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Stimulatory Role for Brain Serotoninergic System on Prolactin Secretion in the Male Rat[1] (39247)

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A large body of evidence has been accumulated in recent years to indicate the existence of an inhibitory role of the dopaminergic system on prolactin (PRL) secretion (1-3). Recent studies have investigated the role of other neurotransmitters that might be also implicated in the regulation of PRL secretion, i.e., serotonin (5-HT) (4-6), norepinephrine (NE) (7), and acetylcholine (8). As far as serotonin is concerned, Kamberi *et al.* (4) have reported that in the rat intraventricular (IVT) injection of 5-HT or its pineal derivative melatonin increased plasma PRL levels, and Lu and Meites (6) observed a prompt rise in plasma PRL levels in the rat after ip administration of L-tryptophan (L-TP) and 5-hydroxytryptophan (5-HTP), the immediate 5-HT precursor. Intravenous infusion of TP or oral administration of 5-HTP were reported also to stimulate PRL secretion in the human (9, 10). While a large number of observations have dealt with the effect of functional activation of 5-HT system on PRL release, fewer and contradictory results have been reported so far on the effect of 5-HT functional suppression (5, 7).

The present investigation was aimed to study the effect of acute or chronic functional ablation of central 5-HT system on circulating plasma PRL levels. Two different approaches were used: the first by using pharmacologic agents which rather specifically affect brain 5-HT neurons, the second by limiting in the diet the presence of the 5-HT precursor amino acid, i.e., TP.

Materials and methods. Animals. In all experiments albino Wistar young adult male rats (kindly donated by Zambon S.p.A., Milano) 120-150 g body wt were used, except for experiments with 5,7-dihydroxytryptamine (5,7-DHT), when Sprague-Dawley male rats (Nossan, Milano) weighing 150-180 g were used. Animals were housed under controlled conditions (22 ± 2°, 65% humidity, and 14 hr per day of artificial light, 06:00-20:00). They were fed a standard laboratory diet with water allowed *ad libitum*, except for rats used in experiments with the TP-deficient diet (see below).

Experimental procedure. In the experiments in which 5,7-DHT was used (see below), a small polyethylene cannula (PE-10) was implanted in the right lateral ventricle of the brain, under barbiturate anesthesia, according to the method of Altaffer *et al.* (11). Animals implanted with the cannula were placed in individual cages and at least 3 or 4 days were allowed for recovery.

Experiments using parachlorophenylalanine. Blockade of 5-HT synthesis was accomplished by the use of parachlorophenylalanine (PCPA). This drug, dissolved in sesame oil, was administered subcutaneously on alternate days for two times at the dose of 100 mg/kg; rats were sacrificed by decapitation 24 hr after last PCPA injection.

Experiments using 5,7-dihydroxytryptamine. Long-lasting depletion of brain 5-HT levels was afforded by the neurotoxic indoleamine derivative 5,7-dihydroxytryptamine, which was administered through the implanted cannula without anesthesia at the dose of 200 μg/20 μl (free base) in 0.1% ascorbic acid. Other animals, 45 min before 5,7-DHT administration, were given desmethylimipramine (DMI, 25 mg/kg ip). Control animals received ascorbic acid 0.1% (20 μl IVT) or DMI alone. The above treatments were given to other rats which were killed by decapitation 12 and 30 days later and were used for brain monoamine determinations (see below).

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After treatments, all rats were weighed at 2- to 3-day intervals, and their food intake was determined daily in the first posttreatment week.

Experiments using a TP-deficient diet. In these experiments, a group of rats was fed for up to 4 days, with a diet made up of maize flour "polenta," the TP content of which is abnormally low (12). Each rat ate approximately 25 g daily of this diet. To compensate for the impairment and gradual loss of nicotinate production due to TP deficiency (13), nicotinic acid (2.65 mg), and, in addition, riboflavin (0.26 mg), pyridoxine hydrochloride (0.13 mg), Vitamin E (0.26 mg), ascorbic acid (83 mg), pantotenic acid (0.26 mg), Vitamin A (1380 IU), Vitamin D_2 (266 IU) were dissolved in 100 ml of the drinking water. A second group of rats received the same diet to which TP (2.0 g/kg of diet) had been added and drank water supplemented with vitamins, as above described. As controls, rats fed *ad libitum* with a standard laboratory diet or undernourished rats, given half their usual food intake (about 10 g per rat per day), were used. During the experiment, body growth was determined by daily weighing.

