INVESTIGATION OF THE EFFECT OF A NEW DOPAMINERGIC AGONIST "PIRIBEDIL" ON OVULATION, IMPLANTATION, LACTATION AND ITS INTERACTION WITH XANTHINES

A THESIS

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BY

GABER ABED EL SABOUR ALY

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DEMONSTRATOR IN PHARMACOLOGY DEPARTMENT

FACULTY OF MEDICINE

AIN SHAMS UNIVERSITY

SUPERVISED BY:

PROFESSOR: MALAK BOTROS IBRAHIM

DR. : ZEINAB M. LABIB

DR. : MAHDY SALAMA

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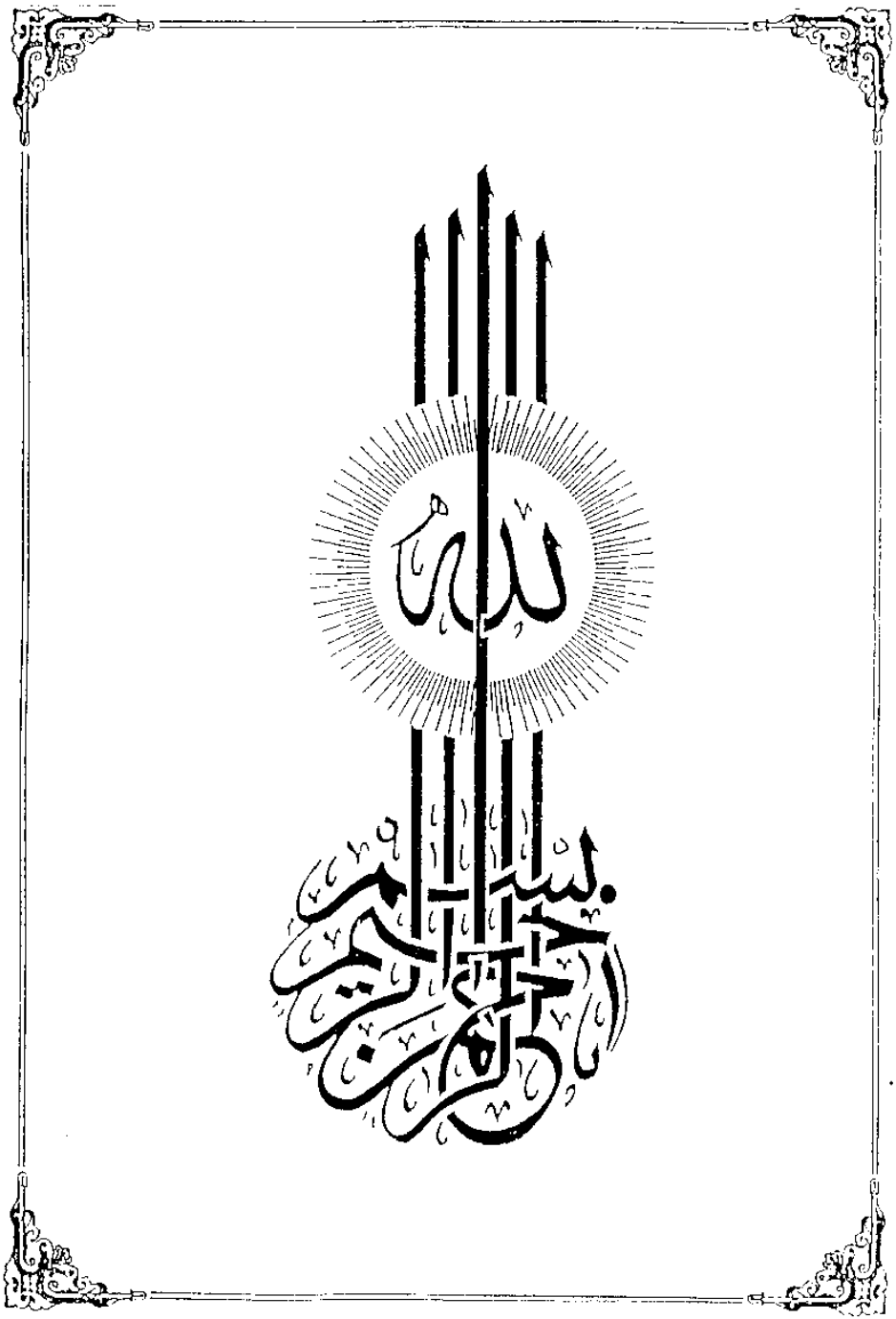

