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THE ROLE OF MONOAMINES IN THE REGULATION OF PITUITARY GONADOTROPINS IN NORMAL AND CASTRATED RATS

NASREEN MASOOD QURESHI AND QAMAR AMIN
Department of Physiology, University of Karachi
Karachi-75270, Pakistan

ABSTRACT:

Despite suggestion of multiplicity of neurotransmitters and neuromodulators contributing in the regulation of pituitary gonadotropin secretion the picture regarding catecholamines in intact and castrated rats is not clear. This study deals with the differential response of pituitary FSH and LH to immediate effect of different adrenergic and non-adrenergic agonist/antagonist (adrenaline, noradrenaline, proparnolol, isoprenaline, clonidine, phenoxybanzamine HCl) in the short-term castrated rat model. The evidence is presented for a direct excitatory or facilitatory effect of adrenaline and noradrenaline for pituitary LH regulation viz-a-viz testosteron feed back mechanism in castrated rats.

INTRODUCTION

Difference in hypothalamic amine concentration between experimental animals (proestrous and short term castrated rats treated with morphine sulphate and its antagonists) and controls indicated the role of aminergic system in the regulation of gonadotropin secretion (Gilmore et al., 1984). Previously, modulatory role for central noradrenergic neurons in the control of LH and ovulation (Sawyer and Clifton, 1980) and excitatory role of Nor-adrenaline (NA) in LH secretion in ovariectomized and ovariectomized-steroid primed rats (Vijayen and McCann, 1978) was shown to be mediated via alpha adrenergic receptors (Ojeda et al., 1982). On the other hand, LH secretion and ovulation in rat can be inhibited by endogenous release of NA elicited by activation of central adrenergic system (Dotti and Taleisnik, 1982). The observed effect of the noradrenergic system on LH secretion whether acting via alpha or beta receptor was previously shown to be only modulatory and not essential (Clifton and Sawyer, 1980).

Adrenaline was found to evoke GnRH release during the critical period of proestrous (Gopalan et al., 1987, 1988). Despite the preliminary evidence for a

stimulatory β adrenergic component on ovulation in proestrous (Al-Hamood et al., 1985), the exact nature of adrenergic involvement in controlling hypothalamic peptidergic neurons and consequently gonadotropin release and ovulation remains warranted.

In males the role of catecholamines in the regulation of gonadotropin secretion remains largely unexplored. Although classical neurotransmitter modulate LHRH-LH release mechanism (Kalra and Kalra, 1983; Taleisnik and Sawyer, 1986), but the precise relationship viz-a-viz feedback mechanism of testosterone via GnRH is not known. Central catecholamine system was shown to mediate the hypothalamic mechanism controlling pituitary gonadotropin release in castrated at (Herdon et al., 1984). Testosterone may exert its negative feedback action by altering hypothalamic catecholamine transmission. NA activity in medial basal hypothalamus and median eminence was reduced by testosterone (Chiocchio et al., 1976; Simpkins et al., 1980). Mediation by alpha adrenergic system (Ojeda and McCann, 1973) as well as by nor-adrenergic system (Herdon et al., 1984) in hypersecretion of LH after castration has been suggested contrary to reports of LH inhibition by nor-adrenergic system (Gallo and Drouva, 1979). Since there is evidence for a possible involvement of a stimulatory B-adrenergic component in the normal regulation of the preovulatory surge (see above) alongwith well documented alpha stimulatory component which together may mask the possible inhibitory effects of a β adrenergic system, it was considered pertinent to investigate the involvement of catecholamine in LH regulation following castration. The castrated model of short term (24-48 hr post-castration) was chosen since previous experiments have demonstrated that rapid rise in plasma LH which follows orchidectomy is sensitive to drugs which alter catecholamine (Ojeda et al., 1982).

