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NEUROENDOCRINE CONTROL OF THE PITUITARY TSH SECRETION

VII. Effects of Intraventricular and Intraarterial Administrations of 5-Hydroxytryptophan (5-HTP) on Rat Pituitary TSH Release

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Masahiro SAKODA, Takaaki KUSAKA, Makoto TATEIWA, Hidetaro MORI and Shigeaki BABA. *Neuroendocrine Control of the Pituitary TSH Secretion. VII. Effects of Intraventricular and Intraarterial Administrations of 5-Hydroxytryptophan (5-HTP) on Rat Pituitary TSH Release.* Kobe J. Med. Sci. 21, 53-59, June 1975——5-HTP in doses of 5 μg were administered into the lateral ventricle and 25, 50 and 100 μg were injected into the carotid artery under pentobarbital anesthesia. Plasma TSH increase was observed at 10, 20 and 30 minutes after injection into the lateral ventricle. Peak value of plasma TSH increase occurred at 20 minutes and this value was significantly high compared to control rats. Arterial administration of 50 and 100 μg of 5-HTP also produced plasma TSH increase to the levels as high as 2 to 3 fold above controls, whereas 25 μg of 5-HTP was ineffective. Saline (0.9%) did not produce any effect on TSH release both in the ventricular and arterial injections. 5-HTP had no effect on the release of TSH from pituitaries incubated in vitro with 5-HTP. 5-HTP had no potentiating effect or inhibiting effect of inactivation on rat TSH under any of the described conditions.

The results provide incidence for the role of 5-HTP in releasing TSH as a possible transmitter for the pituitary TSH secretion.

INTRODUCTION

Recent observations have revealed that the presence of neurons containing dopamine (DA), norepinephrine (NE) and serotonin (5-HT) might play a role in the regulation of the anterior pituitary tropic hormones. A number of studies on hormone releasing effect of biogenic amines were performed in vivo and in vitro: Pituitary GH release is facilitated by the administration of 1-Dopa.[1] Pituitary LH release is also enhanced

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both by the ventricular injection of DA[9] and DA addition to the incubation medium of pituitary in the presence of hypothalamic fragment.[8]

Howerer, roles of biogenic amines in regulation of pituitary TSH secretion are remained in obscure. This paper dealt with the effect of 5-HTP injected into the lateral ventricle and carotid artery on pituitary TSH release of rats.

MATERIALS AND METHODS

Experimental animals.

Male Swiss-Webster rats (body weight 150 g) of 3 week age were offered to ventricle and arterial injections.

All rats were housed in an air-conditioned room with controlled lighting (lights on 7 AM - 7 PM) and were supplied with oriental FM chow and water ad lib.

Female d-d strain mice (body weight 15 g) were used for TSH assay after McKenzie. All mice were housed in constant temperature and constant light (above mentioned) and were fed with low iodine diet and distilled water.

Experimental procedure.

(i) Ventricular cannulation.

Ventricular cannulation was performed with stainless steel cannula. Cannulae were 24 gauge with a mandril to prevent its obstruction. The animals were anesthetized with pentobarbital (4.5 mg/100 g BW) for cannulation, ear plugs were inserted, and the rat's head was fixed in a krieg stereotaxic instrument.

Lateral ventricle was chosen because of its convenience and certainty of insertion. The coordinates used for the lateral ventricle were as follows: anteroposterior (AP)= 5.3 mm anterior from ear bar, lateral=1.3 mm apart from the mid line and vertical= 6.4 mm above the ear bar following the map of Sherwood and Timiras.[10] Location of needle top was ascertained by the following 3 methods, i. e. back flow of CSF, ink filling of 3rd ventricle and histological examination. At the end of the experiments ink filling of ventricle was performed in all animals and histological sections were made in all animals.

(ii) Ventricular 5-HTP administration.

Ventricular 5-HTP administration was performed just after insertion of cannulae and puncture of 0 time blood samples.

After the removing of mandril, the cannula was connected by a polyethylene plastic tube to a syringe filled with 5-HTP for experimental rats and saline for control. The injection time was approximately 60 sec. All injections were performed around noon under pentobarbital anesthesia.

(iii) Blood puncture.

