PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 168, 282–285 (1981)

Inhibition by Naloxone of Prolactin Release Induced by L-5-Hydroxytryptophan in Rats[1] (41273)

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Abstract. Intravenous injection of L-5-hydroxytryptophan (L-5-HTP), but not D-5-HTP, resulted in an increase in plasma prolactin (PRL) levels in anesthetized rats. L-5-HTP-induced PRL release was blunted by naloxone, a specific opiate antagonist. In rats pretreated with either reserpine or α-methyl-*p*-tyrosine (α-MT), basal plasma PRL levels were elevated and L-5-HTP injection caused a further increase in plasma PRL concentrations. However, PRL release induced by L-5-HTP was not blunted by naloxone in these animals pretreated with reserpine and α-MT. These results suggest that PRL release induced by serotonin is modulated, at least in part, by opioid peptidergic mechanisms, which are closely related to brain catecholamines.

It is well known that the secretion of prolactin (PRL) from the anterior pituitary gland is mainly under the tonic inhibitory control of the hypothalamus and that dopamine is at least a major PRL-inhibiting factor (PIF) (1, 2). On the other hand, PRL secretion is stimulated by many substances found in the hypothalamus, which include serotonin (3) and such neuropeptides as TRH (4), VIP (5, 6) and opioid peptides (7, 8). TRH and VIP stimulate PRL release from pituitary cells *in vitro*, whereas serotonin and opioid peptides have no direct stimulatory effect on the pituitary.

It has been reported that PRL secretion induced by opioid peptides may be mediated by hypothalamic dopaminergic neurons (9, 10). It is also suggested that opioid peptides exert their PRL-releasing effect via mediation of the brain serotonin system (11, 12). In the present experiments, we further studied the interaction of serotonin with opioid peptides in regulating PRL secretion in the rat.

Materials and Methods. Male Wistar strain rats weighing 200–220 g (Japan Animal Co., Osaka) were used throughout the experiments. They were maintained in a temperature-controlled room on a 12-hr dark:12-hr light schedule (lights on 0600–1800 hr). Laboratory chow (Oriental Yeast Co., Tokyo) and tap water were given *ad libitum*.

After overnight fasting, the rats were anesthetized with urethane (150 mg/100 g body wt, ip). Test substances were injected intravenously and blood samples of 0.6 ml were withdrawn from the jugular vein immediately before and 10, 20, and 40 min after the injection as previously described (13). Plasma samples were promptly separated and kept at -20° until assayed.

5-Hydroxytryptophan (5-HTP) and naloxone (Endo Laboratories, Garden City, N.Y.) were dissolved in physiological saline. Reserpine was first dissolved in a few drops of chloroform and then diluted in physiological saline. α-Methyl-*p*-tyrosine (α-MT) was dissolved in 0.5 *N* NaOH and then the pH of the solution was brought to 9.0 with 0.5 *N* HCl. Reserpine (0.3 mg/100 g body wt) was given intraperitoneally 14 hr before the experiment. α-MT (10 mg/100 g body wt) was administered intraperitoneally three times: 14, 9, and 5 hr before the experiment.

Plasma PRL levels were measured by radioimmunoassay as previously described (5). NIAMDD rat PRL-RP-1 was used as a standard. Duncan's new multiple-range test was used for statistical evaluation.

Results. Intravenous injection of L-5-HTP (1 and 5 mg/100 g body wt) caused a

[1] Supported in part by grants from the Ministry of Education and the Ministry of Health and Welfare, Japan.

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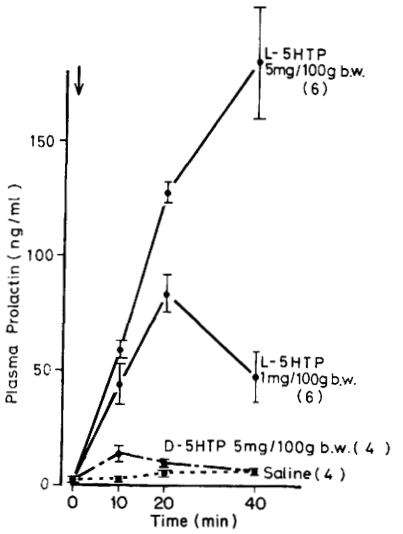

FIG. 1. Effects of L-5-HTP (1–5 mg/100 g body wt, iv) and D-5-HTP (5 mg/100 g body wt, iv) on plasma PRL levels in urethane-anesthetized rats. Means ± SE are shown. Numbers of animals in each group are shown in parentheses. Saline was injected in control rats.