METHODOLOGY

Adult male rats (250-300g, 60 days old, from the Animal House of H.E.J. Inst./PCSIR and kept under controlled temperature and 12:12 hr light: darkness schedule) were maintained in the department for 1-2 days prior to experiments. They were divided into groups of 3-4 animals each in various experimental protocols:-

Intact (no surgery: Group-I): castrated under ether anesthesia via scrotal approach and left for 24-48 hr before administration of either physiological saline (Group II) or the drug under study (Group III).

The drugs to be tested were dissolved in ether (5%; w/v) glucose (to act as anti-oxidant). Phenoxybenzamine was initially dissolved in a few drops of 70% ethanol and made up (1:5) in distilled water immediately before injection or 0.9% NaCl. Their dosages and duration of treatment are given under results.

Blood samples (0.5 ml) were withdrawn from tail vein into heparinized tubes just before injection and at 30, 60 and 180 min. afterwards as given under results for each experimental protocol. Experimental design involves measurement mostly at single/double time points as gonadotropin e.g. LH is released in discrete pulses (Ellis and Desjardins, 1984). Our previous experiments had shown large variations within and between animals, hence, simpler one/two point measure was adopted. It is also known that absolute LH concentrations at any given time must be function of both frequency and amplitude of the secretion. Blood was centrifuged at 400 x g at 5°C for 5 min and plasma stored in eppendorfs at -20°C till the assay was done. Plasma samples for all the rats were assayed at NRIFC laboratory. LH was measured by the ovine-ovine RIA procedure (Niswender et al., 1968), using NIH-LH-S16 as standard and FSH was assayed using the rat-rat NIAMDDK system, using FSH-RP-1 as standard. The kits were gifts from NIH, USA. All samples were measured in duplicate in the same RIA batch.

RESULTS

Plasma gonadotropin levels in intact and castrated rat:

Experiments were conducted to establish the post-castration rise in plasma gonadotropins in 60 days old rats at 24 and 48 hr after castration, since there has been wide range in rise of LH values at different durations following castration (Al-Hamood et al., 1987) and the results obtained are shown in Fig.I. Plasma level of LH was almost X 6-7 more than the values in intact rat at day 1 which increased further though not significantly on day 2. FSH, on the other hand, showed comparatively lesser increase (x 2-2.5) at day 1 following castration and did not show further increase at day 2.

Effect of non-selective B-adrenergic agents:

Adrenaline (20mg/Kg, i.p.) a mixed beta adrenergic ($alpha_1$, $Beta_1$ and $Beta_2$) agonist, was tested in short term castrated rats (24-48 hr post castrated) for its effect on the raised plasma gonadotropins and stimulatory effect was found on plasma LH but not on plasma FSH, which was observed both a 1 and 3 hr. is shown (Fig. II) Another non-selective beta- adrenergic agonist (mixed beta) isoprenaline-HCl, (20mg/Kg i.p) in short term castrated rats was found to have increased levels of plasma LH; maximum at 1 hr. post administration. However, plasma LH levels at 3 hr following drug treatment was though higher than castrated controls but lower than that determined at 1 hr post administration. FSH level remained unaffected in these experiments.

Effect of Beta-adrenergic Antagonist:

The results of non selective antagnoist (mixed beta) propranolol (20mg/Kg i.p.)

demonstrated (Fig. II) that when administrated to 24 hr. castrated rats, plasma LH and FSH levels remained more or less in the same range both at 1 and 3 hr. as that in castrated controls, indicating that this drug does not have any effect on plasma gonadotropins in castrated rats.

Effect of non-selective alpha adrenergic agonist and antagonists:

Noradrenaline, predominantly alpha receptor agonist (Beta$_1$, alpha$_1$, alpha$_2$) when administered (20mg/Kg i.p.) to 24-48 hr castrated rats showed (Fig. III) that the plasma LH level was insignificantly higher at 1 hr post administration but at 3 hr following administration, was comparatively higher than in the castrated controls, indicating thereby a moderate stimulatory effect. Plasma FSH values in these experiments, being in slightly higher range than castrated controls, were not markedly different both at 1 and 3 hr following administration of the drug.