Blood was collected from a cannula inserted into the jugular vein just before and 15, 30 min after intraventricular injection and 30 min after intraarterial

injection. Blood samples from the same experimental group were pooled and freezed until assay.

(iv) Intraarterial injection.

The right carotid artery was exposed and pulled up softly with string. The artery was punctured with caution and test solution was injected within 15 seconds.

(v) Pituitary incubation of rat.

This procedure was previously reported by Sakoda and Nakabayashi.[7] As the donor of pituitary, weighing about 150 mg male rats of Wistar strain were used. They were freshly decapitated and their anterior pituitaries were taken out of the brain and hemisected sharply. Three halves taken from different pituitaries were pooled in one flask and incubated in pH 7.2 Krebs-Ringer-Bicarbonate medium with 200 mg/dl glucose and in an atmosphere of 95% 0_2 and 5% CO_2. One flask containing 3 pituitary halves was used for experiment and the other flask containing 3 concomitant halves was served as control (paired incubation).

As a rule, a preincubation for 60 minutes was carried out at first to standardize the responses of incubated anterior pituitary in estimating the effect of test materials on the response of anterior pituitary. Then the 3 pituitary halves were transmitted to another flask and final incubation for 60 min was carried out with fresh medium containing test preparation for the experiment. As a parameter of the pituitary response, TSH activity released in the incubation medium was estimated.

TSH Assay.

Rat plasma TSH was assayed by McKenzie's method[5] with slight modification.. Precision index was 0.295 and minimum detectable value was 0.05 musp in our laboratory. Significance of differences between control and experimental groups or pre- and post-injection plasma TSH levels was calculated by Student's "t" test.

Materials tested.

1-5HTP: 1-5-Hydroxytryptophan dissolved in saline (source: Aldrich Chemicals Co., Milwaukee).

RESULTS

Injection of saline into the lateral ventricle and carotid artery.

Isotonic saline solution injected into the lateral ventricle or carotid artery in the volume equivalent to the respective test material injection, has no effect on plasma TSH levels in rats (Table 1). These findings indicate that experimental or surgical manipulation of the rats and injection of the diluent for test material had no nonspecific effect on plasma TSH levels.

Table 1 Failure of injection of 2 μl isotonic saline into the lateral ventricle and 0.1 ml of isotonic saline into the carotid artery to influence plasma TSH level in male rats.

Treatment	Rat No.	Rat plasma TSH. ^{131}I release from mouse thyroid at 2 hrs. (%) Mean±S. E.	P
2 μl saline into lat. ventricle	5	96.8±9.2	N.S.
0.1 ml saline into carotid artery	5	94.5±8.8	N.S.
Control (untreated)	4	107.6±7.9	—

Effect of 5-HTP on TSH release of pituitary incubated in vitro.

5-HTP in doses of 1 or 3 μg was added to incubation medium. No significant difference in TSH activity was observed between the medium from experimentals (5-HTP addition) and the medium from controls (saline addition) (Table 2). These findings indicate that 5-HTP has no releasing activity of TSH at pituitary level and that 5-HTP also has no potentiating effect or no inhibiting effect on inactivation of rat TSH in this condition.

Table 2 Effcet of 5-HTP on TSH release of rat pituitary incubated in vitro.

Addition to incubation medium	TSH activity in the medium ^{131}I release from mouse thyroid at 2 hrs. (%) Mean±S. E.	P
Saline	91.9±10.3	—
5-HTP (1 μg/Flask)	112.3±14.1	N.S.
5-HTP (3 μg/Flask)	88.7±11.0	N.S.

Lateral ventricle injection of 5-HTP.

5-HTP was infused in doses of 5 μg dissolved in 2 μl of saline solution. Plasma TSH increase was observed at 15, 20 and 30 minutes after injection into the lateral ventricle.

Maximun increase occurred at 20 minutes and this level was significantly ($P<0.05$) high compared to controls, while the plasma TSH levels were high but not significant at 10 and 30 minutes (Table 3).

Table 3 Effect of 5-HTP injection into the lateral ventricle on plasma TSH in male rats.

