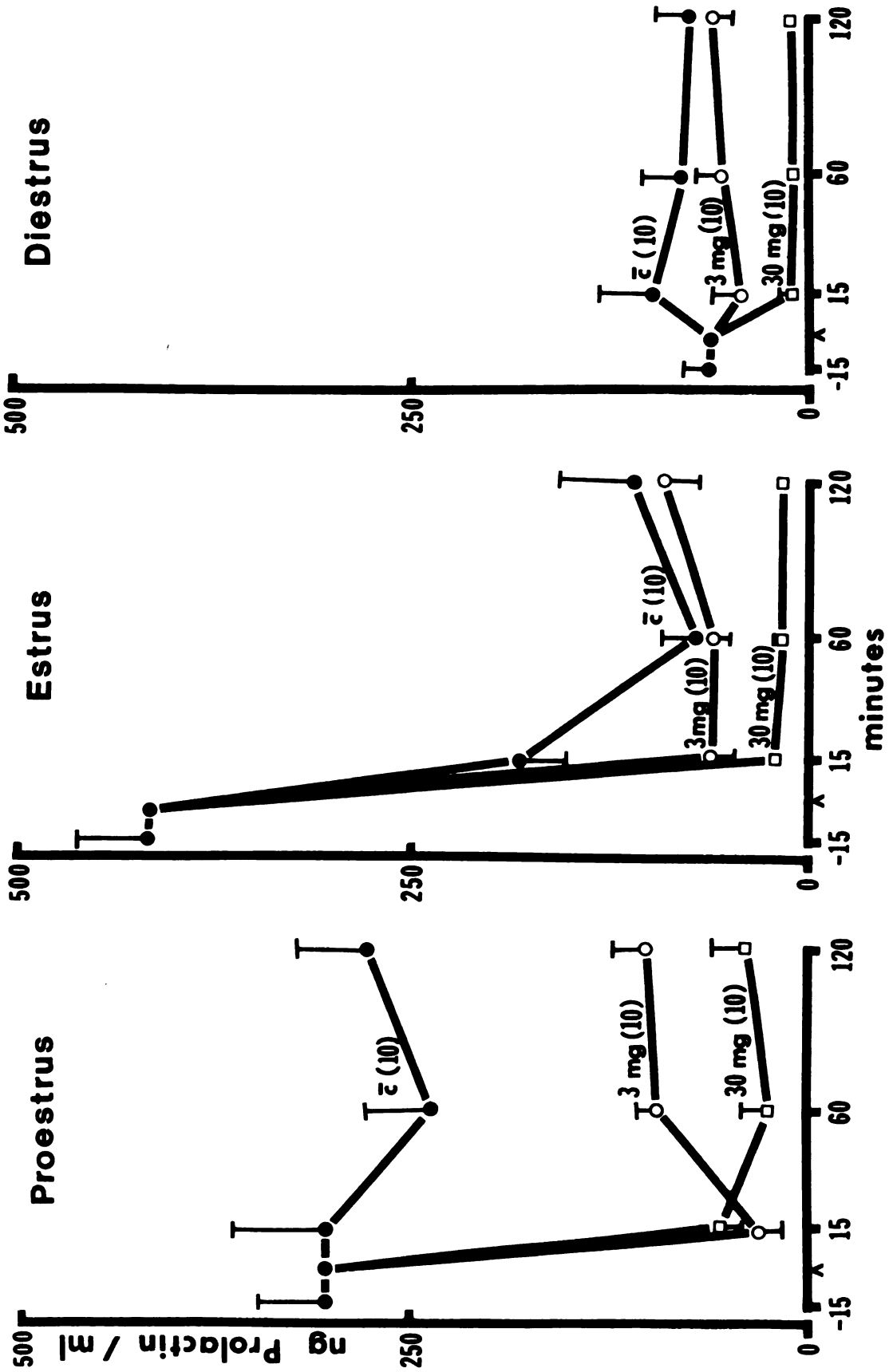
Proestrus
Estrus
Diestrus
ng Prolactin / ml
500
250
0
-15
15
60
120
minutes
x̄ (10)
3 mg (10)
30 mg (10)

Figure 3

Figure 4. Effects of L-dopa therapy on serum prolactin concentrations in aged female rats.

These data illustrate the effects of L-dopa treatment on serum prolactin levels in senescent constant estrous and constant diestrous rats. Mean serum prolactin levels expressed in ng/ml and their standard errors appear on the ordinate as a function of time relative to injection of various L-dopa doses. L-dopa administration is depicted by a caret on the abscissa, with blood samples taken 15 minutes before drug treatment, and at 15, 60 and 120 minutes afterwards. Solid circles represent control values while open circles and open squares signify 3 and 30 mg L-dopa treatment, respectively.

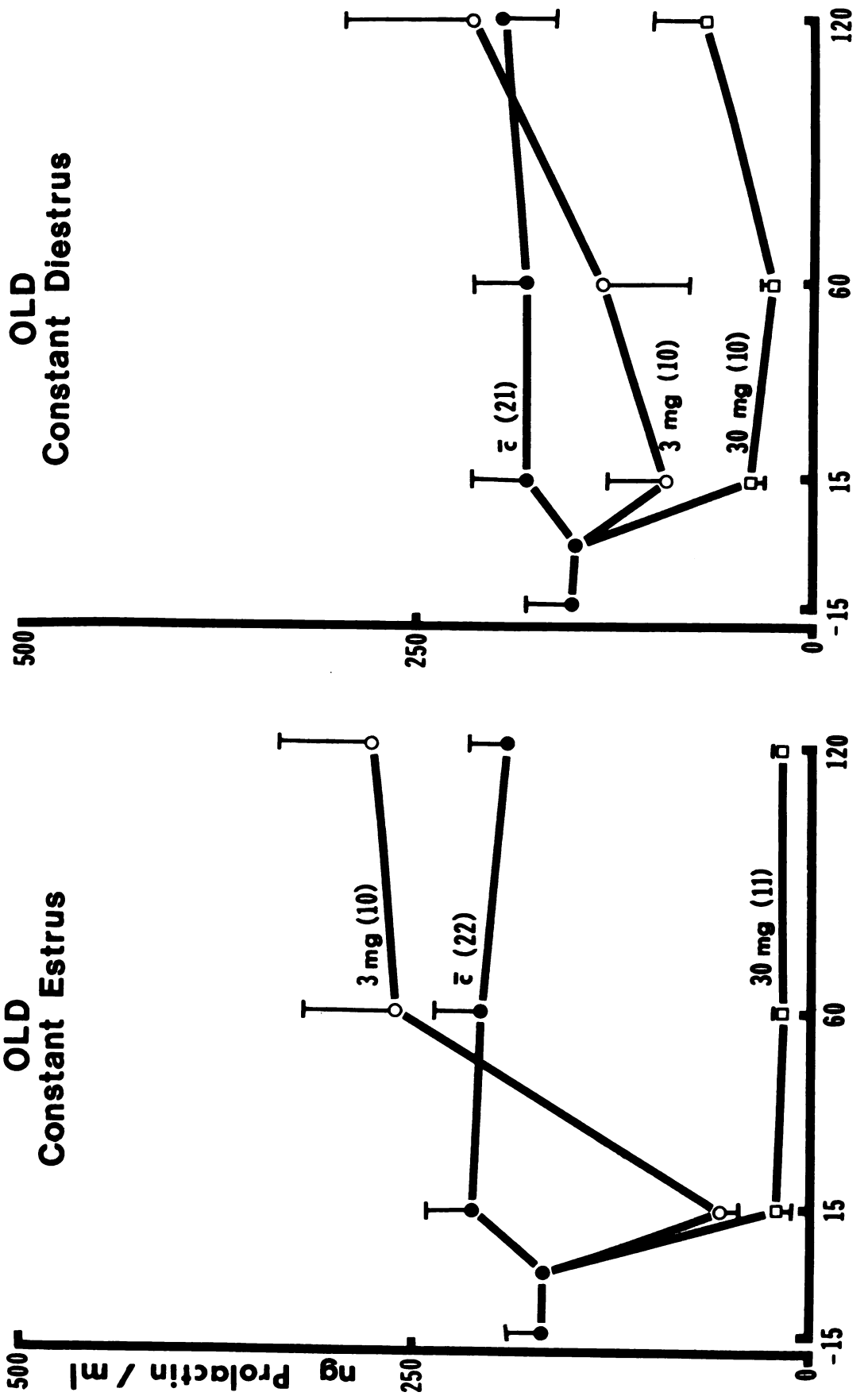
OLD
Constant Estrus
OLD
Constant Diestrus
ng Prolactin / ml
500
250
0
-15
15
60
120
3 mg (10)
ε (22)
30 mg (11)
ε (21)
3 mg (10)
30 mg (10)

Figure 4

Figure 5. Effect of steroid pretreatment on basal serum prolactin concentration in young and aged female rats.

The height of each bar represents mean serum prolactin concentration expressed as ng/ml, with brackets corresponding to the standard error of the mean. Each bar indicates prolactin levels in serum samples taken at about 15 minutes before acute L-dopa treatment. Open bars depict responses in rats receiving no steroid pretreatment while shaded and striped bars correspond to subcutaneous pretreatment with 20 μg estradiol benzoate and 5 mg progesterone, respectively. Two hour progesterone pretreatment was employed in all reproductive states. Similar two hour estrogen priming was performed in all groups except young rats on the second day of diestrus and aged constant diestrous animals, which were given 24 hours of estrogen pretreatment.

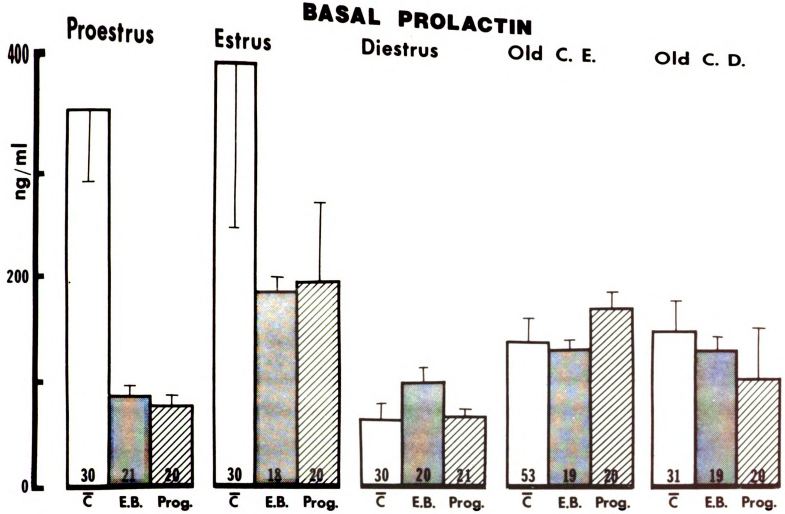
BASAL PROLACTIN
Proestrus
Estrus
Diestrus
Old C. E.
Old C. D.
400
200
0
ng/ml
30
21
20
30
18
20
30
20
21
53
19
20
31
19
20
C
E.B.
Prog.

