Modulation of Luteinizing Hormone(LH) Release by Clonidine and Opioid Peptides in Castrated Male Rats

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Abstract

The effect of clonidine, a central α -adrenergic agonist, on the suppression of LH release induced by β -endorphin or FK33-824, an endogenous opioid peptide or its synthetic analog, was investigated in castrated male rats, with or without pretreatment with reserpine. Pulsatile LH secretion was inhibited by intravenous injection of FK33-824 (400 μ g/kg), or intraventricular injection of β -endorphin (5 μ g). Without pretreatment with reserpine, intraperitoneal administration of clonidine (1 mg/kg) failed to reverse the inhibition of LH release induced by these peptides. However, with pretreatment with reserpine (10 mg/kg), clonidine abolished the inhibitory effect on LH secretion induced by these peptides in castrated male rats. These data indicate that, unlike the results in ovariectomized, steroid-primed rats, pretreatment with reserpine allows the α -adrenergic system to act more peripherally than the opioid neuronal system in a neuronal network-regulating LH release in castrated male rats.

In order to elucidate the regulatory mechanism of pulsatile LH release, various studies have explored the possible involvement of norepinephrine, opioid peptides and other putative neurotransmitters. Accumulating evidence suggests that opioid peptides inhibit LH secretion (Barraclough and Sawyer, 1955; Pang *et al.*, 1977; Bruni *et al.*, 1977; Ieiri *et al.*, 1980), whereas norepinephrine exerts a stimulatory effect (Karla *et al.*, 1972; Krieg and Sawyer, 1976; Gallo, 1980). Opioid peptides are known to affect the

activities of catecholaminergic neurons of the brain (Starke *et al.*, 1977; Meites *et al.*, 1979). In addition to such functional interaction, histochemical studies have revealed a close anatomical interaction between norepinephrine and opioid peptides (Atweh and Kuhar, 1977; Ungerstedt, 1971). However, there are only a few reports dealing with the relationship between norepinephrine and opioid peptides in the regulation of pulsatile LH release, in which ovariectomized, steroid-primed rats are used (Karla and Simpkins, 1981; Karla and Gallo, 1983). Therefore, in order to gain further insight in modula-

tion of LH release by opioid peptides and norepinephrine, we have studied the effect of clonidine on the inhibition of LH release induced by opioid peptides in castrated male rats.

Materials and Methods

Twenty-eight male Wistar rats, weighing 200–250 g, were castrated and kept under conditions of controlled temperature ($25 \pm 1^\circ\text{C}$) and light (12 h light, 12 h dark). Food and water were provided *ad libitum*. More than 4 weeks after castration, a silastic cannula was inserted through the jugular vein into the right atrium, as described previously (Kinoshita *et al.*, 1980). A stainless steel cannula (23 gauge), was stereotactically inserted at a point 1.5 mm lateral to the sagittal suture, 1 mm posterior to the bregma. Several days later, animals were subjected to experiments in conscious, freely moving states.

0.5 ml blood samples were withdrawn from the silastic cannula at 10 min intervals for 1 h before, and 2 h after the initial injection of β -endorphin or FK33-824. An equivalent volume of washed red cells suspended in saline was replaced after each bleeding. After centrifugation, plasma was stored at -20°C until assayed.

FK33-824 ($\{\text{D-Ala}^2, \text{MePhe}^4, \text{Met}(\text{O})^5\text{-ol}\}$ enkephalin, Sadoz, Basel), a potent Met^5 -enkephalin analog (Roemer *et al.*, 1977), and β -endorphin were dissolved in physiological saline at a con-

centration of 4 mg/10 ml and 5 $\mu\text{g}/5 \mu\text{l}$, respectively. Reserpine (Sigma Chemical Co., St. Louis, MO) was dissolved in a few drops of glacial acetic acid and diluted with 5% glucose solution to give a concentration of 1 mg/ml (pH 4). Clonidine hydrochloride (Sigma Chemical Co., St. Louis, MO), a central α -adrenergic agonist, was dissolved in physiological saline at a concentration of 1 mg/10 ml.

The castrated rats were divided into 7 groups. The 1st and 2nd groups were given β -endorphin iv through the stainless steel cannula at a dose of 5 μg twice at 40 min intervals, in combination with clonidine (1 mg/kg, ip) or saline. The 3rd and 4th groups received FK33-824 iv through a silastic cannula at a dose of 400 $\mu\text{g}/\text{kg}$ twice at 40 min intervals, in combination with clonidine or saline. The 5th group was treated with reserpine (10 mg/kg, ip) 24 h before the bleedings, and injected with clonidine (1 mg/kg, ip). The 6th and 7th groups pretreated with reserpine were given clonidine in combination with FK33-824 or β -endorphin.

Plasma LH concentrations were measured by double antibody radioimmunoassay using the kit provided by the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD), the rat Pituitary Hormone Distribution Program. As a reference standard, NIAMDD-rat-RPI was used. Intra- and inter-assay coefficients of variation were within 8% and 15%, respectively. Paired or unpaired *t*-test was used for analysis of the data.

Fig. 1. Effect of repeated intracerebroventricular injections of β -endorphin (β -EP, 5 μg each time) on plasma LH levels in castrated male rats.

Results

Figures 1 and 2 illustrate plasma LH levels before and after treatment with β -endorphin and FK33-824, respectively. All animals before the injection of β -endorphin or FK33-824 showed elevated LH release characteristic of castrated rats. Administration of β -endorphin icv or FK33-824 iv promptly lowered plasma LH levels in all animals tested. The mean plasma LH levels

during the 70 min period after the injection of β -endorphin or FK33-824 were significantly lower than that before the injection ($p < 0.05$).

