Effect of Hypothalamic Surgery on Prolactin Release Induced by 5-Hydroxytryptophan (5-HTP) in Rats*

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Synopsis

Intravenous injections of varying doses of 5-HTP (1, 3 and 5 mg/100 g body wt), a precursor of serotonin, caused a significant and dose-related increase in plasma prolactin concentrations in urethane-anesthetized rats. Increases in plasma prolactin concentrations caused by 5-HTP (1 mg/100 g body wt iv) were abolished by the concomitant administration of L-DOPA (2 mg/100 g body wt iv). Plasma prolactin levels were also significantly elevated following the injection of 5-HTP in rats with complete hypothalamic deafferentation, whereas 5-HTP had no significant effect on plasma prolactin levels in rats with extensive hypothalamic ablation. These results suggest that 5-HTP causes prolactin secretion by stimulating the serotoninergic mechanism in the hypothalamus.

Prolactin secretion from the pituitary is regulated predominantly by tonic inhibition, possibly via hypothalamic prolactin-inhibiting factor (PIF) (Meites et al., 1972). It has also been reported that extracts of the hypothalamus stimulate prolactin release (Dular et al., 1974; Valverde et al., 1972), suggesting the presence of prolactin-releasing factor (PRF) in the hypothalamus. The hypothalamus also contains serotonin in high concentration as well as other biogenic amines (Dahlström and Fuxe, 1964).

Recently, it was demonstrated that the systemic administration of L-tryptophan, a precursor of 5-hydroxytryptophan (5-HTP), or 5-HTP, a precursor of serotonin, raised plasma prolactin levels both in rats (Lu and Meites, 1973) and in man (Kato et al., 1974c; MacIndoe and Turkington, 1973). The intraventricular injection of serotonin also stimulates prolactin release (Kamberi et al., 1971a). These results suggest that a serotoninergic mechanism is involved in prolactin secretion, although the exact mechanism responsible for serotonin-induced prolactin release remains to be clarified.

In the present study, we investigated the effect of 5-HTP on plasma prolactin concentrations in rats with the hypothalamus deafferentated or ablated, in order to elucidate the site of action of the serotoninergic mechanism in regulating prolactin secretion.

Materials and Methods

Animals

Male Wistar rats weighing 180–220 g were housed for 1 week before the experiment under standardized conditions of temperature (22 ± 2°C) and light (7:00–21:00), and were maintained on Oriental Laboratory
Chow (Oriental Yeast Co., Tokyo) and water ad lib. In some experiments hypothalamic surgery was performed two weeks before the experiment as described below.

Experimental procedure

After an overnight fast the animals were anesthetized with urethane (150mg/100g body wt ip). Thirty min later the test materials were injected into an exposed jugular vein in groups of 4 to 6 rats.

In the first experiment, 5-HTP (1, 3 or 5mg/100g body wt) dissolved in physiological saline was injected into a jugular vein as a bolus in a volume of 0.1ml per 100g body wt. Controls were injected with physiological saline only. In the second experiment, L-3, 4-dihydroxyphenylalanine (l-DOPA, 2mg/100g body wt iv) was administered immediately before the injection of 5-HTP (1mg/100g body wt iv). l-DOPA was first dissolved in a warm solution of 0.5 N HC1, to which 0.5 N NaOH was added to bring the pH to 2.8 (Lu and Meites, 1971). In the third experiment, 5-HTP (1mg/100g body wt iv) or physiological saline was injected in rats with complete hypothalamic deafferentation. In the fourth experiment, 5-HTP (1mg/100g body wt iv) or chlorpromazine (200μg/100g body wt iv) was injected in rats with extensive hypothalamic destruction.

Immediately before the injection of test materials or vehicle and at intervals of 10 to 20 min thereafter, blood samples of 0.6ml were withdrawn from a jugular vein as described previously (Kato et al., 1973). Plasma was promptly separated and stored at −20°C until assayed.

Radioimmunoassay of plasma prolactin

Plasma prolactin concentrations were determined in duplicate by specific radioimmunoassay with the kit supplied by the Rat Pituitary Hormone Program, NIAMDD, as described previously (Kato et al, 1974a). NIAMDD Rat Prol-I-1 was iodinated with 125I by either the chloramine-T method (Greenwood et al., 1963) or the lactoperoxidase method (Thorell and Johanson, 1971). NIAMDD Rat Prolactin-RP-1 was used as the standard. The minimum detectable concentration of plasma prolactin was 1ng/ml. The coefficient of variation between assays averaged 9.5%. Statistical analysis was performed by either Student's t test or Duncan's multiple range test (Duncan, 1957).