Figure 5

Figure 6. Effect of steroid pretreatment on the ability of L-dopa therapy to suppress serum prolactin concentration in female rats, without regard to reproductive status.

The height of each bar represents mean serum prolactin concentration expressed as ng/ml, with brackets corresponding to the standard error of the mean. Each cluster of graphs indicates measured prolactin concentration at 15, 60 and 120 minutes after drug injection for untreated rats as well as those receiving pretreatment with 20 µg estradiol benzoate or 5 mg progesterone. Open bars signify prolactin levels in saline treated controls at various times after injection. Serum prolactin levels following 30 mg L-dopa treatment are designated by squared, shaded and striped bars in rats receiving no steroid, estradiol benzoate and progesterone priming, respectively. Two hour progesterone pretreatment was employed in all reproductive states. Similar two hour estrogen priming was performed in all groups except young rats on the second day of diestrus and aged constant diestrous animals, which were given 24 hours of estrogen pretreatment -

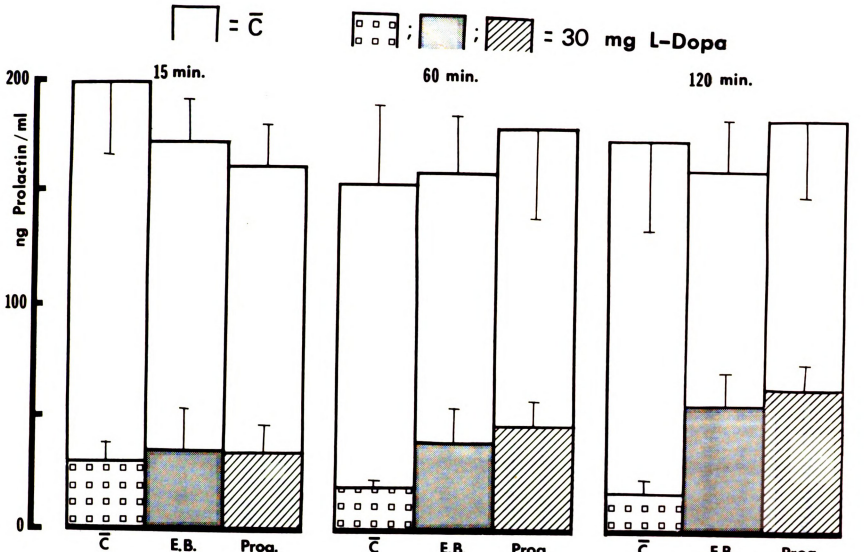
= C̄
; ; = 30 mg L-Dopa
15 min.
60 min.
120 min.
200
100
0
ng Prolactin / ml
C̄
E.B.
Prog.
C̄
E.B.
Prog.
C̄
E.B.
Prog.

Figure 6

Effects of L-Dopa on Serum LH

The series of experiments designed to assess the influence of systemic L-dopa treatment on serum LH levels involved drug administration at about 1:30 p.m. As is seen in Table 5 and Figure 7, basal LH levels at about 10 minutes before L-dopa therapy were markedly elevated and quite variable in proestrous rats. This finding indicates that the preovulatory gonadotropin surge had already begun to occur in several of the rats studied. Of the low levels representative of all other reproductive states, LH concentrations of old constant estrous rats were slightly greater than those of either estrous or diestrous, which in turn exceeded those found in aged constant diestrous animals.

The effect of drug treatment through time depended on dosage used. Figure 8 shows the interaction between L-dopa dose and time after injection as averaged for all reproductive conditions. Although the data depicted in this figure were highly variable, they suggest that the 30 mg L-dopa dose elevated serum LH at 15 and 60 minutes, but did not change LH from corresponding control levels at 120 minutes post-injection. On the other hand, the 3 mg dose appeared to cause moderate LH elevation which was maintained for the entire 120 minute sampling period. Analysis of variance showed that the effectiveness of L-dopa to elevate serum LH depended on the reproductive status of animals studied. Data in Table 5 and Figure 9 indicate that 3 mg L-dopa optimally stimulated pituitary LH release in young diestrous rats, significantly increasing serum LH for at least 120 minutes. However, this drug dose did not cause significant LH changes in any of the other

reproductive conditions studied. The effectiveness of 30 mg L-dopa also varied as functions of time after injection and of reproductive status. Figure 9 shows that the high dose of L-dopa nearly doubled the already greatly elevated serum LH concentrations in proestrous rats at 15 and 60 minutes post injection. Thirty mg of L-dopa also markedly augmented serum LH after 15 minutes in the young estrous group. On the other hand this drug dose caused only moderate LH elevation in young diestrous rats, though the effect was evident for the entire 120 minute sampling period. In contrast, data in Figure 10 indicate that old rats responded differently to 30 mg L-dopa than did younger ones. While L-dopa caused LH enhancement in constant estrous rats which was comparable to that of young rats on the day of estrus, the response of old animals was not significant until 60 and 120 minutes after treatment. Old constant diestrous rats were completely unresponsive to 30 mg L-dopa.

Figure 11 depicts basal serum LH levels as a function of pretreatment with estradiol benzoate or progesterone. Estradiol benzoate therapy suppressed resting serum LH levels below those characteristic of untreated controls when assessed across all reproductive conditions. Evaluation of responsiveness within each reproductive status showed that this depressive effect was significant in proestrous, diestrous and aged constant diestrous rats. Although progesterone priming tended to decrease basal serum LH in all groups, this difference was not significant. Pretreatment with estradiol benzoate blocked L-dopa's ability to elevate serum LH by 15 minutes after injection in

all reproductive conditions other than the young proestrous group (Table 6; Figure 12). Direct comparison of responses between untreated control rats on the day of proestrus and those receiving estradiol benzoate pretreatment was not made because the experiments involving steroid priming were initiated at 10 a.m. in order to avoid the demonstrated early afternoon preovulatory LH rise in proestrous rats. However, response similarity between control and estradiol benzoate pretreated proestrous rats is suggested since in both groups L-dopa caused significant elevation above corresponding saline injected controls at 15 and 60 minutes after administration. On the other hand, prior therapy with exogenous progesterone did not significantly affect the ability of 30 mg L-dopa to alter serum LH at 15 minutes after injection in any of the reproductive conditions studied (Table 7; Figure 12). However, comparison of data in Table 7 with that in Table 5 indicates that progesterone priming increased the duration of L-dopa's effect in young rats since pretreatment resulted in L-dopa induced LH elevation which lasted for the entire 2 hour period in these animals. Conversely, Table 7 shows that progesterone inhibited the effect of L-dopa to raise serum LH levels through time in unprimed constant estrous rats, whose responses are summarized in Table 5.

Table 5. Effects of L-dopa therapy on serum LH levels of young and senescent female rats

Reproductive state	n	Serum LH (ng/ml) Time (minutes) -15	15	60	120
Proestrus:					
Control	21	366.2 ± 100.6[a]	523.3 ± 180.3	296.3 ± 107.0	97.9 ± 39.4
3 mg L-dopa	11		598.6 ± 199.8	386.9 ± 141.8	291.8 ± 156.8
30 mg L-dopa	10		788.7 ± 264.8	778.8 ± 251.7	293.0 ± 96.6
Estrus:					
Control	21	17.3 ± 3.4	22.8 ± 9.0	24.8 ± 9.1	35.3 ± 12.5
3 mg L-dopa	11		48.8 ± 21.5	38.3 ± 14.5	36.1 ± 14.6
30 mg L-dopa	10		56.0 ± 17.0	17.2 ± 4.9	12.7 ± 1.9
Diestrus:					
Control	17	22.8 ± 4.7	12.9 ± 1.9	11.7 ± 0.8	10.1 ± 0.1
3 mg L-dopa	12		29.4 ± 10.1	33.8 ± 9.4	25.2 ± 8.6
30 mg L-dopa	10		22.6 ± 4.9	21.4 ± 6.4	15.4 ± 3.6
Old Constant Estrus:					
Control	22	35.0 ± 4.4	34.9 ± 7.0	33.1 ± 6.6	24.3 ± 5.4
3 mg L-dopa	10		32.8 ± 7.3	28.8 ± 6.8	26.4 ± 7.7
30 mg L-dopa	10		47.1 ± 11.4	66.0 ± 10.3	56.6 ± 12.8
Old Constant Diestrus:					
Control	10	9.6 ± 4.4	6.7 ± 0.2	7.7 ± 1.0	7.2 ± 0.5
30 mg L-dopa	10		6.7 ± 0.2	10.1 ± 3.4	14.7 ± 8.0

Hormone concentrations are shown as mean ± S.E.M.

[a]Pretreatment control values are pooled for all treatments in a given reproductive state.

Table 6. Effects of L-dopa therapy on serum LH levels of young and senescent female rats receiving 20 μg estradiol benzoate pretreatment

Reproductive state	n	Serum LH (ng/ml) Time (minutes) -15	15	60	120
Proestrus:[b]					
Control	11	18.2 ± 3.6[a]	14.4 ± 3.0	22.3 ± 3.4	25.1 ± 6.2
30 mg L-dopa	10		43.5 ± 12.7	47.4 ± 15.9	37.9 ± 5.6
Estrus:[b]					
Control	9	11.0 ± 3.0	11.4 ± 3.8	6.4 ± 2.7	14.2 ± 5.7
30 mg L-dopa	9		9.8 ± 2.6	4.8 ± 0.4	4.1 ± 0.6
Diestrus:[c]					
Control	10	3.2 ± 1.0	6.5 ± 2.2	5.0 ± 2.9	4.0 ± 0.8
30 mg L-dopa	10		7.2 ± 3.1	4.5 ± 1.4	12.8 ± 5.1
Old Constant Estrus:[b]					
Control	10	25.8 ± 9.2	30.5 ± 15.4	11.0 ± 3.9	16.3 ± 6.6
30 mg L-dopa	9		17.3 ± 6.4	23.6 ± 7.6	17.0 ± 6.9
Old Constant Diestrus:[c]					
Control	12	3.0 ± 0.9	8.8 ± 4.6	10.7 ± 6.0	19.6 ± 11.1
30 mg L-dopa	10		8.6 ± 3.0	11.1 ± 3.7	34.8 ± 11.6

Hormone concentrations are shown as mean ± S.E.M.

[a]Pretreatment control values are pooled for all treatments in a given reproductive state.

[b]Two hour estradiol benzoate pretreatment.

[c]Twenty-four hour estradiol benzoate pretreatment.

