Chinese Journal of Physiology 41(4): 235-240, 1998

Effects of Intraventricular Norepinephrine on LH Release in Short- and Long-term Ovariectomized Steroids-primed Rats

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Abstract

This study examined the noradrenergic mechanism in regulation of luteinizing hormone (LH) release in short- and long-term ovariectomized (OVX) steroids-primed rats. All rats were OVX on the diestrous day 1(D1) morning about 1000 h. After OVX, rats in the short-term OVX group were immediately primed with estradiol (E_2, 0.1mg/kg BW s.c.), fitted with atrial Silastic tubing, and a guide cannula in the right lateral cerebroventricle stereotaxically. Rats in the long-term OVX group received the same treatment (E_2, atrial tubing and guide cannula implantation) three weeks later. Rats in both groups received progesterone (2 mg/rat s.c.) at 0930 h on the next day after E_2. At 1000 h, intraventricular administration of norepinephrine HCl (NE, 0.01, 0.1, or 1.0 μg in 2 μl saline) was given. In short-term OVX-steroids-primed rats, NE did not alter LH levels in the peripheral plasma within 60 or 100 min. By contrast, in long-term OVX-steroids-primed rats, 1.0 μg of NE gradually decreased plasma LH concentrations, which became significantly different from the initial value at the 60 min time point after treatment. On the other hand, intraventricular injection of 5 ng of the LH-releasing hormone (LHRH) elevated plasma LH concentrations within 10 min in both groups of rats, but at different efficacy: a brief release of LH in short-term OVX-steriods-primed rats and a prolonged release of LH in long-term OVX-steriods-primed rats. These results indicated that the interval after OVX plays a critical role in modulating the responsiveness to NE and LHRH in the steroids-primed OVX rats.

Key Words: norepinephrine, luteinizing hormone, luteinizing hormone-releasing hormone, ovariectomized-steroids-primed rat

Introduction

The control of the luteinizing hormone (LH) secretion is complicated. Fundamentally, the release of the LH is always associated with release of the hypothalamic LH-relasing hormone (LHRH) and modified by the feedback action of gonadal steroids, In intact cyclic rats, there is an LH surge release before ovulation. This LH surge release, however, can be induced in OVX rats treated with ovarian steroids. On the other hand, LHRH release depends on an integration of central excitatory and inhibitory neuronal singals (for reviews see 3, 21, 37).

As early as 1949, ovulation was found to be inhibited in spontaneously ovulating rats after treating with an alpha-adrenergic blocking agent, dibenamine, before the critical period on the day of proestrus (13). Dibenamine was also capable of inhibiting estrogen-induced (40) and progesterone-induced (14) ovulation in rats. The ability of this drug to block ovulation was ascribed to blockade a neurogenic stimulus involved in activation of the adenohypophysis to release the ovulating hormone. Subsequently, negative results on a prolonged treatment of dibenamine were reported (26). Before indentifying the LHRH, investigators have since focused on neurogenic properties or neurotransmitters involved in the regulatory process of LH secretion. Among the many systems which may affect the activity of the as then to be identified LHRH neurons, the adrenergic system has been most extensively studied in proestrous rats and in ovariectomized (OVX) or steroid-primed OVX rats

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(3,37). Several findings demonstrated that the central adrenergic network are involved in the regulatory mechanism of LH secretons. (1) The hypothalamic areas implicated in LHRH release were extensively innervated by noradrenergic fibres, which are near the brain ventricular regions, from the brainstem (23). (2) An increase of the NE content and the turnover rate of NE or epinephrine (E) in the hypothalamic nuclei and the median eminence were observed to precede the preovulatory LH surge or LH surge induced in steroid-primed OVX rats (10, 38, 42). (3) All NE synthesis inhibitors decreased the hypothalamic NE or E content and inhibited LH surge release as well (1, 9). (4) However, neither surgical interruption of the ascending adrenergic pathway affected ovulation (8) nor electrical stimulation the central adrenergic system in the locus coeruleus or the medullary A1 areas under anesthesia was reported to alter the plasma LH level (11, 17, 18). Furthermore, NE has been intraventricularly injected to explore the role of the central adrenergic system on LH release, and steroid could reverse the LH response to intraventricular NE in primed or unpriming rats, although the exact mechanism is not clear yet (16, 24).