Hypothalamic surgery

All nervous pathways to the hypophysiotropic area were interrupted stereotaxically by a modification of the method described by Halász and Pupp (1965), using a bayonet-shaped knife (vertical 1.5 mm, radius 1.5 mm). The knife was lowered with the tip placed anteriorly through the midline to the base of the brain, 1.5 mm posterior to the bregma at an angle of 10° posterior to the vertical plane. It was rotated 90° to the left, moved posteriorly 2.0 mm, rotated 180° to the left, moved anteriorly 2.0 mm, rotated 90° to the left and then removed from the brain at the site of entry. The medial basal hypothalamus was ablated by a modification of the method described by Dunn and Critchlow (1973), using a stirrup-shaped knife (vertical 2.0 mm, diameter 3.0 mm). The knife was lowered through the midline to the base of the brain in the same manner as described in complete hypothalamic deafferentation. It was then rotated 90° to the left, moved posteriorly 2.0 mm, rotated 180° to the left, moved anteriorly 2.0 mm, rotated 90° to the left and then removed. The details of our surgical procedure were reported previously (Chihara et al., 1975; Kato et al., 1974a; Kato et al., 1974b). Animals subjected to hypothalamic surgery were carefully housed in individual cages. Hypothalamic lesion resulted in a significant suppression of body weight gain when compared with non-operated controls and 10 to 20% of rats operated were dead before the experiment.

After the experiment, the brains were placed in formalin and stained with hematoxylin-eosin. Histological evaluation demonstrated some variations in placements and dimensions of the hypothalamic surgery. Most of the isolated hypothalamic islands included the arcuate nuclei, the periventricular nuclei, the ventromedial nuclei and the premamillary nuclei. These nuclei were destroyed in rats with hypothalamic ablation. The posterior pituitary was considerably atrophied. The anterior pituitary appeared almost normal, but occasionally small central infarcts were observed in rats with hypothalamic destruction. Rats in which the placements and dimensions of hypothalamic surgery were demonstrated to be incomplete were eliminated from the study.

Results

Effect of 5-HTP on plasma prolactin

The intravenous injection of varying doses of 5-HTP (1, 3 and 5 mg/100 g body wt) caused a significant increase in plasma prolactin levels as shown in Fig. 1. Peak levels of plasma prolactin were observed 20 to 40 min after 5-HTP administration. The mean (±S. E. M.) peak value of plasma prolactin after 1 mg of 5-HTP per 100 g body wt was 25.7±7.8 ng/ml, which was significantly higher than the saline control (P<0.01). The mean peak value of plasma prolactin in rats given 3 mg of 5-HTP per 100 g body wt was higher than that in rats
given 1 mg, but lower than that in the group given 5 mg (P<0.05 and P<0.05, respectively). Physiological saline injection as a control had no significant effect on plasma prolactin concentration.

**Effect of L-DOPA on plasma prolactin responses to 5-HTP**

The simultaneous administration of L-DOPA (2 mg/100 g body wt iv), a precursor of dopamine, significantly inhibited plasma prolactin responses to 5-HTP (1 mg/100 g body wt iv) 10, 20 and 40 min following the injection compared with those of a control group (P<0.02, P<0.05 and P<0.05, respectively) (Fig. 2). A single intravenous injection of the vehicle (pH 2.8) had no significant effect on plasma prolactin levels although the data are not shown.

**Plasma prolactin responses to 5-HTP in rats with hypothalamic deafferentation**

The basal plasma prolactin levels were 16.1 ± 3.1 ng/ml (mean ± S.E.M.) in rats with complete hypothalamic deafferentation. Plasma prolactin levels rose significantly following the administration of 5-HTP (1 mg/100 g body wt iv) as shown in Fig. 3, whereas physiological saline injection caused no significant change in plasma prolactin levels in these animals. The
mean peak value of plasma prolactin following 5-HTP administration in these rats was significantly higher than that in intact rats without surgery (103.3 ± 19.7 ng/ml vs 37.8 ± 7.8 ng/ml, P < 0.02).

Plasma prolactin responses to 5-HTP and chlorpromazine in rats with extensive hypothalamic ablation

Basal levels of plasma prolactin in rats with hypothalamic ablation ranged from 14.0 to 165.0 ng/ml with a mean (± S.E. M.) of 76.9 ± 18.0 ng/ml, which were significantly higher than those in intact rats and in rats with hypothalamic deafferentation (P < 0.001 and P < 0.01, respectively). The intravenous injection of 5-HTP (1 mg/100 g body wt) or physiological saline caused no greater increase in plasma prolactin in rats with hypothalamic ablation. In contrast, chlorpromazine (200 µg/100 g body wt iv) resulted in a further increase in plasma prolactin 10, 20 and 40 min after the injection (P < 0.001, P < 0.001 and P < 0.001, respectively) as shown in Fig. 4.

Fig. 3. Effect of 5-HTP (1 mg/100 g body wt iv) on plasma prolactin in rats with complete hypothalamic deafferentation. The number in parentheses indicates the number of animals in each experimental group. The values are given as means ± S.E.M. Statistical differences (vs. saline control) are shown by asterisks: *P > 0.025.