Table 7. Effects of L-dopa therapy on serum LH levels of young and senescent female rats receiving two hour pretreatment with 5 mg progesterone

		Serum LH (ng/ml)			
		Time (minutes)			
Reproductive state	n	-15	15	60	120
Proestrus:					
Control	10	40.1 ± 7.9[a]	45.0 ± 6.3	48.1 ± 5.3	50.9 ± 6.2
30 mg L-dopa	10		60.8 ± 9.4	81.8 ± 16.4	86.9 ± 14.8
Estrus:					
Control	10	11.1 ± 2.5	16.7 ± 3.9	13.2 ± 2.8	29.1 ± 12.6
30 mg L-dopa	10		43.2 ± 20.3	63.5 ± 14.0	70.0 ± 14.6
Diestrus:					
Control	10	11.6 ± 3.6	7.2 ± 2.1	7.3 ± 2.8	12.6 ± 5.6
30 mg L-dopa	11		18.4 ± 4.9	34.7 ± 12.9	31.8 ± 14.0
Old Constant Estrus:					
Control	10	17.8 ± 5.7	15.9 ± 4.8	18.2 ± 4.5	28.7 ± 8.5
30 mg L-dopa	10		27.6 ± 11.8	19.2 ± 6.8	27.6 ± 6.3
Old Constant Diestrus:					
Control	10	10.2 ± 4.1	17.2 ± 10.0	10.2 ± 3.8	6.8 ± 2.2
30 mg L-dopa	10		11.2 ± 3.7	11.2 ± 4.2	10.9 ± 4.3

Hormone concentrations are shown as mean ± S.E.M.

[a]Pretreatment control values are pooled for all treatments in a given reproductive state.

Figure 7. Resting serum LH levels in young and aged female rats.

The height of each bar represents mean serum LH concentration expressed as ng/ml, with brackets corresponding to the standard error of the mean. The number within each bar denotes that group's sample size.

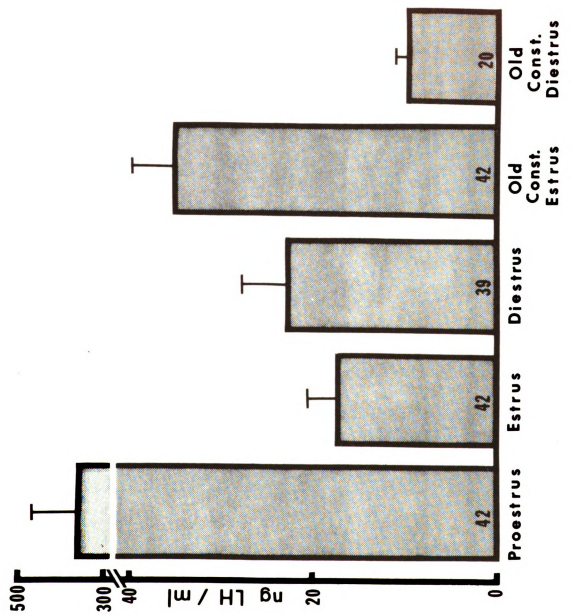
500
300
40
20
0
ng LH / ml
42
42
39
42
20
Proestrus
Estrus
Diestrus
Old Const. Estrus
Old Const. Diestrus

Figure 7

Figure 8. Serum LH levels in female rats after administration of various doses of L-dopa, without regard to reproductive status.

The height of each bar represents mean serum LH concentration expressed as ng/ml, with brackets corresponding to the standard error of the mean. Each group of three bars indicates measured LH concentration at 15, 60 and 120 minutes after drug injection. Open bars depict control responses while striped and shaded bars correspond to 3 and 30 mg L-dopa treatment, respectively.

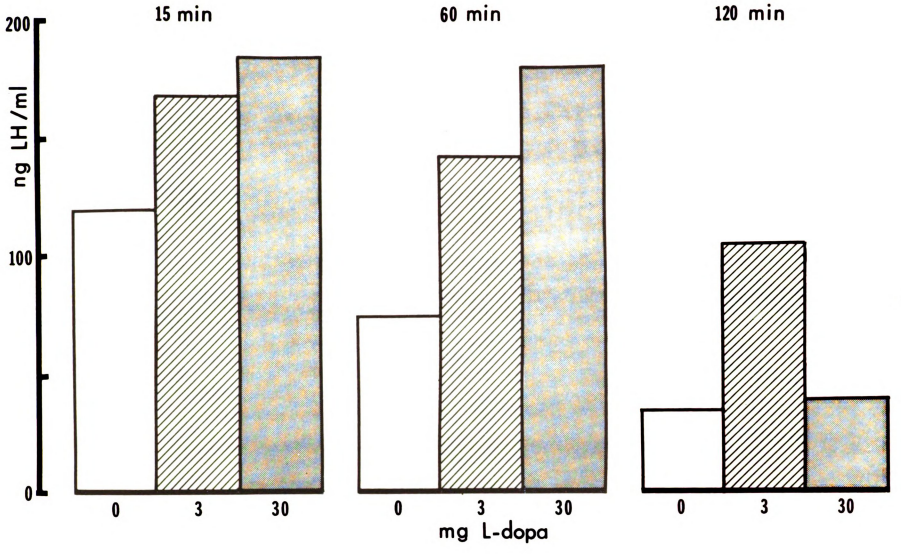

Figure 8

Figure 9. Effects of L-dopa therapy on serum LH concentrations in young cycling female rats.

These data illustrate the effects of L-dopa treatment on serum LH levels in young proestrous, estrous and second day diestrous rats. Mean serum LH levels expressed in ng/ml and their standard errors appear on the ordinate as a function of time relative to injection of various L-dopa doses. L-dopa administration is depicted by a caret on the abscissa, with blood samples taken 15 minutes before drug treatment, and at 15, 60 and 120 minutes afterwards. Solid circles represent control values while open circles and open squares signify 3 and 30 mg L-dopa treatment, respectively.

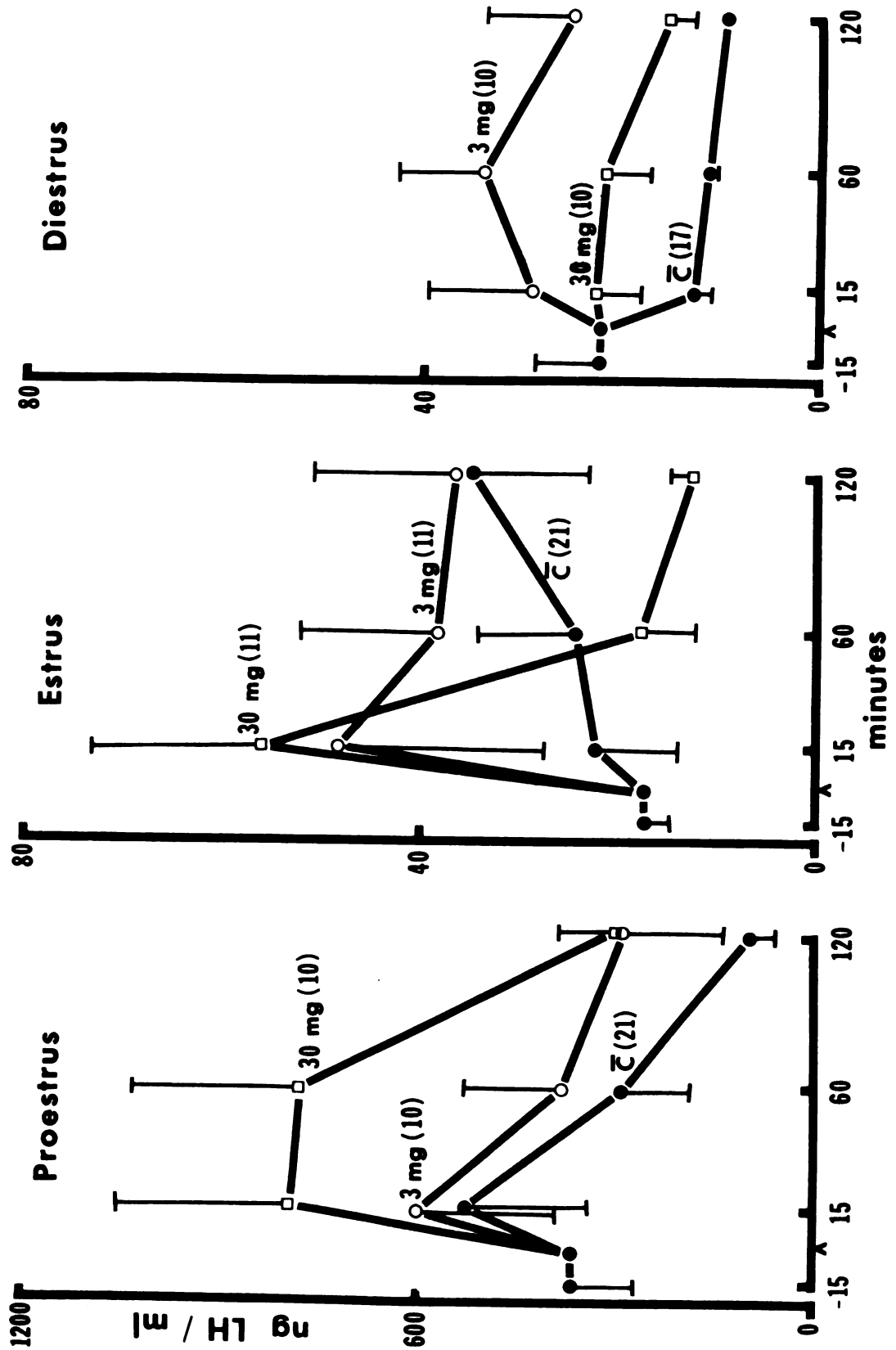
Proestrus
Estrus
Diestrus
ng LH / ml
1200
600
0
80
40
0
-15
15
60
120
minutes
30 mg (10)
3 mg (10)
C̄ (21)
30 mg (11)
3 mg (11)
C̄ (21)
30 mg (10)
3 mg (10)
C̄ (17)

Figure 9

Figure 10. Effects of L-dopa therapy on serum LH concentrations in aged female rats.

These data illustrate the effects of L-dopa treatment on serum LH levels in senescent constant estrous and constant diestrous rats. Mean serum LH levels expressed in ng/ml and their standard errors appear on the ordinate as a function of time relative to injection of various L-dopa doses. L-dopa administration is depicted by a caret on the abscissa, with blood samples taken at 15 minutes before drug treatment, and at 15, 60 and 120 minutes afterwards. Solid circles represent control values while open circles and open squares signify 3 and 30 mg L-dopa treatment, respectively.