Our previous studies indicated that the LH surge release can be induced on the expected diestrous day 2 (D2) afternoon in short-term OVX (S-OVX) steroids-primed rats (33). The pattern of LH surge induced by steroids is similar to that in the preovulatory LH surge. However, such steroids-induced LH surge can not be experimentally brought about in the forenoon. We considered that the endogenous trigger signal or signals for LHRH and LH secretion may not yet be available in the forenoon (34). Though progesterone facilitated LHRH release from the mediobasal hypothalamic tissues in vitro (12,20), but it can not be the trigger factor for an in vivo system.

The present study investigated whether NE, the most extensive studied agent with a facilitatory property on LH secretion, administered intraventricularly can induce LH surge release in S-OVX- and long-term OVX (L-OVX)- steroids-primed rats, Meanwhile administration of LHRH after the NE test in the same site to evaluate the viability of this intraventricular approach. Results of the study have been presented in the American Physiological Society-Chinese Physiological Society Joint Meeting (36).

Materials and Methods

Animal Preparation

Female Sprague-Dawley rats, weighing 220-250 g, provided by the National Yang-Ming University were maintained in a controlled environment (21±2°C, with lights on 0500-1900 h). Food and water were supplied ad libitum. Rats were monitored for their estrous cyclicity by daily vaginal lavage and those having 2 to 3 consecutive regular four-day cycles were used. All rats were OVX on D1 morning about 1000 h. Rats in the S-OVX group immediately received estradiol-17beta (E_2, 0.1 mg/kg BW in 1 ml olive oil s.c., Sigma), implanted atrial tubing (Silastic tube, 605-135, Dow-Corning Co.) for frequent blood sampling and guide cannula for drug injection. The guide cannula (gauge 23 containing a 30-gauge stylet) was stereotaxically implanted into the right lateral cerebroventricle with the following coordinates: 8.2 mm anterior, 1.5 mm lateral, and 3.7-3.8 mm ventral (31) under anesthesia with diethyl ether. The guide cannula was anchored with one screw attached to the cranium with dental cement. Rats in the L-OVX group received E_2, atrial tubing and guide cannula three weeks after OVX. Rats in both groups received progesterone (P_4, 2 mg/rat in 0.2 ml olive oil s.c., Sigma) at 0930 h on the next day after E_2. Both groups of rats were examined for the action of NE at 30 min (at 1000 h) after the treatment of P4. Rats in the control group received saline as vehicle.

Intracerbroventricular (ICV) Microinjection

For ICV microinjection of NE or vehicle, the stylet was removed and an inner injection cannula (gauge 30) connected via a polyethylene tube (PE 10) to a movably driven Hamiltion syringe was inserted into the lateral ventricle through the guide cannula. NE was freshly prepared in saline, and the pH was adjusted to 7.2-7.4 with NaOH (0.4 N, Merck Art. 6466) before use. 0.01, 0.1 or 1.0 μg of NE in 2 μl of saline was injected into the ventricle within one min.

In order to examine the viability of this ICV infusion regimen, 5 ng of LHRH in 2 μl saline was injected into the ventricle at the end of the NE test. Serial blood samples (0.3 ml each) were withdrawn through the indwelt atrial tubing at zero time, 10, 30, 60 or 100 (or 120) min after each ICV injection. Blood samples were centrifuged at 2000 rpm for 10 min at 4°C and plasma was separated and stored in freezer (-20°C) until RTA. Saline in a volume equal to that of the removed plasma was added to the blood cell portion, and injected back to rats before the next blood sampling. At the end of the experiments, fast green solution (1-2 μl, 1%) was injected intraventricularly to examine the position of the cannula of injection. For the subjects of which the lateral ventricle was hit, the third ventricle invariably turned to green seen from the ventral surface of brain tissue after perfusion with saline and formalin. The data from rats showing intraventricular bleeding due to cannula implantation, improper distribution of green