Blood collection and radioimmunoassay (RIA) measurements. Blood was collected immediately from the decapitated trunks (PCPA and TP-deficient diet experiments) or by the orbital bleeding technique (14) (3, 12, and 30 days after drug administration, 5,7-DHT experiments) into heparinized tubes and immediately chilled. The plasma was then separated and frozen until assayed for prolactin by a double antibody immunoassay (15). All results were expressed in nanograms per milliliter in terms of the NIH standard Rat Prolactin, RP-1, whose potency is approximately 11 IU/mg. The sensitivity of this assay is 1.0 ng/ml; intraassay variability is 5%. To avoid possible interassay variation, all samples of each experiment were assayed within the same radioimmunoassay.

Determination of monoamines in the brain. After killing, brains were rapidly removed, weighed, and hemisected; half of the brain was used for fluorometric estimation of brain NE and DA, according to Neff *et al.* (16), and the other half was used for 5-HT and 5-hydroxyindolacetic acid (5-HIAA) estimations, according to Curzon *et al.* (17). Otherwise, brain indole levels were determined in the whole brain.

Statistics. In all experiments, significance of difference between groups was calculated by the Student's *t* test.

Results. Administration of PCPA resulted in a clear-cut reduction of plasma PRL levels, determined 24 hr after last PCPA injection. Brain 5-HT levels were markedly decreased following PCPA administration (31% of control values), while no difference was present in brain NE and DA levels (Table I).

Degeneration of brain 5-HT nerve terminals by IVT injection of 5,7-DHT reduced significantly plasma PRL levels evaluated 3, 12, and 30 days after treatment. Pretreatment with DMI did not modify the PRL-lowering effect of 5,7-DHT at 3 and 12 days after treatment; at 30 days, PRL levels were higher in the 5,7-DHT + DMI group than in 5,7-DHT-treated rats, although in both groups PRL levels were significantly lower than control levels. DMI alone did not affect circulating PRL levels at any time interval. Base line plasma PRL levels increased progressively with age in both control as well in

TABLE I. EFFECT OF PARACHLOROPHENYLALANINE (PCPA) ON PLASMA PROLACTIN AND BRAIN MONOAMINES LEVELS.[a]

Groups	Plasma Prolactin (ng/ml ± SE)	Brain		
		5-HT (μg/g ± SE)	NE (μg/g ± SE)	DA (μg/g ± SE)
Controls (10)	22.6 ± 4.4	0.94 ± 0.05	0.77 ± 0.026	1.33 ± 0.03
PCPA-treated (10)	11.2 ± 1.8[b]	0.29 ± 0.01[c]	0.81 ± 0.03	1.40 ± 0.04

[a] Number of rats in parentheses. PCPA was administered on alternate days at the dose of 100 mg/kg sc × 2, dissolved in sesame oil, and rats were killed 24 hr after last injection. Control rats received sesame oil (0.5 ml/100 g body wt).

[b] $P < 0.01$ vs controls.

[c] $P < 0.001$ vs controls.

5,7-DHT and/or DMI-treated groups (Fig. 1).

In 5,7-DHT-treated rats, brain levels of 5-HT and 5-HIAA were markedly decreased both at 12 and 30 days after treatment (Table II). Brain levels of NE were slightly reduced (28%) at 12 days after treatment, while no significant change was present in brain DA levels at both 12 and 30 days. Pretreatment with DMI partially counteracted at 12 days the NE depletion induced by 5,7-DHT (17%) and induced a slight, although not significant, decrease in brain DA levels. Brain 5-HT and 5-HIAA levels were not significantly different in 5,7-DHT + DMI or 5,7-DHT-treated animals (Table II).

Both 5,7-DHT and 5,7-DHT + DMI treated groups showed an impairment in body growth in the immediate posttreatment period, but the effect did not attain statistical significance (Fig. 2); a slight but not significant decrease in food consumption was also present in these groups in the first 2 days after injection (data not presented).

