Naltrexone-Induced LH Release in Ovariectomized Estrogen-Primed Rats

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Abstract

In order to investigate the physiological role of endogenous opioid substances in the regulation of gonadotropin secretion, we studied the effect of Naltrexone (Nalt), a morphine antagonist, on serum luteinizing hormone (LH) concentrations in ovariectomized estrogen-treated rats. The site of action of Nalt and its interaction with other putative neurotransmitters on LH secretion were investigated in hypothalamic deafferented rats and in animals treated with pharmacological inhibitors of the actions of neurotransmitter substances. Nalt injection increased serum LH levels at doses 0.08 to 2 mg/kg BW. The response was dose-dependent but higher doses of Nalt had less effect. In inducing this response pattern, mediobasal connection between the anterior hypothalamus and the mediobasal hypothalamus was essential, but posterior input to the mediobasal hypothalamus was not necessary. Excepting α -adrenergic blocker, all the blockers used, i.e. β -adrenergic, serotonin, dopamine and acetylcholine blockers were effective in eliminating the LH release evoked by Nalt injection. These results suggest that endogenous opioid substance might inhibit LH secretion tonically through aminergic and/or cholinergic neurons and that the mediobasal neural connections to the mediobasal hypothalamus are indispensable for this inhibition.

In 1955 Barraclough and Sawyer observed inhibition of ovulation by morphine injection in female rats, which was explained as a result of blocking the pituitary gonadotropin release by morphine. Their result was confirmed by the determination of serum gonadotropin concentrations using radioimmunoassay (Muraki *et al.*, 1977). The anti-ovulatory effect of morphine until recently has been attributed to its pharmacological action, like that of such anesthetics as pentobarbital. Since endogenous opioid substances and their receptors were discovered (Hughes *et al.*, 1975) and opioid substances were found to be abundant in the hypothalamus (Hong *et al.*, 1977)

it may be possible that these opioid substances are physiologically involved in the regulation of secretion of pituitary hormones including gonadotropins.

In order to investigate this regulatory aspect of endogenous opioid substances, we studied the effect of a specific antagonist of morphine, naltrexone on serum LH levels in ovariectomized estrogen-treated rats. Furthermore, we attempted to elucidate the site of action of the morphine antagonist using neural deafferentation (Halász and Pupp, 1965) and to clarify its interaction with other putative neurotransmitters reported to be involved in the gonadotropin regulation (Gnodde and Schuiling, 1976, Kawakami *et al.*, 1979; Sawyer, 1977), by using their receptor blockers.

Materials and Methods

Animals

Female Wistar rats (180–200 g BW) were housed in a light-controlled (light on 500–1700), air-conditioned room ($25 \pm 1^\circ\text{C}$) and fed pelleted food (MF, Oriental Kobo, Japan) and tap water *ad libitum*. They were ovariectomized under ether anesthesia and subjected to the brain operation at least one month after spaying.

Hypothalamic deafferentation was performed according to the method described by Halász and Pupp (5). A Halász knife with a 2.0 mm radius and 2.0 mm vertical blade (large knife) or one with a 1.2 mm radius and 1.2 mm vertical blade (small knife) was used. The animals were placed in a 2.5 mm nose down position into the holder of a stereotaxic instrument and the hypothalamus was subjected to the following types of deafferentation. Large anterior deafferentation I: large knife was rotated 180° at the level of the suprachiasmatic nucleus (guided by König and Klippel's rat brain atlas, 9). Anterior deafferentation II: large knife was rotated 180° at the level of posterior border of the anterior hypothalamic area. Small anterior deafferentation: small knife was rotated 180° at the same level as large anterior deafferentation II. Posterior deafferentation: large knife was rotated 180° at the level of mid-mammillary body. Sham operated animals: large knife was inserted down to the base of the skull in the midsagittal plane without being rotated. The operated animals were used for the Nalt experiment at least one month after surgery. All animals were injected with estradiol benzoate sc. (EB, 5 μg). An intraatrial cannula was implanted the next day and these rats were placed in individual cages. Two days after EB injection, the rats were injected with Nalt dissolved in physiological saline through the cannulae. At the termination of the experiment the brains were fixed and frozen sections were cut in the frontal plain at 60 μ and were examined histologically to determine the deafferentation sites.