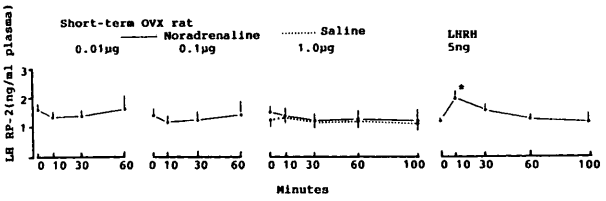

Fig. 1. Effects of ICV NE on plasma LH levels in short-term OVX-steroids-primed rats. Each point represents the mean with the standard error of the mean indicated by vertical bar. Rats were OVX on the diestrous day 1, treated with E_2 immediately, implanted atrial tubing and guide cannula in the right lateral ventricle. They received P_4 at 0930 h and NE at 1000 h next morning (the expected diestrous day 2, 5 rats in each group). LHRH was administered after the NE test. Per cent change of LH levels at 10 min time point after LHRH was significantly different from the zero time value (t=2.3697, p<0.05). LH levels in saline controls were drawn in the dotted line.

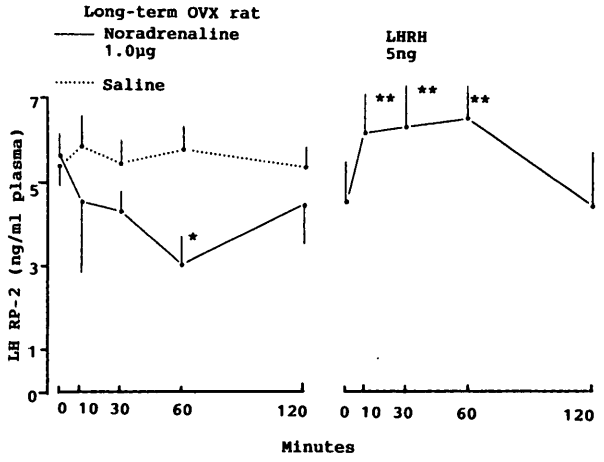

Fig. 2. Effects of ICV NE on plasma LH levels in long-term OVX-steroids-primed rats. Each point represents the mean with the standard error of the mean indicated by vertical bar. Rats were OVX three weeks ago, treated with E_2, implanted atrial tubing and guide cannula in the right lateral ventricle on the 21th day after OVX. They received P_4 at 0930 h and NE at 1000 h next morning (5 rats in each group). LH levels at the 60 time point after ICV NE were significantly different from the zero point (t =2.8964). LH levels at 10, 30, and 60 minutes after LHRH were significantly different from the zero point time value (t=3.4105, t=4.39 and t=4.38, respectively). LH levels of saline controls were drawn in the dotted line. (*p<0.05, **p<0.01)

dye in the ventricular space or lacking LH responses to LHRH testing were excluded from results.

LH Measurement

Plasma LH was measured by RIA with NIDDK rat-LH kits according to the procedure of Dr. A.F. Parlow. The LH value was expressed in terms of rLH-RP-2. Coefficients of variation of intraassay and interassay for LH values were 4.0% and 10%, respectively. Concentrations of the LH in plasma between the experimental group and controls were analyzed by Student t tests. P less than 0.05 was chosen for the level of significance.

Results

As shown in Fig. 1, the basal concentration of plasma LH in S-OVX steroids-primed rats was quite low. ICV administration of 0.01, 0.1 or 1.0 μg of NE in these rats did not affect their plasma LH levels within 60 or 100 min after the treatment, however, injecting 5 ng of LHRH brought up a short-period rise of plasma LH concentrations within 10 min after the treatment. The fractional elevation of LH levels at the ten min point compared with the value at the zero point in terms of changes in percentage was statistically significant (t=2.3697, p<0.05). The plasma LH concentrations in the saline controls were 1.2±0.2, 1.4±0.2, 1.3±0.2, 1.3±0.2 and 1.1±0.2 for the zero time, 10, 30, 60 and 100 min after infusion, respectively.