Plasma PRL levels in animals fed for 4 days with a TP-deficient diet were not different from PRL levels present in normal controls. Addition of TP to the TP-deficient diet caused a striking increase in PRL levels. In undernourished animals on standard laboratory diet, PRL levels were slightly and not significantly lower than in *ad libitum* fed controls (Table III).

Brain 5-HT and 5-HIAA levels were signifcantly decreased in rats on TP-deficient diet (72 and 60%, respectively, of values of normal controls on standard laboratory diet), while no change in brain indole levels was present in the undernourished rats. Addition of TP to the TP-deficient diet increased brain 5-HT and 5-HIAA levels to 135 and 140%, respectively, of values present in normal controls (Table III). Rats fed a TP-deficient diet, supplemented or not with TP, and undernourished rats presented an impaired body growth pattern when compared to fed controls (Fig. 3).

Discussion. Pharmacologic, physical, and biochemical approaches are commonly used to evaluate the functional role of 5-HT in

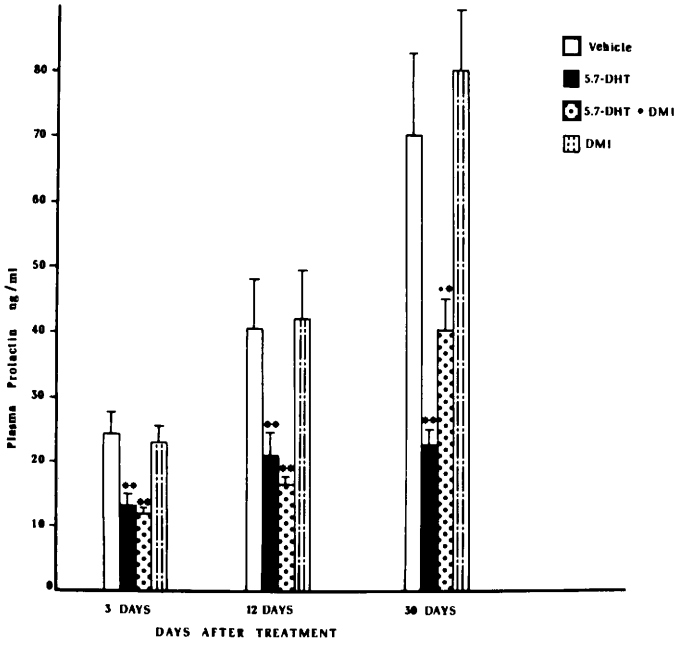

FIG. 1. Effect of intraventricular administration of 5,7-DHT (200 μg/20 μl) alone or plus DMI (25 mg/kg ip) on plasma prolactin levels in young male rats, 3, 12, and 30 days after treatment. Each bar represents mean values + SE of 10 determinations. Asterisks denote P values of * < 0.05, ** < 0.01 between 5,7-DHT or 5,7-DHT + DMI and vehicle-injected controls. The closed circle denotes P values of < 0.05 vs 5,7-DHT-treated rats.

TABLE II. EFFECT OF INTRAVENTRICULAR ADMINISTRATION OF 5,7-DHT ON BRAIN MONOAMINE LEVELS (μg/g).[a]

Treatment	Days after treatment	
	12	30
5-HT		
Vehicle	0.80 ± 0.04	0.73 ± 0.03
5,7-DHT	0.33 ± 0.04[c]	0.42 ± 0.09[b]
5,7-DHT + DMI	0.40 ± 0.03[c]	0.37 ± 0.06[c]
DMI	0.74 ± 0.05	0.77 ± 0.03
5-HIAA		
Vehicle	0.65 ± 0.04	0.78 ± 0.06
5,7-DHT	0.29 ± 0.02[c]	0.29 ± 0.08[c]
5,7-DHT + DMI	0.39 ± 0.01[c]	0.31 ± 0.05[c]
DMI	0.60 ± 0.01	0.69 ± 0.01
NE		
Vehicle	0.64 ± 0.01	0.53 ± 0.04
5,7-DHT	0.46 ± 0.01[c]	0.56 ± 0.03
5,7-DHT + DMI	0.54 ± 0.02[b, d]	0.60 ± 0.09
DMI	0.67 ± 0.03	0.57 ± 0.02
DA		
Vehicle	0.91 ± 0.09	0.57 ± 0.04
5,7-DHT	0.81 ± 0.04	0.59 ± 0.03
5,7-DHT + DMI	0.70 ± 0.07	0.56 ± 0.03
DMI	0.93 ± 0.01	0.67 ± 0.04