Chemicals

Chemicals used in the experiment were obtained from the following sources: naltrexone (Endo Labs., Garden City, USA), phentolamine (Chiba Ltd., Basel, Switzerland), phenoxybenzamine (Tokyo Kasei Ind., Tokyo, Japan), propranolol (Sumitomo Chemical Ind., Osaka, Japan), pimoziide (Fujisawa Pharmaceutical Co., Osaka, Japan), atropine (Wako Pure Chemical Ind. Ltd., Osaka, Japan), methysergide (Sandoz Ltd., Basel, Switzerland), estradiol benzoate (Teikoku Hormone Mfg. Co., Kawasaki, Japan).

Estradiol benzoate was dissolved in sesame oil.

Pimoziide was dissolved in 0.1 M tartaric acid. The other reagents were dissolved in or diluted with physiological saline. These materials were injected through the cannulae except phenoxybenzamine which was administered intraperitoneally and atropine and estradiol benzoate which were administered subcutaneously.

Radioimmunoassay

Blood samples (0.5 ml) were taken through intra-atrial cannulae just before, and 10, 30, 60 min following Nalt injection. The experiment was performed before noon in order to avoid the spontaneous estrogen-induced afternoon elevation of serum LH. Separated serum was stored at -20°C until assayed. Serum LH concentration was determined by double antibody radioimmunoassay (Niswender *et al.*, 1968) using NIAMDD rat LH radioimmunoassay kit and anti-ovine LH serum (R-15, a generous gift from Dr. G. D. Niswender, Colorado State University, USA). LH levels were expressed in terms of NIH-LH-S1. Data were analysed by one way analysis of variance.

Results

Effect of Nalt on serum LH levels

Intravenous injection of Nalt in doses of 0.08 to 10 mg/kg BW induced a significant ($p < 0.01$ or 0.05) increase in serum LH levels in ovariectomized estrogen-treated rats (Table 1). The amount of LH released within 60 min after Nalt injection was estimated by the values summing ΔLH at 10, 30 and 60 min after Nalt injection. It was increased with increasing doses of Nalt injected up to 2 mg/kg BW, but the increment was declined by further increasing the doses of Nalt. Moreover, when a larger dose of Nalt was injected, for example 50 mg/kg BW, the serum LH concentration did not continue to increase.

LH release by Nalt in hypothalamic deafferentated rats

As shown in Table 2 serum LH levels increased following Nalt (2 mg/kg BW) injection in sham operated rats and in those with posterior deafferentation. However, the rats bearing anterior deafferentation II

Table 1. Dose response relationship between injected naltrexone and released LH

Naltrexone (mg/kg BW)	Serum LH (ng/mg)				Significance	Cumulative Δ LH
	Time after naltrexone injection (min)					
	0	10	30	60		
0	2.8±0.7 ^a	3.0±0.9	2.9±0.5	2.7±0.	N.S. ^b	
3.2×10 ⁻³	3.2±1.4	5.1±1.5	4.0±1.0	2.4±0.8	N.S.	1.9±0.4 ^c
1.6×10 ⁻²	2.9±0.5	5.2±0.8	4.7±1.1	3.6±0.6	N.S.	4.8±0.8
8 ×10 ⁻²	2.7±0.3	10.5±2.5	5.2±0.8	3.9±0.8	p<0.01	11.5±3.1
4 ×10 ⁻¹	3.1±0.7	10.6±0.8	6.6±1.1	6.4±0.9	<0.01	14.3±3.2
2	3.1±0.6	11.0±1.1	6.8±0.9	7.4±1.4	<0.01	15.9±4.1
10	1.8±0.6	7.1±1.9	6.9±0.1	4.4±1.5	<0.05	13.0±2.9
50	3.2±1.1	3.3±1.1	6.8±2.7	5.9±2.9	N.S.	6.3±2.0

a: Mean ± standard error of means (n=5)

b: Analysed by one way analysis of variance

c: Estimated amount of LH released in 60 min

Table 2. Naltrexone-induced (2 mg/kg BW) LH release in rats bearing hypothalamic deafferentation