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The dopaminergic tubero-infundibular system has been shown to play a role in regulation of the pituitary gonadotrophins and prolactin secretion in experimental animals (Meites et al. 1972-1976). Recent studies have suggested that dopamine and dopamine agonists also exert an inhibitory effect on gonadotrophin and prolactin release in humans (Le Blanc and Lachelin 1976-1977).

Dopamine was known earlier to function as an intermediary precursor in the biosynthesis of nor-adrenaline and adrenaline in sympathetic neurons and chromaffin cells starting either with tyrosine or with dopa. However, dopamine is known now to play a role as a neurotransmitter exerting its effects via rather specific receptors different from those of nor-adrenaline and adrenaline within the central nervous system.

The detailed morphological description of dopamine neurons were made possible through the histochemical fluorescent technique (Flack et al. 1962). With this technique, it was possible to visualize the dopamine neuron, the cell body and its dendrites, the axon and the dense terminal network ramifying in all directions. Recently, a new immunohistochemical techniques have been developed by which enzymes engaged in the synthesis of dopamine e.g. tyrosine hydroxylase can be visualized. Using the latter

technique, the dopamine neurons can be demonstrated with greater perfection (Hokfelt et al. 1976). The cell bodies of dopamine neurons give rise to long axons and dense networks of nerve terminals. All along the nerve terminals, varicosities occur which synthesize and secrete dopamine in response to nerve impulses. The dopamine synapse is a complicated structure, where the dopamine nerve terminal communicates with the postsynaptic cell by the release of dopamine.

Dopamine is synthesized from tyrosine which is taken up into the dopamine nerve terminal, where it is converted to DOPA and then to dopamine. It is stored in small granules from which it is released in response to nerve impulses. It then affects the post-synaptic cell by stimulating the dopaminergic receptor which in turn leads to changes in the activity of the post-synaptic cell. After stimulating the receptor, dopamine is removed in two ways; metabolized enzymatically to homovanillic acid (HVA) or taken up into the presynaptic nerve terminal to be re-used as neurotransmitter.

While the most important dopamine neuron systems are located within the brain, dopamine containing cells are also present in retina, glomus caroticum, renal and vascular tissues, within sympathetic ganglia. Dopamine nerve terminals are also present in the chemo-trigger

zone in the area postrema where vomiting may be elicited by dopamine agonists (Thorner 1975).

The most important dopamine systems from the functional point of view are the tubero-infundibular system involved in control of pituitary hormone release and the mesencephalic ascending dopamine systems participating in diencephalic as well as cortical function. The cell bodies of the tuberoinfundibular neurons are localized in the hypothalamus, in close vicinity of third ventricle. They send their short axons to the external layer of the median eminence and terminate partly on the portal vessels. These are concerned in pituitary hormone release especially luteinizing hormone-releasing hormone and prolactin (Fuxe et al. 1978).

There are many drugs interfering with various aspects of dopamine neurotransmission. Certain drugs increase the stimulation of dopamine receptors either indirectly by increasing the release of dopamine (like amphetamine) or directly by mimicking the function of dopamine on the pre or post-synaptic receptor (like bromocryptine, apomorphine and piribedil). Other drugs interfere with dopamine neurotransmission either by blocking dopamine synthesis (like Alphamethyl-para-tyrosine), by blocking the uptake of dopamine into the dopamine storage granules (like reserpine) or by having a more direct action leading to blockade of

dopamine receptors e.g. haloperidol, chlorpromazine, thioridazine (anti-psychotic drugs).