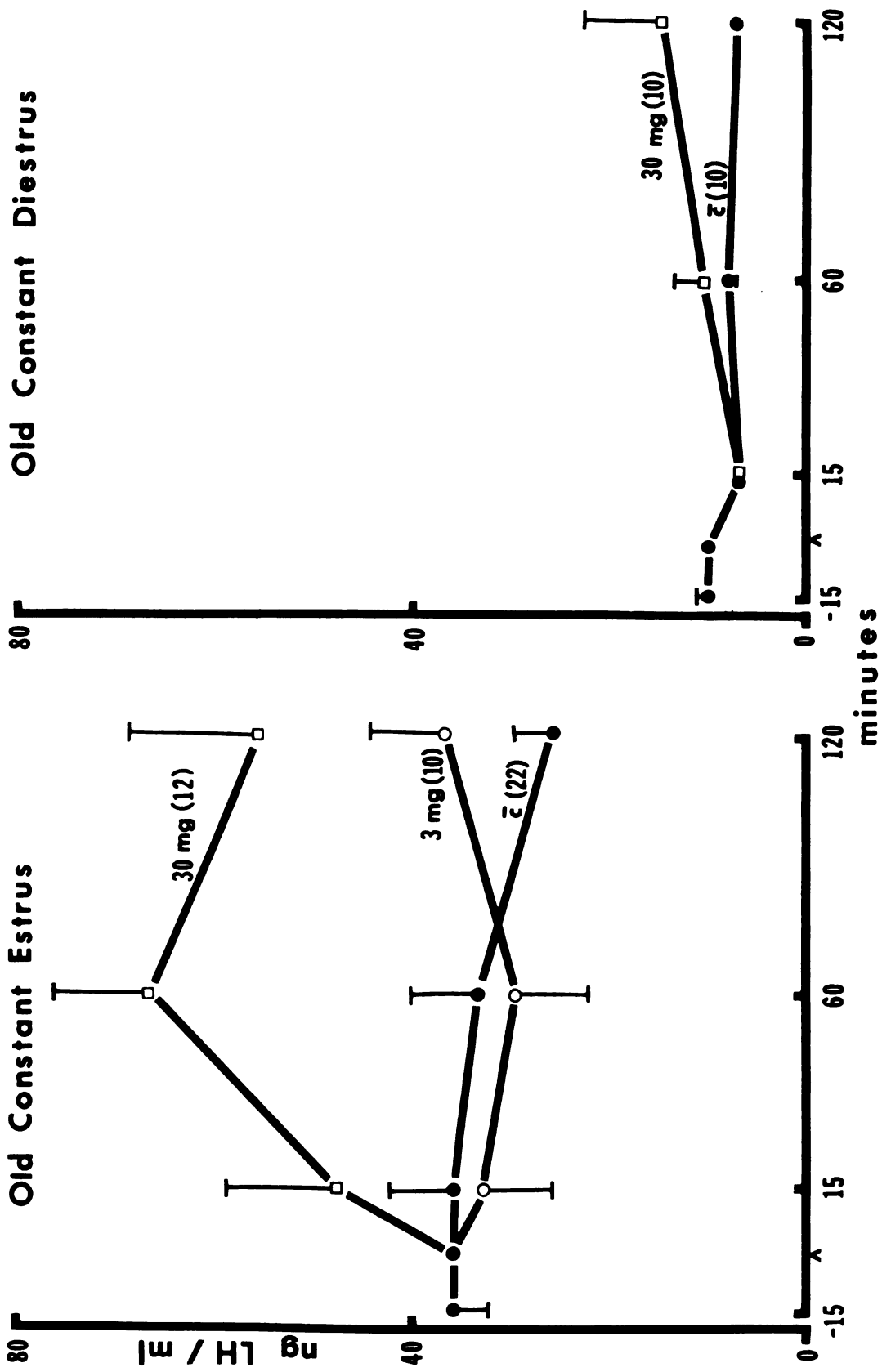
Old Constant Estrus
Old Constant Diestrus
ng LH / ml
80
40
0
-15
X
15
60
120
minutes
30 mg (12)
3 mg (10)
c̄ (22)
30 mg (10)
c̄ (10)

Figure 10

Figure 11. Effect of steroid pretreatment on basal serum LH concentration in young and aged female rats.

The height of each bar represents mean serum LH concentration expressed as ng/ml, with brackets corresponding to the standard error of the mean. Each bar indicates LH levels in serum samples taken at about 15 minutes before acute L-dopa treatment. Open bars depict responses in rats receiving no steroid pretreatment while shaded and striped bars correspond to subcutaneous pretreatment with 20 μg estradiol benzoate and 5 mg progesterone, respectively. Two hour progesterone pretreatment was employed in all reproductive states. Similar two hour estrogen priming was performed in all groups except young rats on the second day of diestrus and aged constant diestrous animals, which were given 24 hours of estrogen pretreatment.

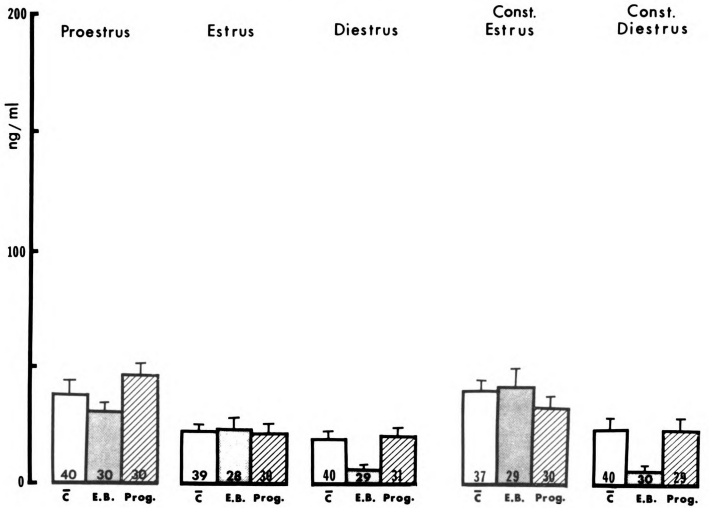
Basal LH
200
100
0
ng/ml
Proestrus
Estrus
Diestrus
Const. Estrus
Const. Diestrus
40
30
30
39
28
30
40
29
31
37
29
30
40
30
29
C̄
E.B.
Prog.

Figure 11

Figure 12. Effect of steroid pretreatment on the ability of L-dopa therapy to increase serum LH concentration in young and aged female rats.

The ordinate portrays the difference in serum LH concentration in ng/ml at 15 minutes following intraperitoneal administration of 30 mg L-dopa or saline. Positive values indicate that serum LH levels in rats receiving L-dopa exceeded those in corresponding controls, while negative values (those falling within the striped area) demonstrate the reverse relationship. Those bars in the left portion of the figure illustrate responses in untreated control rats of all reproductive states tested. The center cluster of bars shows responses following two hour pretreatment with 5 mg progesterone. In the set of bars on the right, 24 hours of pretreatment with 20 μg estradiol benzoate was used in aged constant diestrous rats and young rats on the second day of diestrus, while rats in the remaining reproductive conditions received 2 hours of estrogen priming.

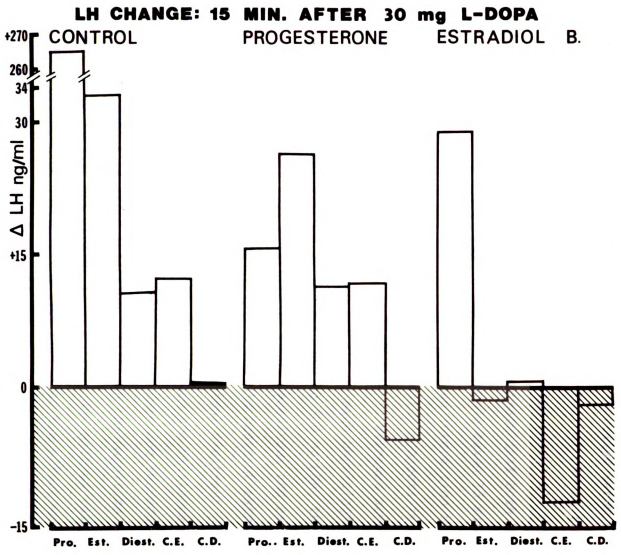
LH CHANGE: 15 MIN. AFTER 30 mg L-DOPA
CONTROL
PROGESTERONE
ESTRADIOL B.
+270
260
34
30
+15
0
-15
Δ LH ng/ml
Pro. Est. Diest. C.E. C.D.
Pro.. Est. Diest. C.E. C.D.
Pro. Est. Diest. C.E. C.D.

Figure 12

Effects of LRH on Serum LH

The responses of the various groups to intravenously administered synthetic LRH are summarized in Table 8. Although the 5 ng dose was ineffective, 50 and 500 ng LRH rapidly increased serum LH in a dose related manner. Figure 13 relates the effect of LRH at the blood sampling times of 15, 30 and 60 minutes as averaged across all reproductive groups. Data in this figure also demonstrate that both 50 and 500 ng LRH caused maximal effect in the blood sample taken 15 minutes following injection. Further, the existence of an interaction between LRH dose and reproductive status of the experimental subjects is depicted in Figures 14 and 15, where serum LH data is averaged across the post-injection bleeding intervals for animals of each physiological status. As is most clearly shown by the response to 500 ng LRH, the synthetic polypeptide was most effective in young proestrous and estrous rats, intermediately so in young diestrous and old constant estrous animals, and minimally effective in the old constant diestrous group. Data in Table 8 and Figures 14 and 15 also suggest the existence of an additional significant interaction between drug dose and reproductive condition through time after injection. Although the magnitude of response at 15 minutes was greater in proestrous and estrous rats than those of the other groups, the time course of the effect was more transient as evidenced in decline of serum by 50% toward baseline levels by 60 minutes after injection. In contrast, the slight elevation characteristic of young diestrous and senescent rats tended to be proportionally more sustained through time. However, even at 60 minutes

after LRH injection, absolute serum LH levels were still higher in young proestrous and estrous rats than in those of the remaining reproductive conditions.

Pretreatment regimes incorporating either progesterone of estradiol benzoate injection (Tables 9 and 10) caused significant changes in pituitary responsiveness to exogenous LRH stimulation as indexed by serum LH levels. Data in Figure 16 describe the interaction of steroid pretreatment on the degree of LRH induced increase of serum LH as averaged through time after injection for all reproductive conditions studied. Statistical analysis shows that although priming with either of the ovarian steroids did not greatly affect basal serum LH, it did significantly enhance the ability of LRH to elevate serum LH. Differences were found in the ability of progesterone to increase pituitary sensitivity to LRH stimulation when administered to rats in different reproductive states. Figure 17 summarizes changes in serum LH occurring at 15 minutes after injection of 50 ng LRH (that time characteristic of maximal serum LH increase) in all experimental groups following steroid pretreatment. Estradiol benzoate therapy increased pituitary responsiveness during estrus and to some degree in diestrous rats, but did not increase the already high responsiveness occurring on the day of proestrus. The steroid also restored pituitary response capability of old constant estrous rats to that characteristic of young proestrous animals. On the other hand, old constant diestrous rats, like young rats during diestrus, did not exhibit greatly enhanced pituitary responsiveness while under the estrogen influence. In young

rats progesterone priming consistently increased pituitary responsiveness to LRH, with the effect being most predominant on the days of proestrus and estrus. However in aged rats progesterone therapy did not significantly alter the ability of LRH to increase serum LH when compared to the response of control animals which received no steroid priming.

