NOTE
The Effect of DL-5-Hydroxytryptophan (5-HTP) on Plasma Prolactin in Pituitary Stalk Sectioned Rats

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Abstract

5-HTP (5 mg/100 g body weight), a precursor of serotonin, was administered intravenously to pituitary stalk sectioned rats. Plasma prolactin levels were initially increased 30 and 60 min after the injection of 5-HTP and thereafter decreased. Pituitary prolactin content was decreased 60 min after the stalk section as compared to those seen following sham operation. The treatment of 5-HTP induced a moderate increase in pituitary prolactin content in the stalk sectioned rats.

In order to confirm the complete disconnection of pituitary stalk after the operation, 2.5 μg of dopamine hydrochloride was given into the lateral ventricle of stalk sectioned rats. Plasma prolactin did not change following the administration.

Our results demonstrate that 5-HTP acts either directly at the pituitary level or via some mechanism yet to be determined.

In mammals, pituitary prolactin secretion is controlled by hypothalamic inhibiting factors. It has been reported that dopaminergic action inhibits prolactin secretion from the pituitary (Lu et al., 1971, 1972; MacLeod et al., 1974; Takahara et al., 1974; Diefenbach et al., 1976; Leblanc et al., 1976; Jimenez et al., 1978), but it is not well understood whether dopamine is a physiological prolactin inhibiting factor or not.

On the other hand, serotonergic agents stimulate prolactin secretion (Lu et al., 1973; Lawson et al., 1976: Ohgo et al., 1976) and the stimulatory effect appears to be more profound when hypothalamic inhibition is removed. This dual control seems to be involved in the prolactin secretion regulating system (Lawson et al., 1976; Ferrari et al., 1978).

The present study was designed to investigate the effects of DL-5-hydroxytryptophan (5-HTP), a precursor of serotonin, on prolactin secretion at the pituitary level by using hypophysial stalk sectioned rats.

Materials and Methods

Animals
Wistar strain female rats (Doken LTD, Shimodate, Japan) weighing 200-250 g and with regular 4 day cycles were used in the experiments. They were housed in a controlled temperature (20-23°C) and illumination (14 h light—10 h darkness) environment. Animals were fed tap water and rat chow (Oriental Kobo LTD, Japan) ad libitum.

Procedures
Hypophysial stalk section was performed under pentobarbital anesthesia (5 mg/100 g body weight ip) at day 1 of the estrous cycle by the temporal approach according to the modified procedure of Harris (1950) and Daniel et al. (1975). The hypo-
physiological stalk was disconnected and a silicon plate (3.0 × 2.0 × 0.5 mm) was inserted between the hypothalamus and pituitary to prevent regeneration of the portal vessels. After the experiments, complete stalk section was confirmed by autopsy. Histological changes in the stalk sectioned pituitary were reported previously (Akabori et al., 1979).

Intraventricular administration of dopamine hydrochloride on the 7th day of stalk section
Dopamine hydrochloride (Tokyo Kasei Kogyo LTD, Tokyo, Japan) was dissolved in saline containing a trace of indigocarmine and injected (2.5 µg/5 µl) into the lateral ventricle with a Hamilton microsyringe.

Blood samples (0.8 ml each) were obtained before and 30 min after injection. If intraventricular injection was successful, the cerebrospinal fluid was stained blue for more than 30 min.

These procedures were performed under ether anesthesia.

Single injection of 5-HTP on the 7th day postsurgical
5-HTP (Tokyo Kasei Kogyo LTD, Tokyo, Japan) was dissolved in 0.5 N-HCl to which 0.5 N-NaOH was added to bring the pH to 2.8. The solution (5 mg/100 g body weight) was injected into the jugular vein of the animals lightly anesthetized with ether.

Blood samples (0.8 ml each) were obtained sequentially from the opposite jugular vein before and after (30 min, 60 min, 120 min) injection under light ether anesthesia.

Administration of 5-HTP shortly after stalk section
5-HTP (5 mg/100 g body weight) was injected into the jugular vein 30 min after stalk section under pentobarbital anesthesia. Blood samples (1.5 ml each) were obtained 30 min after injection from the opposite jugular vein under light ether anesthesia.

These animals were decapitated immediately after blood collection and the pituitaries were removed for the measurement of pituitary prolactin content. Plasma level and pituitary prolactin content were compared with those 60 min after sham operation and 60 min after stalk section with vehicle.

Prolactin assay
Blood samples were centrifuged (3000 rpm × 15 min) and plasma was stored at -20°C until assayed. Pituitaries were homogenized in a teflon homogenizer with 3 ml of saline per gland, and after centrifugation (3000 rpm × 15 min) the supernatant was stored until assayed.

All samples obtained from the present experiments were measured by radioimmunoassay at the same time. Each sample was assayed at proper dilutions in duplicate by double antibody radioimmunoassay and expressed in terms of the purified rat prolactin reference standard, NIAMDD-rat prolactin-RP-1.