Piribedil (a piperonyl-pyrimidine derivative, Et_{495}) is a dopamine agonist exerting its effects via the dopaminergic receptors. It penetrates into the central nervous system. Goldberg (1972, 1975) speculated that piribedil may be active centrally and ineffective peripherally as vasolilator because it is converted to an active metabolite in brain but not in kidney. However, Masala et al. (1978) reported that piribedil is employed in clinical practice in treatment of obstructive arterial diseases and this drug is thought to be a long acting dopamine receptor agonist. It has been also used in treatment of parkinsonism in doses up to 300 mg daily (P. Jenner et al. 1973). The effects of piribedil are easily blocked by pimozide, a specific dopaminergic receptor antagonist (Fuxe et al. 1974), and reduced by $H_{44/68}$ pretreatment (Fuxe 1973).

It was found that theophylline and caffeine markedly enhanced (5 - 10 folds) the effects of DOPA and dopamine receptor agonists such as apomorphine and piribedil (Fuxe et al. 1974). Results suggested that theophylline and caffeine can considerably sensitize dopamine receptors to the action of various dopamine receptor agonists, on the assumption that adenylcyclase is a part

of dopamine receptor (Kebabian et al. 1972) and that xanthines can inhibit cyclic AMP phosphodiestrase enzyme which is known to inactivate cyclic AMP in the central nervous system.

Mammals other than primates do not menstruate and their sexual cycle is called Estrus Cycle, named for the conspicuous period of heat or estrus at the time of ovulation. In spontaneously ovulating species such as the rat, the underlying endocrine events are essentially the same as those in the menstrual cycle in human although the cycle is numbered from the day of estrus.

In hypophysectomized rats, highly purified follicle-stimulating-hormone preparations do not cause estrogen secretion unless a small amount of lentinizing-Hormone is also injected. Leutinizing-hormone and presumbly prolactin stimulate progesterone secretion. It is clear that FSH from pituitary gland is responsible for early maturation of ovarian follicles and that FSH and LH together are responsible for their final maturation and that a burst of LH secretion (Ovulatory hormone release) is responsible for ovulation and initial formation of corpus luteum (Ganong 1977).

Experimental animal studies have shown that the hypothalamus and may be the limbic system are involved in

the control of gonadotrophin secretion from the anterior pituitary gland. The hormonal link in pituitary-gonad regulation is the hypothalamic luteinizing-hormone-releasing hormone (L.H.R.H.) (Schalch 1971; Schally et al. 1973 and Tanner 1975). This substance originally isolated from the porcine hypothalamus by Schally et al. 1972, is a linear decapeptide which has all the hormonal properties of the native hormone isolated from both porcine and ovine hypothalamic tissue. Injection of L.H.R.H. decapeptide brings about the release of both the gonadotrophic hormones LH and FSH. For this reason, most workers now believe that this material is the only hypophysiotrophic factor regulating gonadotrophin release (Besser 1972; Kastin 1972; Franchimant 1974 and Mortimer 1974). Some call this hormone the gonadotrophin-releasing hormone. It has been proposed that there is a second FSH. R.H. distinct from L.H.R.H., but the chemical evidence on which this claim is based is not extensive (Schally 1973 and Vale et al. 1973). The gonadotrophic hormone releasing effects of hypothalamic extracts can be blocked by antibody to the L.H.R.H. decapeptide.

Under basal conditions, pituitary secretion of LH and FSH is episodic, one secretory burst occuring approximately each hour (Boyer et al. 1972; Cramer et al. 1973). The occurrence of an ovulatory surge is widely considered to be characteristic of the female pattern of gonadotrophin

secretion (Rassman et al. 1971). Most workers believe that the ability to release gonadotrophins in an ovulatory surge pattern means that the hypothalamic pituitary axis has a positive feed-back capacity, but the exact site of the positive feedback receptor is still under study. The rise in LH that precedes ovulation is composed of a series of progressively larger surges (Naftolin et al. 1973).

Maintenance of the basal levels of gonadotrophin secretion appears to depend on tonic hypothalamic influences arising from the medial tubero-hypophysial system. In the rat, several workers have proposed on the basis of lesion and electric studies that the medial preoptic region is the site of origin of neurons responsible for the midcycle ovulatory surge (Clemens et al. 1971; Cramer et al. 1973). In another study, the hippocampus was shown to exert an inhibitory effect on gonadotrophin secretion (Kawakami et al. 1973). Cann 1974 reported that impulses arising in the limbic system may alter L.H.R.H. and thereby, the gonadotrophin secretion. He also pointed out that the hypophysiotrophic neurons which release L.H.R.H. are in turn regulated by biogenic amines. Lesions of arcuate nucleus in ventral hypothalamus caused ovarian atrophy in experimental animals.