Table 8. Effects of LRH therapy on serum LH levels of young and senescent female rats

Reproductive state	n	Serum LH (mg/ml) Time (minutes) -15	15	30	60
Proestrus:					
Control	9	38.4 ± 6.8[a]	51.0 ± 7.5	47.5 ± 6.5	68.0 ± 10.0
5 ng LRH	9		41.4 ± 9.0	48.7 ± 15.5	69.0 ± 16.9
50 ng LRH	12		104.8 ± 14.8	73.0 ± 8.4	70.0 ± 11.5
500 ng LRH	9		611.0 ± 112.0	421.0 ± 66.6	250.0 ± 72.0
Estrus:					
Control	10	22.6 ± 1.6	33.1 ± 8.8	35.8 ± 11.5	50.1 ± 12.1
5 ng LRH	10		47.2 ± 13.2	28.1 ± 7.4	15.4 ± 4.9
50 ng LRH	10		87.8 ± 12.8	64.6 ± 13.0	22.8 ± 7.1
500 ng LRH	9		800.0 ± 196.0	433.0 ± 67.5	334.0 ± 85.8
Diestrus:					
Control	10	18.1 ± 5.2	8.8 ± 3.0	4.9 ± 0.3	9.5 ± 2.2
5 ng LRH	10		6.3 ± 0.5	7.5 ± 1.2	8.3 ± 1.7
50 ng LRH	10		26.0 ± 5.8	11.5 ± 4.2	9.7 ± 2.6
500 ng LRH	10		292.0 ± 44.7	236.0 ± 68.5	135.0 ± 29.8
Old Constant Estrus:					
Control	9	37.9 ± 2.3	25.7 ± 4.9	40.6 ± 16.1	57.4 ± 18.3
5 ng LRH	9		41.4 ± 15.4	15.9 ± 5.0	13.3 ± 3.7
50 ng LRH	9		59.4 ± 12.6	38.6 ± 16.2	35.3 ± 16.1
500 ng LRH	10		172.0 ± 21.5	174.0 ± 13.6	171.0 ± 41.8
Old Constant Diestrus:					
Control	10	22.0 ± 3.7	17.5 ± 7.6	12.2 ± 6.6	22.2 ± 10.0
5 ng LRH	10		7.8 ± 1.4	14.0 ± 7.0	16.7 ± 6.6
50 ng LRH	10		41.0 ± 18.5	25.8 ± 6.1	18.7 ± 7.0
500 ng LRH	10		83.6 ± 6.3	83.9 ± 14.5	66.0 ± 12.8

Hormone concentrations are shown as mean ± S.E.M.

[a]Pretreatment control values are pooled for all treatments in a given reproductive state.

Table 9. Effects of LRH therapy on serum LH levels of young and senescent female rats receiving 20 μg estradiol benzoate pretreatment

Reproductive state	n	Serum LH (ng/ml) Time (minutes) -15	15	30	60
Proestrus:[b]					
Control	11	32.1 ± 18.0[a]	14.4 ± 3.0	--	22.3 ± 3.4
50 ng LRH	10		120.0 ± 10.1	80.0 ± 4.0	97.7 ± 8.4
Estrus:[b]					
Control	9	28.3 ± 16.2	11.4 ± 3.8	--	6.4 ± 2.7
50 ng LRH	10		142.6 ± 21.5	86.3 ± 12.2	49.3 ± 6.4
Diestrus:[c]					
Control	10	3.8 ± 1.4	6.5 ± 2.2	--	5.0 ± 2.9
50 ng LRH	10		63.4 ± 11.4	47.8 ± 15.1	29.6 ± 11.3
Old Constant Estrus:[b]					
Control	10	44.4 ± 23.0	30.5 ± 15.4	--	11.0 ± 3.9
50 ng LRH	10		110.9 ± 16.8	112.2 ± 22.1	92.6 ± 24.2
Old Constant Diestrus:[c]					
Control	10	4.0 ± 1.6	8.8 ± 4.6	--	10.7 ± 6.0
50 ng LRH	10		57.6 ± 13.5	41.9 ± 12.6	25.7 ± 11.6

Hormone concentrations are shown as mean ± S.E.M.

[a]Pretreatment control values are pooled for all treatments in a given reproductive state.

[b]Two hour estradiol benzoate pretreatment.

[c]Twenty-four hour estradiol benzoate pretreatment.

Table 10. Effects of LRH therapy on serum LH levels of young and senescent female rats receiving two hour pretreatment with 5 mg progesterone pretreatment

Reproductive state	n	Serum LH (ng/ml) Time (minutes) -15	15	30	60
Proestrus:					
Control	10	50.7 ± 6.1	45.0 ± 6.3	--	48.1 ± 5.3
50 ng LRH	10		193.0 ± 34.0	100.0 ± 15.0	104.0 ± 18.0
Estrus:					
Control	10	26.5 ± 10.0	16.7 ± 3.9	--	13.2 ± 12.6
50 ng LRH	10		140.0 ± 40.7	92.0 ± 30.0	56.0 ± 14.5
Diestrus:					
Control	10	21.3 ± 10.6	7.2 ± 2.1	--	7.3 ± 2.8
50 ng LRH	10		54.0 ± 17.5	40.2 ± 7.9	21.0 ± 5.2
Old Constant Estrus:					
Control	10	21.1 ± 5.3	15.9 ± 4.8	--	18.2 ± 4.5
50 ng LRH	10		88.9 ± 24.7	78.8 ± 27.9	82.1 ± 32.6
Old Constant Diestrus:					
Control	10	31.8 ± 16.7	17.2 ± 10.0	--	10.8 ± 3.8
50 ng LRH	9		64.1 ± 16.5	52.0 ± 20.7	37.0 ± 9.1

Hormone concentrations are shown as mean ± S.E.M.

[a]Pretreatment control values are pooled for all treatments in a given reproductive state.

Figure 13. Serum LH levels in female rats after administration of various doses of LRH, without regard to reproductive status.

The height of each bar represents mean serum LH concentration expressed as ng/ml, with brackets corresponding to standard error of the mean. Each cluster of bars indicates measured LH concentration at 15, 30 or 60 minutes after LRH injection. Open bars depict control responses. Horizontally striped, diagonally striped, and shaded columns correspond to 5, 50 and 500 ng LRH treatment, respectively.

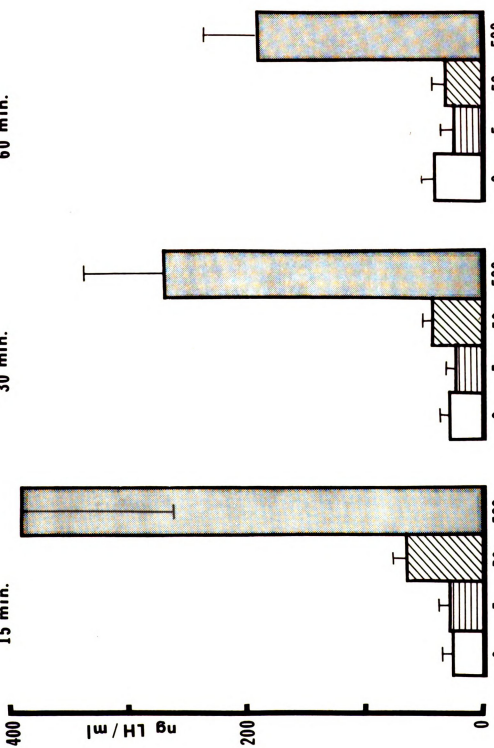

Figure 13

Figure 14. Effects of LRH therapy on serum LH concentrations in young cycling female rats.

These data illustrate the effects of LRH treatment on serum LH levels in young proestrous, estrous and second day diestrous rats. Mean serum LH levels expressed as ng/ml and their standard errors appear on the ordinate as a function of time relative to injection of various LRH doses. LRH administration is depicted by a caret on the abscissa, with blood samples taken about 10 minutes before hormone treatment, and at 15, 30 and 60 minutes afterwards. Solid circles represent control values while open circles and open squares signify 50 and 500 ng LRH treatment, respectively.

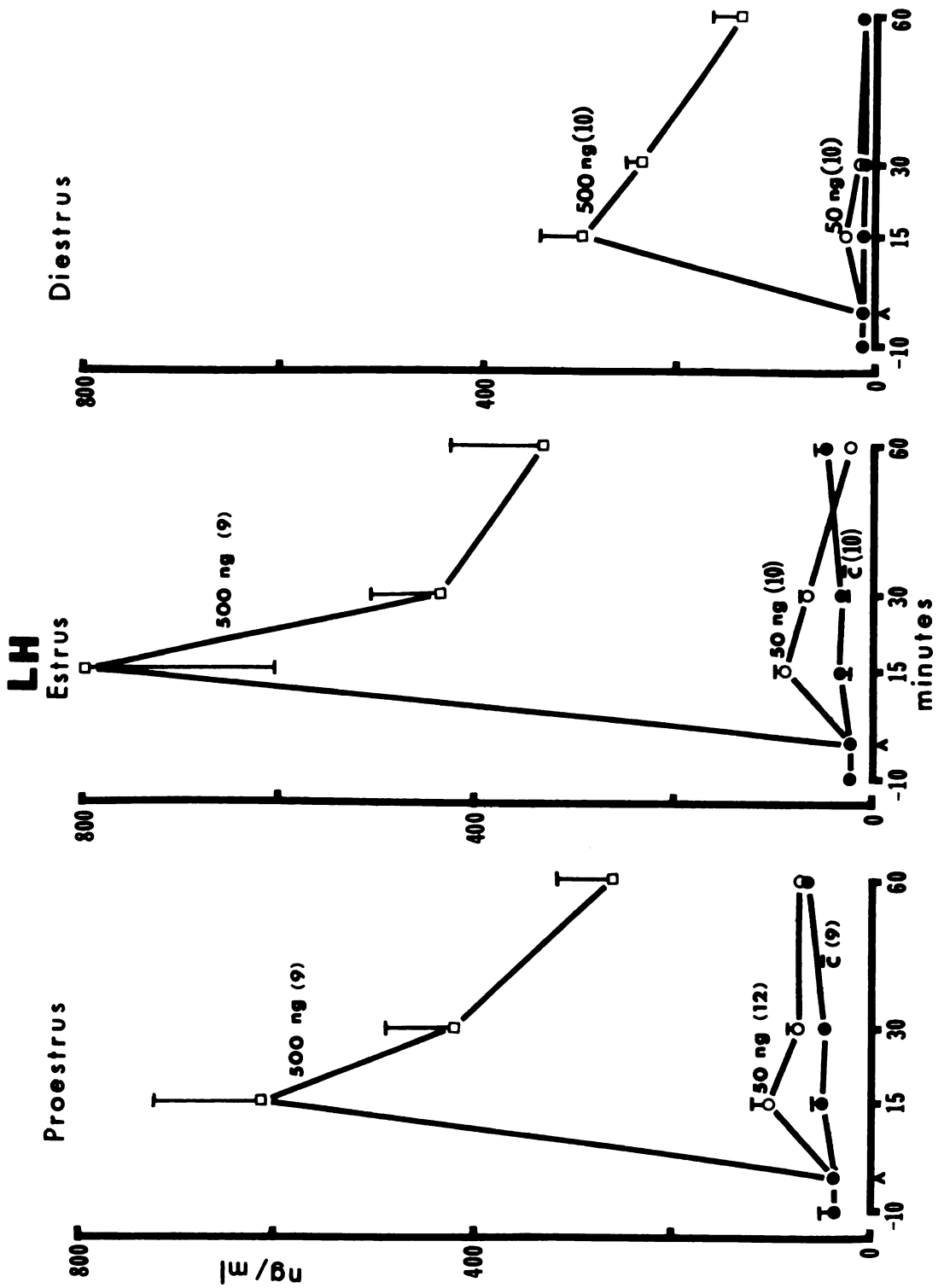
LH
Proestrus
Estrus
Diestrus
ng/ml
800
400
0
-10
0
15
30
60
minutes
500 ng (9)
50 ng (12)
500 ng (9)
50 ng (10)
500 ng (10)
50 ng (10)

Figure 14

Figure 15. Effects of LRH therapy on serum LH concentrations in aged female rats.