[a] Six to ten animals per group were used. 5,7-DHT was given intraventricularly at the dose of 200 μg in 20 μl of 0.1% ascorbic acid. Vehicle-injected rats were given intraventricularly 20 μl of 0.1% ascorbic acid. DMI was administered ip at the dose of 25 mg/kg, 45 min before 5,7-DHT or vehicle administration.

[b] $P < 0.01$ vs vehicle.

[c] $P < 0.001$ vs vehicle.

[d] $P < 0.05$ vs 5,7-DHT.

the mammalian central nervous system (CNS). Data derived from each of these strategies have their own characteristic set of strengths and weaknesses (18).

In the present study, the effect of functional suppression of central 5-HT system on tonic secretion of PRL was investigated in the male rat by means of two different approaches. Impairment of central 5-HT function was first obtained by the use of PCPA, a blocker of 5-HT biosynthesis (19). Following PCPA, there was a concomitant depletion of brain 5-HT and a significant lowering of baseline plasma PRL levels. Inhibition of the suckling-induced rise in plasma PRL has been observed in the lactating rat following acute PCPA administration by Kordon *et al.* (5). These authors, however, failed to observe in this situation reduction in baseline PRL levels; also, Donoso *et al.* (7) were unable to show such an effect when PCPA was given acutely to castrated male rats. Quite recently, Chen *et al.* (20) observed a decrease in baseline serum PRL levels following administration to estrogen-primed ovariectomized rats of parachloroamphetamine, a 5-HT depletor (21). The exact reason(s) for these discrepant results escapes our attention, although it might be ascribed to the different physio-

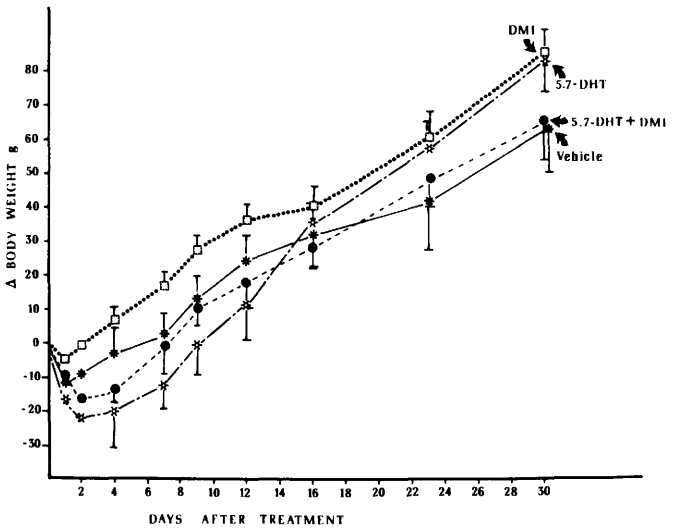

FIG. 2. Effect of intraventricular injection of 5,7-DHT or 5,7-DHT + DMI on Δ body weight during 30 days after treatment. Each point represents mean values + SE of 10 determinations. For the sake of clarity some SE's have been omitted.

TABLE III. EFFECT OF A TP-DEFICIENT DIET ALONE OR SUPPLEMENTED WITH TP ON PLASMA PROLACTIN AND BRAIN INDOLE LEVELS.[a]

Groups	Plasma prolactin (ng/ml ± SE)	Brain	
		5-HT (μg/g ± SE)	5-HIAA (μg/g ± SE)
Standard pellet diet	14.9 ± 2.5	0.65 ± 0.01	0.46 ± 0.01
TP-deficient diet	14.4 ± 1.9	0.47 ± 0.01[d]	0.27 ± 0.01[d]
TP-deficient diet + TP	35.0 ± 4.7[c]	0.88 ± 0.03[d, e]	0.64 ± 0.07[b, e]
Underfed controls	10.4 ± 0.9	0.66 ± 0.02	0.46 ± 0.01

[a] Ten animals per group were used (see text for details).
[b] $P < 0.02$ vs standard pellet diet.
[c] $P < 0.01$ vs all other groups.
[d] $P < 0.001$ vs standard pellet diet.
[e] $P < 0.001$ vs TP-deficient diet.