On the contrary, basal levels of plasma LH in L-OVX steroids-primed rats were considerable high. Administrating 1.0 μg of NE into the lateral ventricle caused a gradual decline of plasma LH concentrations and the LH level at the 60 min point was significantly lower than the LH concentration seen at the zero time (t=2.8964, p<0.05). Plasma LH levels recovered thereafter (Fig. 2). Plasma LH levels in the saline controls were 5.4±0.5, 5.9±0.6, 5.5±0.6, 5.8±0.5 and 5.4±0.5 for the zero time, 10, 30, 60 and 120 min after infusion, respectively. Intracerebroventricular injection of 5 ng of LHRH caused a sustained elevation in the concentration of plasma LH from the 10 min time point up to 60 min after the treatment. Plasma levels of LH returned to OVX basal levels about at 120 min after the treatment (Fig. 2). The LH response to LHRH as quantified by the area under the curve was significantly larger in L-OVX steroids-primed rats than that in S-OVX steroids-primed rats (t=64, p<0.001).

Discussion

In the present study, the influence of intraventricular NE on LH release was examined in both S-OVX- and L-OVX-steroids-primed rats. NE at dosages ranging from 0.01 μg up to 1.0 μg did not change plasma LH levels in S-OVX-steroids-primed rats, but decreased plasma LH levels in L-OVX-steroids-primed rats. Intraventricular injection of 5 ng of LHRH elicited LH release in both groups but the pattern of LH responses was different. Results suggested the number of days after OVX may alter the responsiveness of LH release to ICV injection of NE or LHRH in OVX rats received the identical steroid

priming.

In fact, S-OVX rats primed with steroids were ready to display a surge release of LH on D2 afternoon (33), but the effect can not be advanced from the afternoon to the forenoon (34). According to two in vitro experiments, P_4 facilitated LHRH release from the mediobasal hypothalamic tissue of estrogen-primed OVX rats after a brief incubation (12, 20). However, results from in vivo studies indicated that an endogenous trigger factor or factors other than P4 would be essential for a surge release of LH, which may not be available in the earlier light period. Earlier work indicated that LH release could be facilitated by activating the central adrenergic system (1, 2, 6, 9, 22, 28, 39). Specifically, previous studies demonstrated that administration of P_4 to estrogen-primed OVX rats produced a surge in plasma NE preceding pituitary release of LH (27) and most of NE came from the brain when arteriovenous differences in concentrations of NE in plasma across the brain was measured (28).

To identify the central action of a drug, ICV microinjection has been a commonly adopted approach (15). In this study, NE was administered 30 min after P4 to examine its influences on LH release. Third ventricle was chosen for ICV administration in most studies, but implantation of a guide cannula (gauge 22 or 23) and infusion of drugs into the third ventricle by themselves may cause considerable damage to the hypothalamus as this ventricle is no more than a slit-like cavity and thus results in uncontrollable effects. In terms of the present study, implantation of a guide cannula into the lateral cerebroventricle seems to be reasonable. Presumably the natural flow of the cerebrospinal fluid (CSF) would carry the injected solutions to the third ventricle where they could exert their effects at the hypothalamic neurons. The pH of NE solutions used in the study was adjusted to around 7.2 to 7.4 closed to physiological states, instead of acidified solution (4).

Concerning the dosage of NE used in the study, the NE level in the CSF fluid obtained from the cistern magnum is about 10 pg/μl CSF (Pi, Wang and Tsai, submitted to publish), as a result, 0.01 μg up to 1.0 μg of NE were used in trials and 1.0 μg of NE was physiologically concerned as a quite large dose to ascertain its function in the CSF space. Additionally, administration of LHRH was used to assess the general effectiveness of ICV administration and to correlate between the effects generated by the two experimental agents.