These data illustrate the effects of LRH treatment on serum LH levels in aged constant estrous and constant diestrous rats. Mean serum LH levels expressed in ng/ml and their standard errors appear on the ordinate as a function of time relative to injection of various LRH doses. LRH administration is depicted by a caret on the abscissa, with blood samples taken about 10 minutes before hormone treatment, and at 15, 30 and 60 minutes afterwards. Solid circles represent control values while open circles and open squares signify 50 and 500 ng LRH treatment, respectively.

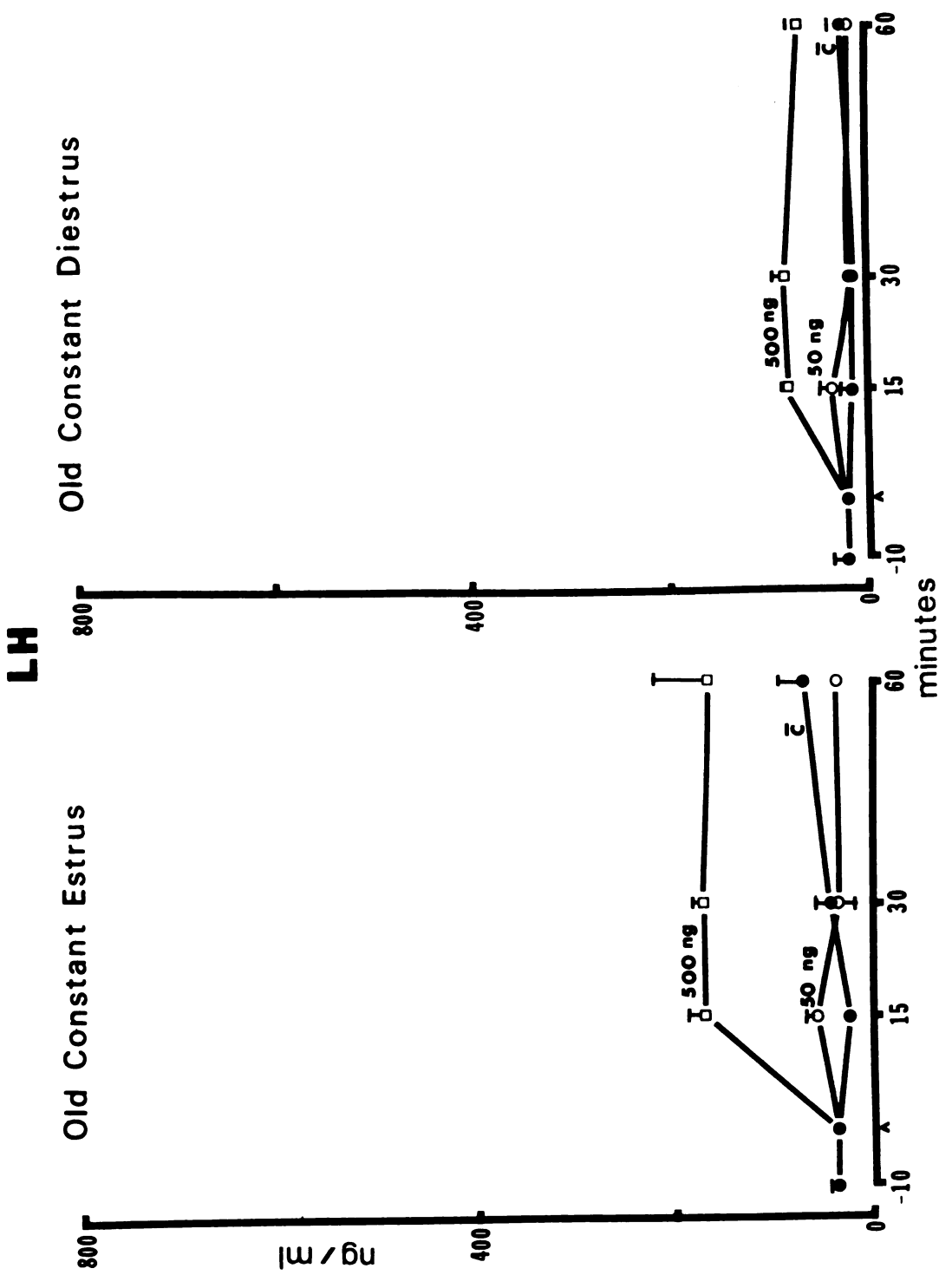
LH
Old Constant Estrus
Old Constant Diestrus
ng / ml
800
400
0
-10
15
30
60
minutes
500 ng
50 ng

Figure 15

Figure 16. Effect of steroid pretreatment on the ability of LRH therapy to elevate serum LH concentrations in female rats, without regard to reproductive status.

The height of each bar represents mean serum LH concentrations expressed as ng/ml, with brackets corresponding to the standard error of the mean. Each cluster of bars indicates the average LH concentration measured between 15 and 60 minutes after LRH injection for untreated rats as well as those receiving pretreatment with 20 μg estradiol benzoate or 5 mg progesterone pretreatment. Two hour progesterone priming was employed in all reproductive groups. Similar two hour estrogen pretreatment was performed in all groups except young rats on the second day of diestrus and aged constant diestrous animals, which were given 24 hours of estrogen pretreatment. Open bars signify LH levels in saline treated controls while shaded bars correspond to LH titers measured in rats having received 50 ng LRH.

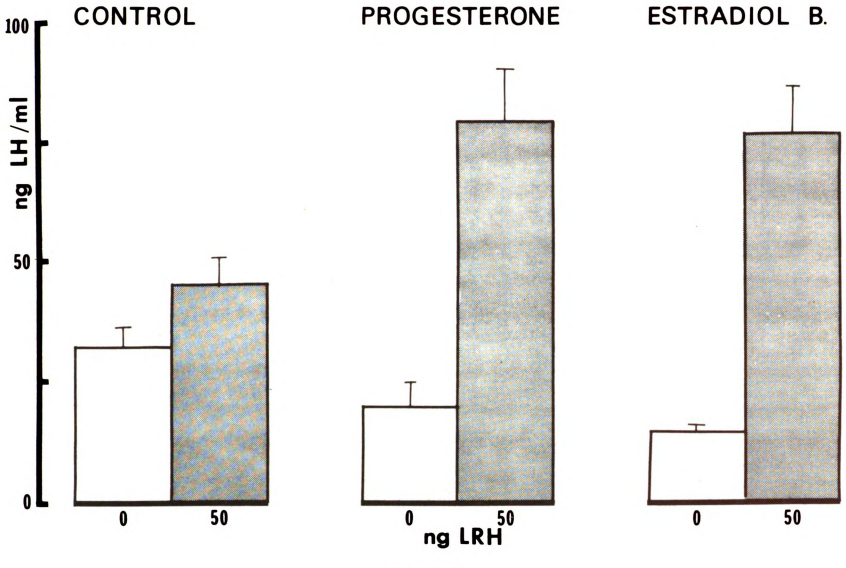
CONTROL
PROGESTERONE
ESTRADIOL B.
100
50
0
ng LH/ml
0
50
0
50
0
50
ng LRH

Figure 16

Figure 17. Effect of steroid pretreatment on the ability of LRH therapy to elevate serum LH concentrations in young and aged female rats.

The height of each bar represents mean serum LH concentration expressed as ng/ml, with brackets corresponding to the standard error of the mean. Each group of bars indicates measured LH concentration at 15 minutes after intravenous injection of 50 ng LRH for each of the reproductive states studied. Open bars depict responses in rats receiving no steroid pretreatment while shaded and striped bars correspond to subcutaneous pretreatment with 20 μg estradiol benzoate and 5 mg progesterone, respectively. Two hour progesterone pretreatment was employed in all reproductive states. Similar two hour estradiol priming was performed in all groups except young rats on the second day of diestrus and aged constant diestrous animals, which were given 24 hours of estradiol benzoate pretreatment. Superimposed on each bar is the corresponding 15 minute control LH level for untreated rats as well as for those receiving estradiol or progesterone pretreatment. Unless otherwise shown, there is a sample size of 10 rats in each group.

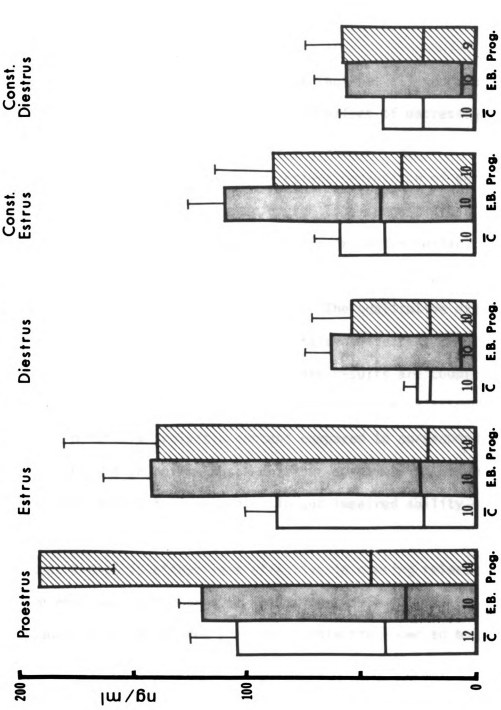
LH: 15 Min. after LRH
Proestrus
Estrus
Diestrus
Const. Estrus
Const. Diestrus
ng/ml
200
100
0
♀ E.B. Prog.
12
10
10
10
10
10
10
10
10
10
10
10
10
10
10
10
10
10
10
10

Figure 17

DISCUSSION

Data in Figures 2 and 3 demonstrate that not all reproductive states are equally susceptible to the L-dopa effect of depressing serum prolactin. The most striking response difference among the groups studied is that L-dopa caused a more transient depression of serum prolactin in old rats than in younger ones. This suggests that perhaps old animals are less capable of releasing PIF or that pituitary responsiveness to PIF decreases with senescence. The possibility of age related changes in PRF control also exists. The qualitative extent of blunted responsiveness within the hypothalamo-pituitary axis in aged animals is made even more apparent when these results are coupled with the report by Clemens and Meites (1971) that old constant estrous rats have elevated pituitary prolactin content compared to corresponding young animals on the day of estrus. In other words the simultaneous existence of elevated pituitary prolactin and impaired ability of L-dopa to maintain suppression of serum prolactin in old constant estrous rats suggests that L-dopa is either less effective in causing release of PIF or that the endogenous PIF released following L-dopa therapy is less able to induce secretion of the pituitary prolactin known to be present in aged constant estrous rats.