Figure 3 shows the result of intraperitoneal injection of clonidine in combination with β -endorphin. The extent and duration of LH suppression were not significantly different from those induced by a combination of β -endorphin and saline (Fig. 1 vs Fig. 3). Similar results were obtained with FK33-824, as shown in Fig. 4. The extent

Fig. 2. Effect of repeated intravenous injections of FK33-824 (400 μ g/kg each time) on plasma LH levels in castrated male rats.

Fig. 3. Effect of intraperitoneal injection of clonidine (CL, 1 mg/kg) on the suppression of LH release induced by β -endorphin (β -EP, 5 μ g twice).

and duration of LH inhibition after the injection of FK33-824 and clonidine were almost the same as those induced by FK33-824 and saline (Fig. 2 vs Fig. 4).

As shown in Fig. 5, pretreatment with reserpine suppressed elevated LH levels characteristic of castrated rats. Clonidine induced marked elevations of LH levels within 30 min in reserpinized rats. The peak plasma LH value after the clonidine injection was significantly higher than the mean preinjection LH value ($p < 0.05$), and was not significantly different from that induced by clonidine alone (Fig. 6 or 7 vs Fig. 5).

tion LH value ($p < 0.05$). In such reserpinized rats, the concurrent ip injection of clonidine with the administration of β -endorphin icv (Fig. 6) or FK33-824 iv (Fig. 7) resulted in a rise in plasma LH levels within 30 min. Each peak plasma LH value was significantly higher than the mean preinjection LH value ($p < 0.05$), and was not significantly different from that induced by clonidine alone (Fig. 6 or 7 vs Fig. 5).

Fig. 4. Effect of intraperitoneal injection of clonidine (CL, 1 mg/kg) on the suppression of LH release induced by FK33-824 (400 µg/kg twice).

Fig. 5. Effect of intraperitoneal injection of clonidine (CL, 1 mg/kg) in castrated male rats pretreated with reserpine (10 mg/kg, ip).

Fig. 6. Effect of the concurrent ip injection of clonidine (CL, 1 mg/kg) with the icv administration of β -endorphin (β -EP, 5 μ g twice) in castrated male rats pretreated with reserpine (10 mg/kg, ip).

Fig. 7. Effect of the concurrent ip injection of clonidine (CL, 1 mg/kg) with the iv administration of FK33-824 (400 μ g/kg twice) in castrated male rats pretreated with reserpine (10 mg/kg, ip).

Discussion

In the present study, we show that the administration of reserpine, β -endorphin or FK33-824 suppresses episodic LH release characteristic of castrated male rats in agreement with earlier reports (Kinoshita *et al.*, 1980; Kato *et al.*, 1982; Kinoshita *et al.*, 1981).

We find that the intraperitoneal administration of clonidine, a central α -adrenergic agonist, fails to reverse the inhibition of LH release induced by opioid peptides in castrated male rats. Our results disagree with the earlier reports by Karla and Simpkins (1981), and by Karla and Gallo (1983); the former states that clonidine ip raises plasma LH levels in morphine-treated, ovariectomized, estrogen-progesterone-primed rats, and

latter that intraventricular infusion of norepinephrine readily elicits well defined episodes of LH hypersecretion in morphine-treated, ovariectomized, estrogen-progesterone-primed rats. A possible explanation for the discrepancy between their results and ours is that our experimental design is different from theirs. It could be speculated that the relationship between norepinephrine and opioid peptides in the regulation of pulsatile LH release in castrated male rats is different from that in ovariectomized, estrogen-progesterone-primed rats.

We also observe that clonidine abolishes the inhibitory effect of opioid peptides on LH secretion in castrated male rats pretreated with reserpine. Such an apparently discordant action of clonidine on LH release, although depending on the pretreatment with reserpine, is difficult to comprehend. It is known that clonidine can stimulate both postsynaptic α_1 -receptor and presynaptic α_2 -receptor, and that activation of α_2 -receptor results in a decrease in the amount of norepinephrine released and a reduction in the firing rate of noradrenergic neurons (Svensson *et al.*, 1975). Moreover it is also reported that the affinity of clonidine for α_1 -receptor is much weaker than its affinity for α_2 -receptor (Haga and Haga, 1980). Therefore, one explanation is that pretreatment with reserpine, which can deplete brain norepinephrine levels, might diminish the inhibitory effect mediated via presynaptic α_2 -receptor, thus promoting the action of clonidine on postsynaptic α_1 -receptor and resulting in the stimulation of LH release. At the same time, without pretreatment with reserpine, clonidine can activate α_2 -receptor, thus resulting in an auto-feedback regulation of norepinephrine and a failure to stimulate LH release.

However, in the brain, reserpine is also able to deplete dopamine and 5-hydroxytryptamine which are known to suppress pulsatile LH release (Drouva and Gallo, 1977; Gallo and Moberg, 1977). Another

explanation is that without pretreatment with reserpine, the administration of clonidine could activate the noradrenergic, dopaminergic and serotonergic neuronal system, and fail to stimulate LH secretion.

In summary, unlike results in ovariectomized, steroid-primed rats, pretreatment with reserpine may allow the α -adrenergic system to act more peripherally than the opioid neuronal system in a neuronal network regulating LH release in castrated male rats.

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