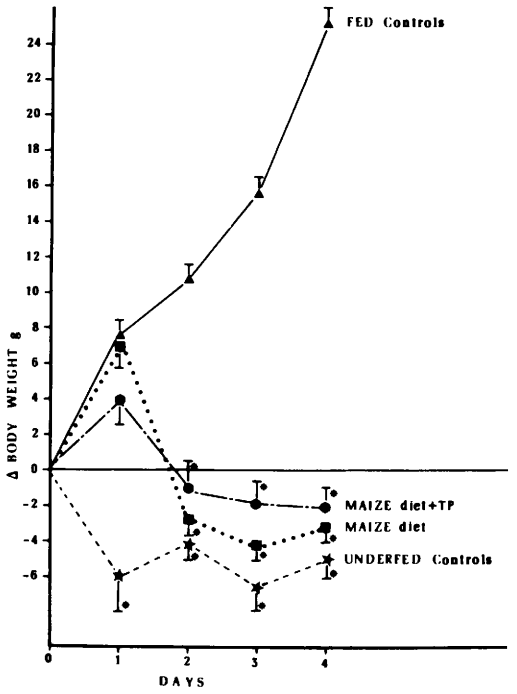

FIG. 3. Effect of the different diet regimens used on △ body weight of male rats. Each point represents mean values + SE of 10 determinations. Asterisks denote values significantly different from those present in fed controls.

logic conditions of the animals during the experiments.

With the aim to give further demonstration of the role of 5-HT on tonic PRL release, it was decided to use another pharmacologic approach. The neurotoxic tryptamine derivative 5,7-DHT induces a long-lasting depletion of 5-HT content by degeneration of indoleamine-containing nerve terminals (22). In our experiments, IVT administration of this drug induced a clear-cut and persistent depletion of brain 5-HT and 5-HIAA levels, and was accompanied by a marked and sustained reduction in plasma PRL levels.

The specificity of the action of 5,7-DHT with regard to the 5-HT system is open to criticism since a slight depleting effect on brain NE content has been reported in the rat following intracisternal (ic) administration of the drug (23). In our experiments a slight depletion of brain NE occurred 12 days after injection.

Brain NE has been proposed by some authors to play a stimulatory role in the mechanism(s) which regulates the secretion of prolactin (7, 24). However, the possibility that the reduced PRL levels found after 5,7-DHT might be attributed to reduction of a central NE stimulatory tone appears to be unlikely. In fact, despite complete recovery of brain NE levels 30 days after the injection, plasma PRL levels were still considerably reduced; moreover, no difference in plasma PRL levels at 3 and 12 days post-treatment was present between animals treated with 5,7-DHT or 5,7-DHT + DMI, a drug which partially counteracted the NE depletion (see also 25). In 5,7-DHT + DMI treated rats, PRL levels were higher than in 5,7-DHT treated rats only at 30 days, at a time when there was no difference in brain NE levels between the two groups.

A transient but significant retardation in body growth due to anorexia has been reported in rats treated ic with 5,7-DHT at birth (26). However, the young adult rats used in the present study appeared to be less

susceptible to the anorexigenic effect of 5,7-DHT then newborn animals. This makes unlikely the possibility that the reduced PRL levels present after 5,7-DHT may merely result from the toxic effect of 5,7-DHT and from the ensuing anorexia. Moreover, as it is evident from the experiments performed in TP-deficient animals (see below), underfeeding did not affect significantly plasma PRL levels.