As shown in Fig. 1, after steroid primings, the basal levels of plasma LH at 1000 h on D2 in S-OVX rats stayed at a low average. By contrast, the ability of steroid treatments to suppress basal LH levels in L-OVX rats was abated. S-OVX rats were less responsive, whereas L-OVX rats were highly responsive to both NE and LHRH. All of these disparities can be interpreted as a result of the different interim after OVX. Ovariectomy results in LH hypersecretion with a time course of rise and changes of pituitary LH content, numbers of LHRH receptors on gonadotropes and neurotransmitters involved (7, 29, 30).

Numerous investigators proposed a stimulatory role of ICV NE in the third ventricle on LH release. Accordingly, 20 μg to 45 μg of NE were required to induce an LH surge release in proestrous rats to overcome the action of barbiturate (39) and in steroid-primed OVX rats (22), or to elevate LHRH levels in the hypophyseal portal blood and peripheral plasma LH (6) and to rise LH concentrations in steriod-primed OVX rats under chloral hydrate (19).

However, evidence for an inhibitory effect of NE on LH release is also available (41). Intraventricular administration of NE or other adrenergic agonists, phenylephrine and methoxamine, alpha-1-adrenergic agonists, and isoproternol, beta-adrenergic agonist, suppressed an on-going LH surge induced by steroids in OVX rats (4, 24).

To complicate the picture further, a stimulatory beta-adrenergic component of the preovulatory LH surge in proestrous rats was reported (2), and an inhibitory effect induced by electrical stimulation of the ascending noradrenergic pathway in the midbrain can be prevented by alpha-adrenergic receptor blocker, phenoxybenamine, but not by a beta-adrenergic blocker, propranolol (5). Therefore, the central adrenergic system appears to exert both stimulatory and inhibitory influences on the release of LH in proestrous rats or in OVX rats primed with steroids, but results to date have not been consistent and are very difficult to interpret.

From an anatomical point of view, after disruption of the central adrenergic pathway, no synapses were observed between tyrosine hydroxylase-immunopositive degenerated axons and LHRH-immunopositive perikaryon. The interneurons intercalated between the noradrenergic nerve endings and LHRH neurons are GABA-immunopositive neurons (23). This fact may explain why electrical stimulation of the central adrenergic system, such as locus coeruleus and the medullary A1 areas, did not affect plasma LH levels in anesthesized proestrus or steroids-primed OVX rats (11, 17, 18). Thus, it is suggested that the central adrenergic pathway may simply have a modulatory, instead of mandatory, role on the regulation of LH surge release.

In the present study, another possibility of the inhibitory action of NE in L-OVX group rats was that NE was administered 30 min after P_4. In a study of immatured OVX rats primed with steroids, plasma LH declined significantly in 30 min after P4 and

returned to control levels within the next 30 min (32). Thus the result of L-OVX steroids-primed rats may indicate either an interactive effect of P4 and NE on LH release or an inhibitory action of NE alone. On the other hand, the LH surge on D2 afternoon in S-OVX-steroids-primed rats, can be suppressed by both alpha-1- and alpha-2-adrenergic antagonists, prazosin and yohimbine, respectively, but potentiated by beta-adrenergic receptor blocker, propranolol (35). Ovariectomized rats primed with steroids would turn on the central adrenergic system in mediation of LH surge release through three different subtypes of adrenergic receptors: alpha-1- and alpha-2- facilitation and beta-inhibition, simultaneously (35). Further studies with appropriate designs to elucidate the precise role of NE on the regulation of LHRH and LH secretion are needed. Taken together, the present data indicated that the apparent responsiveness of OVX rats primed with steroids to NE infused into the cerebroventricle is modulated by the interval after OVX.

Acknowledgements

This research was supproted by grants from the National Science Council, Republic of China (NSC-76-0412-B002-30). We thank the NIDDK, U.S.A. for the rat LH kit and Dr. P.S. Wang for sheep anti-RGG.

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