Edwardsson (1968); Fluckiger et al. (1968) and Hokfelt et al. (1972) have pointed out that blockade of

ovulation, inhibition of implantation and reduction of lactation after ergocornine, bromoergocryptine and agroclavine could be due to activation of dopamine receptors in median eminence of hypothalamus (Hokfelt et al. 1972; Fuxe 1973 and Stone 1973) thereby inhibiting LRF secretion and enhancing prolactin inhibitory factor (PIF) secretion (Wuttke et al. 1971). However, a dopamine receptor may also exist on the prolactin-containing cells in the pituitary gland (Birge et al. 1970; Tasteels et al. 1971 and Quijada et al. 1973/1974).

Craven and McDonald (1973) reported that the infusion of dopamine into the arcuate nucleus median eminence area of proestrus rats actually blocked subsequent ovulation. Uemura and Kobayashi (1971) implanted dopamine into the arcuate nucleus median eminence and noted that animals subjected to such treatment experienced prolonged anovulatory periods of diestrus or estrus depending on the dosage applied. Schneider and McCann (1969) demonstrated the increase in LH, FSH release following intraventricular injection of dopamine, however similar doses had no effect when injected into the portal vessels.

Data concerning catecholamine stimulation of LH release are ambiguous and controversial. Fuxe et al. (1970, 1972) suggested that dopamine is inhibitory to LH release. In those studies, evaluation of changes in histochemical fluorescence indicated that the tubero-infundibular

dopaminergic neurons were relatively inactive during the proestrus surge of LH, but increased their amine turnover rate during steroid-induced negative feedback. Schneider et al. (1970), and Kamberi et al. (1970) using radio-immunoassay methods to measure plasma LH, have reported that dopamine is stimulatory to LH release in anaesthetized rats. On the other hand, Sawyer et al. (1974) have found that intraventricular nor-epinephrine stimulated the release of LH in the unanaesthetized estrogen-primed rabbit, whereas dopamine not only failed to release LH, but actually blocked the subsequent LH response to a previously effective dose of nor-epinephrine. The inhibitors of enzymatic synthesis of nor-adrenaline from dopamine such as diethyl dithiocarbamate, blocked the LH release by progesterone in the proestrus rat (Kalra et al. 1972), as well as the LH response to electrochemical stimulation of the preoptic area (Kalra et al. 1973).

Laverty (1965); Donso et al. (1967) and Kavanagh (1973/1974) reported that the concentration of catechdamines in hypothalamus fluctuates during the estrus cycle of rats. The concentration of dopamine in portal plasma from cyclic rats was highest during estrus and lowest during proestrus periods. Moss et al. (1975) reported that part of activation of L.H.R.H. neurons

might result from the depression of inhibitory dopaminergic tubero-infundibular neurons in the arcuate nucleus. Nor-epinephrine and dopamine were applied iontophoretically to neurons in the arcuate nucleus identified antidromically as projecting to the median eminence. Those workers noted that such L.H.R.H. neurons, when activated by nor-epinephrine were usually depressed by dopamine, whereas neurons activated by dopamine were often depressed by nor-epinephrine and none was stimulated by both nor-epinephrine and dopamine.

Using quantitative microfluorimetry to study the catecholamine turnover changes in relation to critical period of experimental ovulation, Lofstrom et al. (1977) found that there was an inhibitory dopaminergic and facilitatory nor-adrenergic mechanism in controlling pregnant-mare-serum (PMS) induced ovulation. This might be partly exerted via nor-adrenergic terminals in medial preoptic area and via dopamine terminals in lateral palisade zone. Fluckiger (1978) demonstrated that bromocryptine, a dopamine agonist could inhibit ovulation in mature female rats as well as prolactin secretion.

A variety of in-vitro studies were performed to elucidate the possible physiological role of monaminergic pathways in the release of LH and FSH (Schneider 1969 and Kamberi 1970). In the presence of median eminence tissue,