Treatment	Rat No.	Rat plasma TSH ^{131}I release from mouse thyroid at 2 hrs. (%) Mean±S. E.	P
Saline inj.	5	94.0±6.3	—
10 min. after 5-HTP inj. (5 μg/100 g B.W.)	4	139.0±11.2	N.S.
20 min. after 5-HTP inj. (5 μg/100 g B.W.)	5	199.7±8.1	<0.05
30 min. after 5-HTP inj. (5 μg/100 g B.W.)	4	142.9±12.1	N.S.

Intraarterial injection of 5-HTP.

5-HTP was injected in doses of 25, 50 and 100 μg dissolved in 0.1 ml of saline solution. 5-HTP in doses 50 and 100 μg raised plasma TSH to the levels as high as 2 or three fold above controls ($P<0.05$) at 30 minutes after injection. 25 μg of 5-HTP has no effect on plasma TSH levels (Table 4).

Table 4 Effect of 5-HTP injection into the carotid artery on plasma TSH in male rats.

Treatment	Rat No.	Rat plasma TSH ^{131}I release from mouse thyroid at 2 hrs. (%) Mean±S. E.	P
Saline inj.	4	97.4±7.1	—
30 min. after 5-HTP inj. (25 μg/100 g B.W.)	4	134.7±10.7	N.S.
30 min. after 5-HTP inj. (50 μg/100 g B.W.)	5	242.3±11.1	<0.05
30 min. after 5-HTP inj. (100 μg/100 g B.W.)	5	242.4±12.2	<0.05

DISCUSSION

Effects of serotonergic agents on pituitary tropic hormone release were studied by several investigators. Depletion of pituitary GH contents was observed after administration of catecholamine into rat lateral ventricle, while serotonin did not show any GH releasing activity.[6] Serotonin has possible inhibitory effect on pituitary LH release.[9] Also, Kordon[4] had reported that serotonin acts centrally to inhibit gonadotropin release.

However, effects of serotonergic agents on pituitary TSH release are not clear. As described before, intraventricular 5-HTP was effective in releasing TSH from the anterior pituitary in normal male rats. The response of plasma TSH to ventricular 5-HTP was rapid and reached peak value within 20 minutes. This time-response relation is in good agreement to the report by Shneider and McCann[9] in which intraventricular administration of dopamine induced significant increase of rat serum LH. Our observations mean from the viewpoint of time-response relation that hypothalamic response was triggered by 5-HTP itself rather than by its metabolite such as 5-HT (serotonin). Additional observations are required whether serotonin (5-HT) has specific effect on pituitary TSH release or not. These results are observed in above mentioned experimental conditions, i. e. under pentobarbital anesthesia and surgical procedure for intraventricular or intracarotid injection. Thus, the pentobarbital anesthesia or experimental manipulation of the rats has no nonspectific influence on plasma TSH levels or pituitary responsiveness to 5-HTP.

However, further observations on the influences of anesthesia and surgical procedure are now being scheduled by another procedure such as transient anesthesia by ether or pseud-implantation of canunla for ventricular injection. Other problems to be elucidated are the interplay between 5-HTP and TRH in which 5-HTP might facilitate the release of TRH. Reichlin and his colleagues proposed that release of TRH is regulated by a double monoaminergic system, with CA as a promoter and serotonin as an inhibitor of TRH release.[3]

The mechanism responsible for this transmission remains to be elucidated. Intraarterial injection of 5-HTP also evokes plasma TSH increase in rats, and this effect was not induced by potentiating or inhibiting inactivation of rat TSH. The TSH responses obtained in both rats receiving 50 μg and 100 μg of 5-HTP were almost the same. This finding may be due to the maximum response of mouse in TSH assay system. The effective dose of 5-HTP in arterial injection was as much as 10 fold in those of ventricular injection. These findings may relate to the permeability of blood brain barrier of rats.

Recently Chen and Meites[2] have reported that 5-HTP produced a significant rise in serum TSH and prolactin of rats. The dose of 5-HTP was extremely large (30 mg/rat, i. p.) and effect of low dose was not observed. Further observations are required with special reference to the dose-response relation between dose of 5-HTP and TSH response as well as the route of administration such as intravenous, intra-arterial, intraperitoneal or intraventricular injection.

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