Figure 4 demonstrates that pretreatment with either estradiol benzoate or progesterone did not affect resting hormone levels in any

of the rats other than those of young proestrous or estrous groups. Since the steroid priming protocol included blood samplings beginning at 10 a.m., the low resting serum prolactin levels on the mornings of proestrus and estrus likely occur before the time of endogenous preovulatory prolactin surges.

As is shown in Figure 6, estradiol benzoate or progesterone priming generally decreased the duration of 30 mg L-dopa's ability to suppress serum prolactin when compared to rats that did not receive comparable steroid pretreatment (Table 2; Figure 1). This steroid effect was observed regardless of the reproductive status of the rats tested. The precise site of this interactive effect of exogenous steroids on L-dopa induced inhibition of prolactin secretion is not known. However there are several potential explanations for the observed phenomenon. It is possible that the decreased response duration under the conditions of ovarian steroid pretreatment is due to altered kinetics of L-dopa absorption or metabolism to decrease its effective availability within the CNS. Estradiol benzoate and progesterone may also act within the CNS to inhibit activation of the hypothalamic dopaminergic mechanisms which are responsible for PIF secretion. The possibility also exists that the steroids may act centrally to facilitate hypothalamic release of a PRF. The steroids may also act at the hypophysial level to repress pituitary responsiveness to endogenously released PIF following L-dopa administration. Conversely, estrogen and progesterone might directly sensitize the pituitary to enhance responsiveness to endogenous PRF. Of course the net result of decreased

L-dopa effectiveness when accompanied with exogenous steroid priming could be a function of more than one of these processes acting simultaneously. Further investigation is needed to clarify the exact mechanism of the steroid effect demonstrated by these studies.

Several investigators have presented substantial support for the concept that systemic administration of L-dopa depresses serum prolactin levels by enhancing hypothalamic synthesis and secretion of PIF (Lu *et al.*, 1970; Lu and Meites, 1971; Lu and Meites, 1972). Results of the present report are in agreement with these studies, showing a dose related reduction of serum prolactin with L-dopa treatment. As is shown in Figures 1 and 3, 30 mg L-dopa maximally depressed prolactin for 120 minutes regardless of prevailing reproductive condition, while 3 mg L-dopa was only effective at 15 minutes when assessed without regard to animal status. However in interpreting the results of these studies it must be borne in mind that the report by Donoso *et al.* (1973) provided evidence that high circulating catecholamine levels may act directly at the pituitary to inhibit prolactin release. It has also been recently shown that physiological levels of dopamine and norepinepherine can sharply reduce prolactin secretion by incubated rat pituitaries (Shaar and Clemens, 1974).

In these studies, basal serum prolactin concentrations were found to be markedly elevated only on the days of proestrus and estrus (Table 2; Figure 4). Although there is ample experimental evidence that normal prolactin elevation in the cycling rat occurs on the day of proestrus (Wuttke and Meites, 1970; Neill *et al.*, 1971), there are also

reports of prolactin elevation which extends to the day of estrus (Arimura *et al.*, 1970; Uchida *et al.*, 1972; Taya and Igarashi, 1973). The experiments described in the present research indicate that the elevated basal prolactin level on the day of estrus is not evidenced in subsequent serial bleedings of saline injected control animals (Table 2). In agreement with these findings, Euker and Riegle (1974) reported that the high serum prolactin encountered in the afternoon of estrus was sharply reduced by serial bleeding or restraint stress. These observations suggest the possibility that on the day of estrus, nonspecific stressors associated with experimental manipulation resulted in the reduced serum prolactin in our saline injected control group. The present data suggest that serum prolactin on the day of estrus is more affected by stress than prolactin on the afternoon of proestrus.

In young adult rats basal serum prolactin levels were highest during times of elevated availability of endogenous estrogens, as indexed by the degree of vaginal cornification. This positive relationship between estrogen and serum prolactin levels has been well documented in castrate rats (Kalra *et al.*, 1973; Arimura *et al.*, 1970) as well as with *in vitro* work at the hypophysial level (Nicoll and Meites, 1964; Ieiri *et al.*, 1972). The neuroendocrine status of rats is apparently altered during the course of ageing. In the present report serum prolactin concentration in senescent rats, regardless of their vaginal cytology, was maintained at a level different from any of those observed in young cycling rats (Table 2; Figure 4). Thus during senescence, endogenous estrogen availability appears to become less

precisely related with normally regulated serum prolactin levels. These data do not indicate whether these differences are due to alterations in estrogen secretion or tissue responsiveness to estrogen in aged rats.

The findings concerning basal serum LH concentrations in female rats are in general agreement with the well accepted concept that serum LH levels remain low throughout the estrous cycle except for a large transient surge of a few hours duration which occurs during the late afternoon or evening of proestrus. However basal serum LH levels were significantly elevated by 1:00 p.m. on proestrus in the study which was designed to assess the effects of various doses of L-dopa on serum LH in cycling rats (Figure 7). The exact onset time of the preovulatory LH surge could vary among different strains of rats, or may be influenced by characteristics of the prevailing diurnal lighting pattern to which rats are subjected. Rats in our colony were under a 12 hour light/dark schedule with lights on at 6:00 a.m. In contrast, investigators using a 14 hour light cycle starting at 5:00 a.m. report increasing proestrous LH levels which begin at between 3:00 and 5:30 p.m. (Goldman *et al.*, 1969; Neill *et al.*, 1972; Taya and Igarashi, 1973).

Aside from the elevation of serum LH in the early afternoon of proestrus, there was also significant variation in LH levels among the remaining reproductive states, being measurably higher in aged constant estrous rats. Likewise, Bishop *et al.* (1972) reported that experimental production of constant estrus by electrically lesioning

the suprachiasmatic area or the paraventricular nucleus of adult female rats caused slight elevation of serum LH. These findings, coupled with those in the present report, suggest that slightly elevated serum LH titers are functionally correlated with continuance of the constant estrous state by maintaining large estrogen secreting ovarian follicles.

Experimental regimes routinely included taking 4 blood samples of about 1.5 ml each in a period ranging from about 1 to 2 hours. This bleeding schedule alone did not generally cause significant changes in serum LH concentrations through time. These findings indicate that the blood hypovolemia associated with these experiments was not of sufficient magnitude to induce increased pituitary LH secretion as has been described by Seyler and Reichlin (1973) under conditions involving a slightly greater degree of blood volume depletion.

Several *in vitro* and *in vivo* experiments have provided strong evidence that dopamine acts within the hypothalamus as a central nervous system neurotransmitter to stimulate LRH release leading to elevation of serum LH levels (Schneider and McCann, 1969; Kamberi *et al.*, 1969 and 1970b). Data included in Table 5 and Figure 5 show that systemic administration of the immediate metabolic precursor of dopamine, L-dopa (which is recognized to be diffusable across the blood-brain barrier) is also generally effective to increase circulating LH concentrations in a dose related manner. Although these data do not identify the site or mechanism of action of L-dopa, available literature implies that the drug diffuses into the CNS where it is decarboxylated to form dopamine. Although the possibility that L-dopa acts directly at the adenohypophysis

to enhance LH secretion cannot be completely ruled out, it has been shown that dopamine does not specifically affect LH secretion from incubated pituitary halves (Schneider and McCann, 1969; Kamberi *et al.*, 1970b).

The finding that not all female rats were equally responsive to L-dopa induced augmentation of serum LH levels (Table 5; Figures 9 and 10) is in accord with the results of Schneider and McCann (1970b). They demonstrated that intravenous dopamine administration caused greater serum LH increases during proestrus and the second day of diestrus than was evident in either estrous rats or those on the first day of diestrus. The present investigation indicates that L-dopa was maximally effective on the afternoon of proestrus, causing a twofold increase in the existing high LH levels characteristic of proestrus. The drug also markedly increased serum LH on the day of estrus, while it was less effective in diestrous rats. The 30 mg L-dopa treatment increased serum LH in old constant estrous rats similar to the magnitude of response found on the day of estrus. Conversely, serum LH levels of old constant diestrous rats were completely unaffected by this level of L-dopa. Comparison of temporal response profiles in Figures 9 and 10 demonstrates that although the magnitude of response to L-dopa is similar in old constant estrous rats to that of young estrous rats, the elevation is delayed in the aged animals. There are several mechanisms which could be involved in this delayed response to L-dopa in senescent rats. The drug may have diffused more slowly into the CNS, delaying the activation of dopaminergic mechanisms responsible for inducing LRH release. However the observation

that L-dopa can rapidly depress serum prolactin in senescent rats (Figure 4) makes this explanation unlikely. The actual kinetics of neuroendocrine activation of LRH release could also be impaired in old rats. It is also possible that pituitaries of old rats exhibit delayed responsiveness to endogenous LRH perfusion. Finally, the observed response differences between young estrous and old constant estrous rats could be a function of some combination of these alterations. The total lack of responsiveness in old constant diestrous rats may result from an intensification of one or several of the functional impairments described for old constant estrous rats. Alternatively, these rats may be completely refractory to L-dopa therapy. The observed response difference between aged constant estrous and constant diestrous rats might reflect different prevailing levels of endogenous steroid feedback interactions which could also conceivably affect L-dopa effectiveness.

The increased responsiveness to L-dopa in young proestrous and estrous rats over that of young diestrous animals, and in old constant estrous rats compared to those in constant diestrus could be interpreted to suggest a positive correlation between presumable endogenous estrogen availability and the subsequent degree of L-dopa effectiveness. It has been shown that estradiol can directly augment basal pituitary LH release, as well as to increase pituitary sensitivity to endogenous LRH (Schneider and McCann, 1970c; Arimura and Schally, 1971; Debeljuk *et al.*, 1972c). However estrogen apparently interferes with dopamine's ability to enhance LH release through LRH mediation (Schneider and

McCann, 1970a and 1970c). This blocking effect of estrogen is thought to require synthesis of an inhibitory peptide or protein which in some manner interferes with LRH releasing mechanisms (Schneider and McCann, 1970c). In the present experiments, prior administration of estradiol benzoate acted to inhibit L-dopa's ability to stimulate pituitary LH secretion in all reproductive conditions except proestrus (Table 12). The depressed responsiveness to L-dopa following estrogen treatment in these groups could be a result of steroid induced inhibition of hypothalamic dopaminergic pathways thought to facilitate LRH secretion. Alternatively these results themselves do not preclude the possibility that estradiol benzoate provided negative feedback at the hypophysial level to suppress pituitary responsiveness to that LRH which may have been released by L-dopa's successful activation of dopaminergic synapses in the hypothalamus. On the other hand, estradiol benzoate pretreatment to young proestrous rats did not depress the ability of L-dopa therapy to enhance serum LH levels (Figure 12). These findings suggest that the inhibitory effect of estradiol benzoate on L-dopa induced gonadotropin release is attenuated only during proestrus, the day of anticipated endogenous gonadotropin surging. The possibility thus exists that in normally cycling rats the negative influence of estrogen, presumably acting at the hypothalamic level, becomes less effective on the day of proestrus. In addition estrogen availability at this time may be actively involved as a facilitory factor for preovulatory surging of LH release, as has been formally hypothesized (Ying and Greep, 1972; Legan *et al.*, 1973). Support for this concept can also

be derived from the report that an antiserum to estradiol given on the second day of diestrus prevents preovulatory LH secretion (Neill *et al.*, 1971). Progesterone pretreatment did not interfere with L-dopa's induction of serum LH elevation in any of the reproductive states tested (Table 12). This lack of effect indicates that under the conditions of these experiments, progesterone does not influence the ability of L-dopa treatment to alter the functioning of the hypothalmo-pituitary axis as regards gonadotropin control.