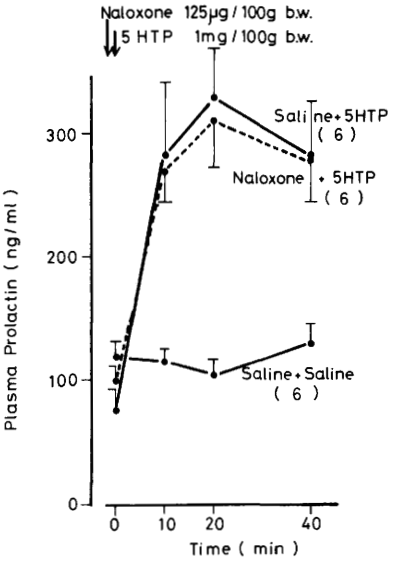

FIG. 3. Effect of naloxone (125 μg/100 g body wt, iv) on PRL release induced by L-5-HTP (1 mg/100 g body wt, iv) in rats pretreated with reserpine (0.3 mg/100 g body wt, ip). Reserpine was injected 14 hr before the experiments. Means ± SE are shown.

dose-related increase in plasma PRL levels in urethane-anesthetized male rats (Fig. 1). D-5-HTP (5 mg/100 g body wt, iv) did not change plasma PRL levels. PRL release induced by L-5-HTP was significantly suppressed by naloxone (125 μg/100 g body wt, iv), a specific opiate antagonist (14), which was injected 3 min before the injection of L-5-HTP (Fig. 2).

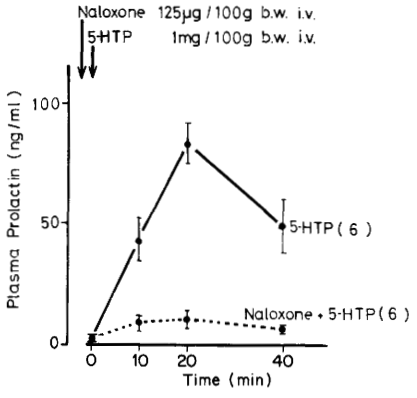

FIG. 2. Effect of naloxone (125 μg/100 g body wt, iv) on PRL release induced by L-5-HTP (1 mg/100 g body wt, iv) in the rat. Means ± SE are shown.

Pretreatment with reserpine or α-MT raised basal plasma PRL levels. Intravenous injection of L-5-HTP (1 mg/100 g body wt) further increased plasma PRL levels in these animals. However, naloxone did not inhibit PRL release induced by L-5-HTP in the rats pretreated with either reserpine or α-MT (Figs. 3 and 4).

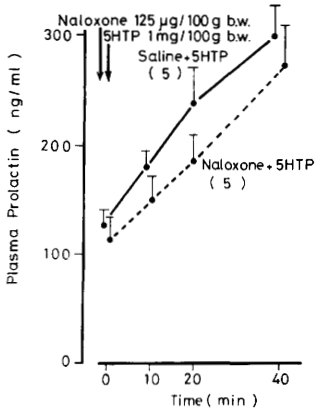

FIG. 4. Effect of naloxone (125 μg/100 g body wt, iv) on plasma PRL increase induced by L-5-HTP (1 mg/100 g body wt, iv) in rats pretreated by α-MT. α-MT (30 mg/100 g body wt, ip) was injected in three divided doses: 14, 9, and 5 hr before the experiments.

Discussion. The administration of 5-HTP, a precursor of serotonin, stimulates PRL secretion possibly by stimulating the serotonergic mechanism in the central nervous system in rats (15, 16) as well as in man (17). It has been considered that the action of serotonin is mediated by a PRL-releasing factor (15). The present study supports this hypothesis, since 5-HTP stimulated PRL release in rats even after the pretreatment with reserpine or α-MT, which is known to deplete brain dopamine, an important PIF.

We found also that 5-HTP-induced PRL release is inhibited by naloxone, a specific opiate antagonist. The inhibiting effect of naloxone on PRL release induced by 5-HTP was abolished in rats pretreated with reserpine or α-MT. It has been reported that opioid peptides stimulate PRL secretion (7, 18) and that they decrease the turnover of dopamine in the rat median eminence (9, 10). More recently, it was demonstrated that dopamine release from the rat median eminence into the hypophysial portal vessel was inhibited by opioid peptides (19). Therefore, opioid peptides may stimulate PRL secretion by inhibiting dopamine release from the median eminence. Naloxone may inhibit 5-HTP-induced PRL release by blocking the inhibitory action of endogenous opioid peptides on dopamine secretion, since dopamine is known to inhibit PRL secretion induced by 5-HTP (16). This may be an explanation for the failure of naloxone to influence 5-HTP-induced PRL release in rats pretreated with reserpine or α-MT.

On the other hand, PRL release induced by morphine or opioid peptides is antagonized by antiserotonergic agents in the rat (11, 12). Opioid peptides increase the turnover of brain serotonin (20). These findings suggest that opioid peptides have a stimulatory effect on PRL release by enhancing the serotonergic activity in the hypothalamus. Our findings suggest that the serotonergic mechanism might influence the opioid peptidergic mechanism.

These data could be explained by postulating that serotonin stimulates PRL release in the normal animal by increasing the activity of opioid systems which in turn inhibit dopamine thereby relieving PRL release from inhibition. On the other hand, in the absence of dopaminergic inhibitory tone serotonin appears to stimulate PRL-releasing factor discharge by a nonopioid dopaminergic mechanism. It is possible, therefore, that interrelation among serotonin, dopamine, and opioid peptides in regulating PRL secretion is more complicated than originally thought. Further studies are required to elucidate the precise mechanism.

We are indebted to the NIAMDD and Endo Laboratories for the gift of the rat PRL radioimmunoassay kit and naloxone, respectively.

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Received April 29, 1981. P.S.E.B.M. 1981, Vol. 168.