Fig. 4. Effects of 5-HTP (1 mg/100 g body wt iv) and chlorpromazine (200 µg/100 g body wt iv) on plasma prolactin levels in rats with hypothalamic destruction. The number in parentheses represents the number of animals in each group. All values are means ± S.E.M. Statistical differences (vs. basal prolactin levels) are shown by asterisks: *P < 0.001.
Discussion

Lu et al. (1973) reported that a large dose of 5-HTP (12.5 mg/100 g body wt) given intravenously raised serum prolactin levels in female rats. It was also reported that the intraperitoneal injection of 5-HTP (30 mg/rat) raised serum prolactin levels in ovariectomized estrogen primed rats (Chen and Meites, 1975). However, Smythe and Lazarus (1973) described that a smaller dose of 5-HTP (0.8 mg/100 g body wt ip) caused a slight but non-significant rise in plasma prolactin levels in male rats. We have demonstrated in this study that a single intravenous injection of 5-HTP (1, 3 and 5 mg/100 g body wt) caused a marked and dose-related increase in plasma prolactin concentrations in urethane-anesthetized male rats.

Kamberi et al. (1971a) reported that the injection of serotonin into the third ventricle increased prolactin levels in the blood, and suggested the possible role of a serotoninergic mechanism in stimulating prolactin release. We have previously shown that the oral administration of 5-HTP raised plasma prolactin in man and concomitant intravenous infusion of cyproheptadine, a serotonin antagonist, significantly blunted the plasma prolactin response to 5-HTP (Kato et al., 1974c). An increase of brain serotonin can be expected following the systemic administration of 5-HTP, a precursor of serotonin known to enter the brain (McBride et al., 1974). It can be assumed, therefore, that 5-HTP causes prolactin release by stimulating the serotoninergic mechanism. However, the exact site of action of serotonin in stimulating prolactin release remains to be clarified.

We then studied the effect of 5-HTP on plasma prolactin levels in rats with hypothalamic deafferentation or extensive hypothalamic destruction. The intravenous injection of 5-HTP caused a marked rise in plasma prolactin levels in rats with complete hypothalamic deafferentation. However, no significant change in plasma prolactin was observed in rats with hypothalamic ablation.

It is known that interruption of the portal vasculature results in extensive necrosis of the pituitary (Daniel and Prichard, 1965; David et al., 1965). However, the anterior pituitaries were kept intact except occasional small central infarct following hypothalamic destruction in the present experiment. These findings were in agreement with other reports (Arimura et al., 1972; Dunn and Critchlow 1973), in which similar hypothalamic surgery was performed. The ability of chlorpromazine to increase prolactin release in rats with hypothalamic ablation also indicates that this animal preparation can release prolactin in response to stimuli, despite the elevated control levels. The inability of 5-HTP to increase prolactin release is, therefore, not due to the damage of the pituitary caused by the surgery.

These results suggest that 5-HTP does not act directly on the pituitary gland but exerts its prolactin-releasing activity at the level of the hypothalamus. Talwalker et al. (1963) also reported that the direct action of serotonin in stimulating prolactin release from the pituitary gland was ruled out by an in vitro experiment. It appears, therefore, that 5-HTP causes prolactin release by stimulating the serotoninergic mechanism in the hypothalamus.

Basal plasma prolactin levels were much higher in rats with hypothalamic ablation than in intact rats as well as in rats with complete hypothalamic deafferentation. These results are in agreement with those reported by Krulich et al. (1975), suggesting a tonic inhibitory regulation by the isolated hypothalamic islands. We have found that 5-HTP (1 mg/100 g body wt iv) administration caused a much higher increase in prolactin release in rats with
complete hypothalamic deafferentation than in intact animals. Serotonin input to the hypothalamus originating in the raphe nuclei in the midbrain (Fuxe and Hökfelt, 1964) is interrupted by complete hypothalamic deafferentation and the isolated hypothalamus may become hypersensitive to serotonin, which may potentiate plasma prolactin response to 5-HTP in rats with complete hypothalamic deafferentation.

We have also demonstrated that plasma prolactin responses to 5-HTP were significantly suppressed by L-DOPA, a precursor of dopamine. Evidence has been reported suggesting that dopamine is related to prolactin-inhibiting factor (PIF) (Kamberi et al., 1971b; VanMaanen and Smelik, 1968) and that prolactin release is regulated by a dual, serotoninergic and dopaminergic, mechanism (Kamberi et al., 1971a). Our results suggest that there exist some interactions between serotoninergic and dopaminergic mechanisms, although the exact nature of the interactions is not clear. Some recent studies (MacLeod and Lehmeier, 1974; Takahara et al., 1974) have provided evidence suggesting a direct inhibitory action of dopamine on pituitary prolactin release. Our observation that chlorpromazine produced a significant increase in plasma prolactin levels in rats with extensive hypothalamic destruction also indicates a possible direct action of the drug on the pituitary. Since chlorpromazine is well-known as a dopamine antagonist, it might stimulate prolactin release by antagonizing the inhibitory action of dopamine at the pituitary level, although other actions of the drug (Anden et al., 1967) cannot be ruled out.

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