Kind of deafferentation	No. of rats	Serum LH (ng/ml)				Significance
		Time after naltrexone injection (min)				
		0	10	30	60	
Anterior I	5	0.7±0.3 ^a	8.0±2.2	4.8±1.0	3.7±0.9	p<0.05 ^c
Anterior II	6	<0.3 ^b	<0.3	<0.3	<0.3	N.S.
Small Anterior	6	0.4±0.1	1.2±0.6	0.7±0.4	0.8±0.3	N.S.
Posterior	7	2.6±0.4	9.2±2.1	8.6±3.0	6.0±1.9	<0.05
Sham	6	2.9±0.5	8.5±2.0	7.8±1.1	5.7±0.7	<0.05

a: Mean ± standard error of means

b: Below the sensitivity limit of LH radioimmunoassay

c: Analysed by one way analysis of variance

or small anterior deafferentation did not respond to Nalt injection with an increase in serum concentrations. Nalt evoked LH release in the rats with anterior deafferentation I comparable to that seen in controls.

LH release by Nalt in rats treated with blockers of neurotransmitters (Table 3)

Ovariectomized estrogen-treated rats were injected with receptor blockers of putative neurotransmitters 1 to 2 hrs before Nalt injection. In animals treated with α -adrenergic blockers (phentolamine, 10 mg/kg BW, phenoxybenzamine, 10 mg/kg BW)

Nalt injection (2 mg/kg BW) induced a significant (p<0.05) increase in serum LH concentrations, though smaller than in saline treated controls. Other receptor blockers; propranolol (β -adrenergic blocker, 5 mg/kg BW), pimozide (dopamine receptor blocker, 1 mg/kg BW), methysergide (serotonin receptor blocker, 10 mg/kg BW) and atropine (acetylcholine receptor blocker, 500 mg/kg BW), were effective in blocking the LH release induced by Nalt injection.

Table 3. Naltrexone-induced (2 mg/kg BW) LH release in rats treated with the receptor blockers of putative neurotransmitters

Receptor blockers	Serum LH (ng/ml)				Significance
	Time after naltrexone injection (min)				
	0	10	30	60	
Phentolamine	1.6±0.2 ^a	4.0±0.6	3.2±0.6	3.0±0.4	p<0.05 ^b
Phenoxybenzamine	1.7±0.2	4.8±1.2	4.4±0.7	2.7±0.4	<0.05
Propranolol	1.8±0.3	1.9±0.3	1.9±0.2	2.4±0.8	N.S.
Pimozide	1.5±0.4	3.7±0.6	1.7±0.3	1.8±0.3	N.S.
Atropine	1.8±0.2	2.1±0.3	3.4±0.5	1.9±0.4	N.S.
Methysergide	1.9±0.3	2.2±0.5	2.1±0.2	1.9±0.2	N.S.
Control	2.5±0.4	11.5±3.1	7.1±2.1	5.1±1.1	<0.01

a: Mean ± standard error of means (n=6)

b: Analyzed by one way analysis of variance

Discussion

Observations made in this study confirmed the stimulatory effect of opioid antagonist on LH release in estrogen-treated ovariectomized rats as reported in human males (Mendelson *et al.*, 1978), male rats (Bruni *et al.*, 1977) and infantile female rats (Blank *et al.*, 1979; Ieiri *et al.*, 1979). The opioid antagonist, naloxone, however, failed to facilitate LH secretion in adult female rats at any stage of the estrous cycle (Blank *et al.*, 1979). The reason why opioid antagonist can induce LH release in ovariectomized estrogen-treated rats or intact males, but not in intact female rats, is not clear. Estrogen treatment blocks the LH response to naloxone in prepubertal female rats (Blank *et al.*, 1979), whereas estrogen in adult ovariectomized rats in this study sensitized them to respond to naltrexone. Developmental changes in the brain or gonads may explain these differences. Further study is needed to clarify these points.