Statistical analysis was carried out by Student's t-test.

Results
Intraventricular administration of dopamine hydrochloride did not affect the plasma prolactin level in stalk sectioned rats, as shown in Fig. 1. There is no significant difference between the two groups.

On the 7th day after stalk section, the injection of 5-HTP significantly raised the plasma prolactin level 30 (p<0.01) and 60 min (p<0.01) after the administration (Fig. 2) but the vehicle (pH 2.8) did not change the plasma prolactin level in stalk sectioned rats (Akabori et al., 1979).

5-HTP also induced a remarkable increase in plasma prolactin in the experiments performed shortly after stalk section. Plasma prolactin level was 48.8 ± 7.3 ng/ml (mean ± SE) in the sham operation group and 366.7 ± 80.0 ng/ml after stalk section. The value in the stalk sectioned group treated with 5-HTP reached 1050.0 ± 75.9 ng/ml (Fig. 3).

Pituitary prolactin content was significantly decreased after stalk section (34.5 ± 4.9 µg/gland : mean ± SE) and that of the sham operation group was 95.8 ± 7.3 µg/gland. The administration of 5-HTP induced a significant increase (p<0.01) in pituitary prolactin content shortly after stalk section (58.5 ± 2.5 µg/gland). These results are shown in Fig. 4.
Fig. 1. Effect of intraventricular injection of dopamine hydrochloride (2.5 µg/5 µl) on prolactin secretion on the 7th day of stalk sectioned rats.

Fig. 2. Effect of 5-HTP on prolactin secretion on the 7th day of stalk sectioned rats.

Fig. 3. Effect of 5-HTP on prolactin secretion shortly after stalk section in rats: Prolactin level was compared with sham operation and stalk section with vehicle.

Discussion

It is well known that prolactin secretion from the pituitary is regulated by an hypothalamic inhibitory factor. In the stalk sectioned or pituitary grafted hypophysectomized rats, the plasma prolactin level was raised by the lack of transport of an inhibiting factor to the pituitary from the hypothalamus (Nikitovitch-Winer, 1965; Lu et al., 1972; Kanematsu et al., 1973; Diefenbach et al., 1976).

The direct action of dopamine on the pituitary gland was demonstrated in in vivo studies. Infusion of dopamine into the portal vessels lowered the plasma prolactin level in rats (Takahara et al., 1974)
Fig. 4. Effect of 5-HTP on pituitary prolactin content shortly after stalk section in rats: Prolactin content was compared with sham operation and stalk section with vehicle.

and intravenous injection of L-DOPA lowered the plasma prolactin level in the stalk sectioned rhesus monkey (Diefenbach et al., 1976).

On the other hand, the direct action of serotonergic agents on prolactin secretion remains to be clarified. Dahlström and Fuxe (1966) reported that serotonin existed in the adenohypophysis and 5-HTP was decarboxylated to serotonin in the pituitary gland. Saavedra et al. (1975) also suggested the existence of serotonin in the anterior pituitary lobe. These studies suggested that a serotonergic mechanism might be involved in the pituitary hormone secretion.

Earlier, Lu and Meites (1973) showed that 5-HTP induced a significant rise in the plasma prolactin level in pituitary grafted hypophysectomized rats. Recently, Wehrenberg et al. (1980) reported the possibility of direct serotonergic action on prolactin secretion in stalk sectioned monkeys. While, Ohgo et al. (1976) demonstrated the opposite, i.e. that 5-HTP did not act on prolactin secretion in the hypothalamic ablation rats, and Lamberts et al. (1978) reported that neither 5-HTP nor serotonin had a direct action on prolactin secretion in in vitro studies.

In our present study, 5-HTP (a precursor of serotonin) stimulated prolactin secretion on both the 7th day in stalk sectioned rats and in rats shortly after stalk sectioning. These results suggest that 5-HTP stimulates prolactin secretion by acting directly on the pituitary gland or by discharging a prolactin releasing factor from the hypothalamus to the peripheral blood in stalk sectioned rats. The latter possibility would be consistent with the results by Ohgo et al. (1976).

Since 5-HTP not only raised the plasma prolactin level but also increased the pituitary prolactin content, it seemed that 5-HTP enhanced prolactin production in the pituitary gland of stalk sectioned rats. Satisfactory disconnection of pituitary stalk using our surgical procedure (Akabori et al., 1979) was proved by intraventricular administration of dopamine, which induced no effect on the stalk sectioned rats, while the same treatment brought on a significant decrease in plasma prolactin in rats (Kamberi et al., 1971).

From our results, it is concluded that the serotonergic agent (5-HTP) affects not only the plasma prolactin level but also the pituitary prolactin content.

The effect of 5-HTP on prolactin secretion may be manifested directly in the pituitary gland, but an undetermined releasing factor induced by 5-HTP cannot be ruled out.
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References