The studies designed to assay pituitary responsiveness to exogenous LRH demonstrate the expected dose related serum LH enhancement for all groups tested (Figures 14 and 15). Maximal LH elevation occurred at 15 minutes after injection and tended to diminish thereafter (Figure 13). This type of response profile implies that the pituitary reacts rapidly to stimulation by LRH and that the effect is rather transient. These findings are in agreement with those of Rippel *et al.* (1973) who reported maximal LH elevation by their first sampling time at 30 minutes post-injection, followed by a decline of peripheral LH to basal concentrations at 4 hours after administration. The results of this current study also show that the effectiveness of LRH to stimulate pituitary LH secretion varies as a function of reproductive status of the animals treated. Young rats were more responsive during the mornings of proestrus and estrus than on the second day of diestrus (Figure 14). Although responsiveness of old constant estrous rats to LRH was significantly depressed below that corresponding even to young diestrous rats, they still showed greater LH elevation following

exogenous LRH than did those senescent rats in constant diestrus (Figure 15). These results suggest that pituitaries of old rats, especially those in the constant diestrous state, are less capable of secreting LH in response to exogenous LRH. The low pituitary responsiveness to LRH in old constant estrous rats, which presumably have substantial serum estradiol levels as indexed by vaginal cytology, suggests that estradiol's recognized augmentation of pituitary response to LRH may be less effective in old animals.

The observed senescent impairment of pituitary responsiveness to synthetic LRH may involve functional deterioration at one or more of the following sites. The decreased responsiveness could be due to recognized decrease in hypophysial LH content (Clemens and Meites, 1971), restricting the total LH pool available for secretion. Senescent rats might also have less efficient hypophysial portal circulation which would decrease effectiveness of LRH perfusion. Old rats may also experience alterations in supportive endocrine milieu which is responsible for optimal pituitary functioning. For instance, there could occur important age related changes in circulating steroid levels, or similar decreases in the tonic tropic stimulus of endogenously released LRH. Finally, pituitary responsiveness may be directly impaired. This deterioration could be a function of lowered hypophysial binding of LRH, or of decreased activation of adenyl cyclase to enhance LH synthesis and secretion. Further experiments are necessary to determine the precise mechanism of senescent decline of pituitary responsiveness to LRH.

Similar findings of increased pituitary responsiveness to LRH in young cycling rats on the day of proestrus have previously been

reported (Cooper *et al.*, 1972; Rippel *et al.*, 1973; Kalra and Kalra, 1974). The recent publication by Kalra and Kalra (1974) shows that the ability of LRH to enhance pituitary LH release was also markedly elevated during the first day of estrus, which they identified as the first day of cornified vaginal cytology. They further demonstrated the existence of a positive relationship between endogenous serum estradiol concentration and pituitary sensitivity to LRH. The pituitary response to exogenous LRH therapy has also been shown to be enhanced by 24 and 48 hours pretreatment with estradiol benzoate (Arimura and Schally, 1971; Debeljuk *et al.*, 1972c). Collectively these data suggest that estrogen acts directly at the pituitary to increase its responsiveness to LRH stimulation. Incorporation of 20 μg estradiol benzoate priming in the present experiments also increased pituitary responsiveness to 50 ng LRH (Figure 16). These results are in agreement with generally accepted concepts of estrogen action at the hypophysial level, but additionally show that estradiol benzoate can sensitize the pituitary to LRH within 2 hours after initial steroid administration. As is seen in Tables 6 and 9 and in Figure 11, estradiol benzoate pretreatment did not generally affect basal serum LH concentrations. Although estradiol benzoate may have depressed resting LH levels in both young and aged rats having leucocytic smears, the significance of this effect is questionable since these hormone levels are near the lower limits of assay sensitivity. The failure of estradiol benzoate to generally influence pre-LRH serum LH levels indicates that these hormones do not greatly affect basal hypothalamic secretion of LRH.

As Figure 17 portrays, estradiol benzoate pretreatment increased pituitary responsiveness during estrus and caused slight response augmentation in the young diestrous and old constant estrous groups. However the steroid priming was ineffective in altering the response of proestrous or old constant diestrous rats. These results, coupled with the demonstration that estrogen pretreatment does not greatly alter resting serum LH levels, indicate that steroid induced increases in response capacity to LRH therapy are mainly implemented at the hypophysial level. The inability of exogenous estradiol benzoate priming to further enhance the already marked pituitary response in proestrous rats may be a consequence of endogenously high circulating estradiol concentrations in those animals which may have already maximally sensitized the pituitaries to LRH stimulation. On the other hand, even 24 hour priming with 20 μg estradiol benzoate was unable to increase the low pituitary responsiveness characteristic of old constant diestrous rats. This finding provides evidence that the impaired functional capacity of pituitaries in old constant diestrous rats abolishes the ability to secrete LH in response to LRH.

Progesterone pretreatment of 2 hour duration invariably increased pituitary responsiveness to LRH in young rats but was ineffective in the old animals. These results are in disagreement with those of Arimura and Schally (1970) who indiscriminately administered 25 mg progesterone subcutaneously to female rats that were not in proestrus. Forty-eight hours after steroid injection, the ability of purified porcine LRH to induce serum LH elevation was depressed.

The differences in response profiles between their report and that of this study could be due to differences in the dose of progesterone used, to the interval between steroid administration and subsequent assessment of pituitary sensitivity, or to the basic LRH preparation used. However a recent report by Libertun and Lipton (1974) demonstrated that progesterone given to estrogen primed ovariectomized rats can sensitize the pituitary to synthetic LRH in a manner similar to that of this report.

Results of these experiments demonstrate a dual role of estradiol in providing feedback information for control of LH secretion by the hypothalamo-pituitary axis. Estradiol benzoate pretreatment augmented the ability of the pituitary to secrete LH while under the stimulatory influence of exogenous LRH (Table 9). On the other hand, the same estradiol treatment regime blunted L-dopa's effect to elevate serum LH through presumed dopaminergic activation of hypothalamic LRH release (Table 6). Results from the L-dopa studies further indicate that the estrogen implemented inhibition of endogenous LRH secretion was physiologically of greater import than the steroid's enhancement of pituitary responsiveness since the net result of the two opposing steroid actions during L-dopa therapy was to block serum LH activation.

Simultaneous assessment of the experiments incorporating L-dopa and LRH therapy (Tables 6 and 9; Figures 10 and 15) generally suggests that with age, functional deterioration of gonadotropin control occurs both within the hypothalamus and at the pituitary. The elevation of serum LH following L-dopa injection was delayed in aged rats while there was no evidence of temporal response retardation in old rats receiving

the LRH regimes. These observations imply that hypothalamic dopaminergic mechanisms mediating LRH release are activated more slowly in senescent rats, and that this delay does not occur at the hypophysial level. On the other hand, results showing a depressed magnitude of serum LH increase in old rats following LRH treatment supports the concept of senescent functional pituitary lesioning as regards capacity for LH secretion. However it has yet to be elucidated whether the major cause of the response impairment is a function of decreased pituitary LH storage as was suggested by Clemens and Meites (1971) or of impaired activation of pituitary mechanisms responsible for LH secretion. The observation that estradiol benzoate treatment can enhance pituitary responsiveness in constant estrous rats (Table 10) suggests that alterations in steroid feedback characteristics can still affect the ability of LRH to induce LH secretion in some aged rats.

Analysis of the patterns of vaginal cytology in young rats shows that acute treatment with L-dopa or moderate LRH doses did not alter cycling patterns when compared to saline injected controls (Table 1). Of these animals, the 13% that experienced altered patterns of vaginal cytology went into pseudopregnancy. The onset of pseudopregnancy in these rats was likely a consequence of changes in gonadotropin control due to nonspecific stressors associated with experimental manipulation. However administration of 500 ng LRH on the morning of the second day of diestrus or of proestrus apparently successfully induced precocious ovulation as indexed by abbreviated duration of the estrus type smears. The succeeding estrus was also usually delayed by 1 or 2 days in the

animals that had presumably been induced to prematurely ovulate under these experimental conditions. Thus the suspected advancement of ovulation induced by administration of high levels of LRH at about 1 day before the expected gonadotropin surge may not have affected the endogenous timing of LH surging since the onset of the following period of estrus was not similarly advanced. Old rats that received L-dopa or LRH did not show greater recovery of vaginal cyclicity than did corresponding control rats. In this case nonspecific stressors restored cyclicity in 76% of constant estrous rats and 43% of those in constant diestrous. This summary of the inability of acute restoration of CNS catecholamine availability with L-dopa therapy to restore estrous cycling in old rats contrasts with the report by Quadri *et al.* (1973) which showed that long term L-dopa therapy can restore vaginal cycling in constant estrous rats. Thus the acute treatment regimes employed in these experiments were ineffective to restore estrous cycling although significant alterations in neuroendocrine control of gonadotropin and prolactin secretion were evidenced.

Steroid hormone pretreatment was also generally not effective in altering vaginal cytology patterns in either young or old rats regardless of acute administration of L-dopa or LRH. However estradiol benzoate priming invariably caused onset of pseudopregnancy in all young rats studied whether or not they received acute treatment with L-dopa or LRH. These results are in accord with those of Ying and Greep (1972) which demonstrated that various doses of estradiol benzoate given on day one of diestrus could advance ovulation by 24 hours and also induce

pseudopregnancy. Twenty-four hour progesterone pretreatment to rats on the second day of diestrus also caused changes in vaginal cyclicity in young rats. These findings are similar to those reported by Paup (1973) who reported that progesterone in a dose of 0.5 mg/100 g body weight given on the day before estrus could advance the estrous activity patterns by 24 hours. Similar actions of exogenous progesterone therapy to advance ovulation have been reported by Redmond (1968). However assessment of vaginal cytology patterns in aged rats showed that steroid priming, as well as acute L-dopa and LRH treatment, was not able to restore vaginal cyclicity.

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