Morphine is known to inhibit proestrous LH surge and ovulation (Barraclough and Sawyer, 1955; Muraki *et al.*, 1977). This inhibitory effect of morphine appears to be due to its suppressing action on LH-RH release through endogenous opioid receptors. In addition to these results, present observa-

tions indicate that endogenous opioid substance tonically suppresses LH release and that Nalt antagonizes the opioid substance to raise serum LH levels. The dose response relationship between the amount of LH released and the amount of Nalt injected was not simple. The released amount and peak level of LH after Nalt injection increased dose-dependently up to 2 mg/kg BW but declined thereafter. A higher doses of Nalt may have other effects than blocking opioid receptors. We observed seizure activity lasting several seconds in rats injected with 50 mg/kg BW Nalt, which may be related to the inability of large doses of Nalt to increase in serum LH concentrations.

From the results obtained in the rats with partial neural deafferentation of the hypothalamus, Nalt-induced LH release does not occur following anterior deafferentation II or small anterior deafferentation but occurs following anterior I or posterior deafferentation. Posterior afferents to the mediobasal hypothalamus therefore seem to be unnecessary for the LH release by Nalt, but the neural connection between the anterior hypothalamus and the mediobasal region is indispensable. Since the small anterior cut was as effective as anterior deafferentation II in blocking Nalt-induced LH release, the indispensable neural connection to the mediobasal hypothalamus for the LH

release may lie in the medial basal part of this area. Since anterior deafferentation II reduced LH concentrations in serum and pituitary and LH responsiveness to LH-RH stimulation (Table 2 and unpublished data), and that immunohistochemical study revealed LH-RH perikarya in the preoptic area and the septum (Barry *et al.*, 1973), these cuts may interrupt the axons of the LH-RH neurons, having their perikarya anterior to the deafferentation II. The opioid antagonist might act at the level of the anterior hypothalamus or the posterior part of the medial preoptic area. And after neural deafferentation between the anterior and the mediobasal hypothalamus, the stimulatory input for LH-RH release induced by the opioid antagonist was unable to reach the mediobasal hypothalamus any more. But there is a possibility that Nalt might act at the mediobasal hypothalamus to induce LH-RH release without a consequent LH rise because of the lower responsiveness of the pituitary to LH-RH or lower releasable content of LH-RH in the mediobasal hypothalamus due to anterior deafferentation (Kalra *et al.*, 1977).

Several putative neurotransmitters are thought to be involved in the regulation of LH secretion. The receptor blockers of these neurotransmitters were reported to suppress the elevated serum LH in ovariectomized rats (Gnodde and Schuiling, 1976) and to inhibit LH release induced by sex steroid hormones in ovariectomized rats (Kawakami *et al.*, 1979). In our study all the blockers, except α -adrenergic blocker, eliminated the facilitating effect of Nalt on the release of LH. Since endogenous opioid substances were reported to influence the release or metabolism of noradrenaline (Taube *et al.*, 1976), dopamine (Subramanian *et al.*, 1977; Biggio *et al.*, 1978), serotonin (Van Loon and De Souza, 1978) and acetylcholine (Subramanian *et al.*, 1977; Van Loon and De Souza, 1978; Moroni *et al.*, 1977), it seems possible that

endogenous opioid substances exert their inhibitory effect on LH secretion via these aminergic or cholinergic neurons.

In summary our results suggest that endogenous opioid substances tonically inhibit LH release via aminergic and/or cholinergic neurons and that the anterior-mediobasal neural connections to the mediobasal hypothalamus are indispensable for this inhibition.

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