Phenoxybenzamine-HCl and clonidine were selected as non-selective alpha-adrenergic antagonist and agonist respectively. Phenoxybenzamine-HCl when administered (20 mg/Kg i.p.) to 24-48 hr. castrated rats clearly demonstrated a significant fall in the level of plasma LH at 3 hr. post injection (Fig. III). FSH levels though not significantly effected, were rather in a lower range as compared to castrated controls if the values from other experiments were also taken into account. On the other hand, clonidine (mixed, alpha$_1$ and beta) when administered (0.2 mg/kg s.c.) to the short term castrated animal model, seemed to have a stimulatory effect on LH at 30 mins. following administration whereas FSH level remained unaffected (Fig. III).

DISCUSSION

In the present study, involvement of the central catecholaminergic system in the neural mechanism responsible for the increased level of plasma LH in particular and of plasma gonadotropins in general in response to castration in male rats, has been studied (McCann, 1973; Lorenzen and Ramley, 1981; and Ringstrom and Schwartz, 1983). Increased levels of LH and FSH following castration were established as in previous reports on adult rats (McCann, 1973; Lorenzen and Ramley, 1981; Ringstrom and Schwartz, 1983) and demonstrated further that a plateau is attained between 24 and 48 hr following castration in rats of 60 days age group. However, wide difference in the degree of increase of plasma LH levels at various durations following castration have been reported i.e. for example, within 9 hr (Schwartz and Justo, 1977) 16 hr (Herdon et al., 1984; Al-Hamood et al., 1987) between days 5-15 (Badger et al., 1978; Hetzel et al., 1981); at 30 (Linkie et al., 1981) or 40 post castration day (Herdon et al., 1984). Further, in males increase in LH levels at 48 hr after castration and no change in serum FSH level at either 12, 24 or 48 hr post castration with increasing age has also

been reported (Lorenzen and Ramely, 1981). This finding may account for the controversial findings regarding the involvement of hypothalamic adrenergic system in the neural mechanism leading to the acute raise of LH in castrated males. Wide range of pre and post infusion values of LH after the administration of substances including adrenergic agents, glucose and saline were found (Al-Hamood et al., 1987). Hence, there seems importance of selection of post castrational day, for studying the catecholaminergic involvement in gonadotropin rise. Such a rise is well known to be sensitive to drugs that alter catecholamine activity (Ojeda and McCann, 1973; Herdon et al., 1984) but it is also known that a few days after castration the system no longer remains sensitive to drugs that effect catecholamine activity in brain (Herdon et al., 1984). Our results of both FSH and LH rise at either 24 or 48 hr post castration in 60 days old rat demonstrate that experimental model seems acceptable for the undertaken study as plateau is obtained between 24-48 hr following castration.

The documentation of stimulatory effect of adrenaline and isoprenaline within 3 hr of i.p. administration in our study indicates that beta adrenergic system plays a role in post castrational LH regulation. Stimulatory effect of adrenaline has been well demonstrated in female rats: prevention of plasma LH rise at proestrous or in orchidectomized rats after treatment by inhibitors of adrenaline synthesis (Crowely and Terry, 1981; Crowley et al., 1982; Coombs and Coen, 1983; McKinnon et al., 1983). The only study on short term castrated males (16 hr postcastration) did record an insignificant rise in circulating LH level brought about by i.v. infusion of adrenaline. Our study using pharmacological dosage was probably significant enough to result in marked change, though it is known that adrenaline acts on both alpha and beta receptor sites and its action is, therefore, more complex than that of a selective drug. The mixed beta adrenergic agonist, isoprenaline, stimulated LH release, thus confirming previous intra ventricular infusion study demonstrating a significant rise in LH within 15-30 minutes in males (Al-Hamood et al., 1987). thus, beta adrenergic component seems a part of the mechanism involved in the neural regulation of LH in short term castrated rats as has been shown for preovulatory LH surge alongwith well demonstrated stimulatory component (Al-Hamood et al., 1985, 1987). Mixed beta antagonist propranolol remained ineffective on either of gonadotropin levels. Previously, beta adrenergic system was shown inhibitory to the release of LH pulses in ovariectomized rats (Leung et al., 1982a, b; Uang and McCann, 1983) but perhaps their effects were due to an action on β_1 receptors. Another study found possible inhibitory effect of β_1 adrenergic system in the neural regulation of the preovulatory LH surge in proestrous rats (Al-Hamood,1985) as well as β_1 adrenergic agonist drug inhibiting LH release and there are evidences which indicate that secretion of LH appears to be a combination of stimulatory and inhibitory influences exerted upon the central catecholaminergic system (Al-Hamood et al., 1987).