Recently, it has been reported that in the male rat PRL levels exhibit a progressive rise in plasma during the period of sexual maturation (27). In agreement with these findings, baseline plasma PRL levels progressively increasing with age were present in our experimental animals. It is of note, in this context, that also in 5,7-DHT treated groups, plasma PRL levels, although constantly lower than in control or DMI-treated rats, showed an age-related increase. This seems to indicate that brain 5-HT system does not play an important role in the mechanism(s) responsible for the increased PRL secretion which occurs during sexual maturation.

It is generally recognized that the rate of 5-HT formation in the brain is regulated principally by the availability of TP (28). Plasma receives TP from two sources only, dietary TP and secretion from the free amino acid pools of various tissues (28). Rats given free access to a TP-deficient diet develop decreased plasma and, ultimately, brain TP levels (13); this results in a decreased brain 5-HT turnover (29). In our experimental conditions, feeding of maize, a staple which is deficient in TP (12) decreased in 4 days brain 5-HT and 5-HIAA levels. In spite of this there were no changes in baseline PRL levels in the maize-fed rats. This might perhaps be due to the small and slowly occurring depletion of brain indole levels induced by the diet regimen. However, ingestion of the maize diet supplemented with TP, at a dose roughly corresponding to the daily intake of TP by rats fed with a standard diet (Zambotti, unpublished observations), resulted in a striking increase of baseline PRL levels over those present in both normal control and TP-deficient rats and in a concomitant rise in brain 5-HT and 5-HIAA levels. These data might be interpreted to mean that addition of TP to the maize diet elicited in the recipient rats an activation of 5-HT receptor sites, which was responsible for the stimulatory effect on PRL secretion.

Since the maize diet is an unbalanced diet (12) and its ingestion for 4 days resulted in a clear-cut reduction in body growth, the possibility was envisaged that underfeeding per se might have altered plasma PRL levels. However, an artificially induced state of undernourishment did not affect plasma PRL levels; moreover, as already pointed out, in TP-deficient rats baseline plasma PRL levels were unchanged, and, in spite of a similar impairment in body growth, they were increased in TP deficient rats supplemented with TP.

Collectively, the data here reported support the thesis that, aside from being involved in the mechanism of PRL-stimulated release (5), the central serotoninergic system plays an important role in the maintenance of tonic PRL secretion.

Although the anatomical site for the interaction of 5-HT and PRL is not firmly established, it may be hypothesized that this interaction takes place in the CNS. This hypothesis is especially supported by the findings here obtained after central administration of 5,7-DHT. The use of neutoxic dihydroxytryptamine compounds, such as 5,7-DHT and 5,6-dihydroxytryptamine (22, 23) has allowed recently to obtain neuroanatomical information in the rat on the medial ascending 5-HT pathway, which primarily innervates the hypothalamus and the preoptic area (30). It is of note, in this context, that in the anterior hypothalamus, the suprachiasmatic region, a typical area of distribution of serotoninergic terminals (30), is certainly involved in the control of PRL secretion (31).

It cannot be excluded "a priori," however, that similarly to the adrenergic compounds (30), 5-HT may directly act on the pituitary to induce its PRL-releasing effect. In this regard, Lu and Meites (6) have observed that 5-HTP administered to hypophysectomized rats bearing an anterior pituitary graft doubled serum PRL levels.

Summary. Systemic administration of parachlorophenylalanine (PCPA, 100 mg/kg sc on alternate days × two times), a blocker of serotonin (5-HT) synthesis, con-

siderably decreased brain 5-HT and plasma prolactin (PRL) levels in young male rats. Intraventricular (IVT) administration of 5,7-dihydroxytryptamine (5,7-DHT, 200 μg/20 μl), a neurotoxic drug which destroys 5-HT nerve terminals, induced, 3, 12, and 30 days after treatment, a marked depletion of brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) and considerably reduced plasma PRL levels at each time interval. Feeding of rats for up to 4 days with a tryptophan (TP)-deficient diet, caused a depletion of brain 5-HT and 5-HIAA contents and did not modify plasma PRL levels. Addition of TP (2 g/kg of diet) to the TP-deficient diet resulted in increased brain 5-HT and 5-HIAA contents and significantly increased PRL levels. These data provide evidence for the role of the 5-HT system in the maintenance of tonic PRL secretion.

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