Our results of mixed adrenergic agonist clonidine and antagonist, phenoxybenzamine support the original suggestion of involvement of adrenergic system in mediating the increased secretion of LH after castration (Ojeda and McCann, 1973) and is consistent with those reported in female rats (Estes et al., 1982; Leung et al., 1982) and in short term castrated rats (Herdon et al., 1984; Almedia et al., 1988). Though a modulatory role of central noradrenergic neurons in the control of LH secretion and in ovulation has been extensively documented (Drouva and Gallo, 1976, 1979; Sawyer, 1979; Vijayan and McCann, 1978; Sawyer and Clifton, 1980), probably via adrenergic receptors (Ojeda et al., 1973; Gallo and Kalra; 1983, Al-Hamood et al., 1987; Almedia et al., 1987, 1988), but there have been controversy regarding their involvement in castrated males. For example, acute inhibition of the nor adrenergic system by receptor blockers or inhibitors of synthesis as well as chronic depletion of hypothalamic nor adernaline by 6 hydroxydopamine, did not effect the normal rise in LH levels seen on days 10 and 40 after castration although at 16 hr after castration adreno receptors blockers did reduce LH levels indicating that nor adrenergic system is likely to be involved in the short term response to castration (Herdon et al., 1984). Later, in another study adrenergic antagonists caused significant reduction in circulation levels of LH in short term castrated and long term overietomized rats but none of the compounds suppressed LH in long term castrated males (Almeida et al., 1988). There appears thus a sex difference in response of hypothalamic noradrenaline system to gonadectomy (dePaolo, 1982) and failure of this system to function in androgen sterilized rats.

It seems that monoamine regulation of gonadotropin secretion is complex but much evidence points towards a direct excitatory or facilitatory effect of adrenaline and noradrenaline on Gn-RH secretion at least during LH surge (See: Ramirez et al., 1984). Further, gonadal steroids may also input to these cells which are under opioid influence (Johnstone et al., 1984). Thus, in one opinion alterations of the opioid adrenergic input to Gn-RH neurons may be part of the mechanism by which gonadal steroids exert their central feedback effects on gonadotropin secretion (See: Bicknell, 1985). Further experiments involving effect of catecholamines on Gn-RH-antagonists treated castrated rats are needed to enhance our understanding of the regulation of gonadotropins in short term castrated rats, since postulated roles for both adrenaline and noradrenaline in the events of pituitary gonadotropin controlling mechanism has been re-emphasized (Gopalan et al., 1987).

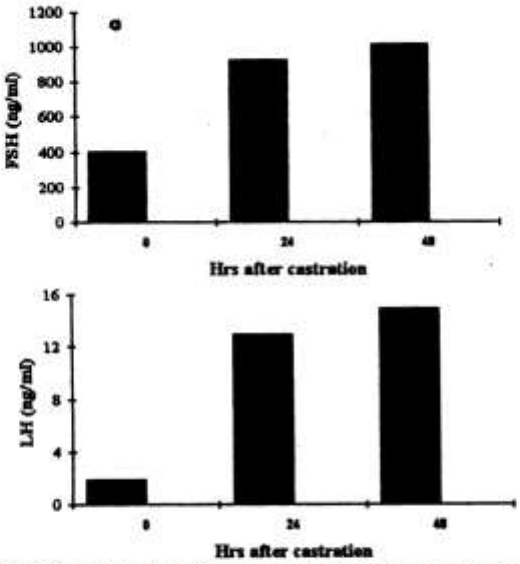

Fig. I: Plasma FSH (fig. a) and Lh (fig. b) levels in 60 days old rats at 0 (intact), 24 and 48 hr following castration.

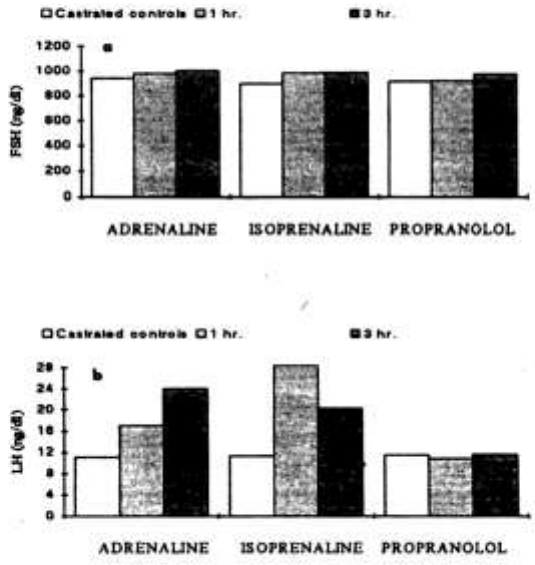

Fig. II: Effect of non-selective mixed beta adrenergic agonists (adrenaline, isoprenaline) and antagonist (propranolol) on plasma FSH (fig. a) and LH (fig. b) levels in short-term castrated rats at 1 and 3 hr following administration of the drug.

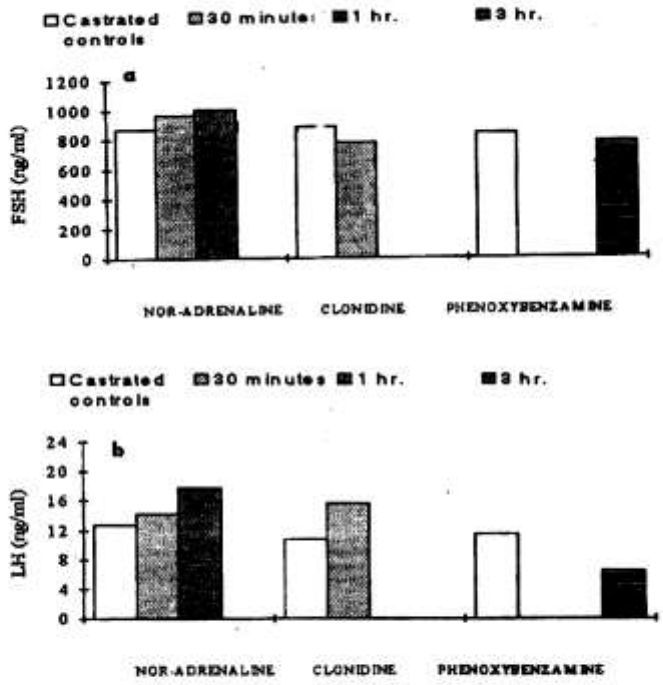

Fig. III: Effect of non-selective alpha-adrenergic agonists (nor-adrenaline and clonidine) and antagonist (Phenoxybenzamine on plasma FSH (fig. a), LH (fig. b) levels in short-term castrated rats at 30 minutes, 1 and 2 hr following the drug administration.

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