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BRIEF COMMUNICATION

Effects of Tianeptine on 5-HTP- and Dextrofenfluramine-Induced Hypophagia in the Rat

FRANCIS CHAOULOFF

Laboratoire de Pharmacologie, Groupe Neuropharmacologie, CNRS, CHU Necker, 156 rue de Vaugirard, 75015 Paris, France

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CHAOULOFF, F. *Effects of tianeptine on 5-HTP- and dextrofenfluramine-induced hypophagia in the rat.* PHARMACOL BIOCHEM BEHAV 44(4) 989–992, 1993.—The aim of this work was to analyze whether tianeptine, a 5-hydroxytryptamine (5-HT) reuptake enhancer, opposes the loss in food intake elicited by 5-HT release. Rats were food deprived for 20 h, pretreated either with saline or 7-[3-chloro-6-methyl-5, 5-dioxo-6,11-dihydro-(*c,f*)-dibenzo-(1,2-thiazepine)-11-*yl*-amino]-heptanoic acid (tianeptine, 10 mg/kg, IP), and injected 1 h afterwards either with saline, with the 5-HT precursor L-5-hydroxytryptophan (5-HTP, 20 or 40 mg/kg, IP), or with the 5-HT reuptake inhibitor/5-HT releaser dextrofenfluramine (d-FEN, 1.5 or 3 mg/kg, IP). Diets were then presented 30 min later, and individual food intakes were measured 1, 2, 3, and 4 h after food presentation. Saline- and tianeptine-pretreated saline-injected rats ate the most, and to a similar extent, during the first hour of food presentation; however, in these animals tianeptine pretreatment tended to diminish rates of eating throughout the last 3 h of analysis. Administration of 5-HTP or d-FEN triggered immediate dose-dependent decreases in food intake that were not affected by tianeptine pretreatment. In addition, tianeptine pretreatment did not alter either brain d-FEN or *d*-norfenfluramine levels, thereby suggesting that the lack of effect of tianeptine against d-FEN-elicited hypophagia could not be accounted for by changes in d-FEN metabolism. This study opens the possibility that tianeptine counteracts the effects of 5-HT release in some paradigms but not in others.

Dextrofenfluramine Feeding L-5-Hydroxytryptophan Serotonin release Tianeptine

TIANEPTINE [7-[3-chloro-6-methyl-5, 5-dioxo-6,11-dihydro-(*c,f*-dibenzo-(1,2-thiazepine)-11-*yl*-amino]-heptanoic acid (S-1574)] is a novel tricyclic agent endowed with antidepressant properties in humans (6,15) and provided with antistress properties in animals (i.e., tianeptine opposes stress-induced deficits in exploratory behaviors) (4,5,23). Interestingly, tianeptine selectively enhances the reuptake of serotonin [5-hydroxytryptamine (5-HT)], an effect opposite to that encountered with other tricyclic antidepressants. Thus, administration of tianeptine has been reported to increase 5-HT reuptake into hippocampal and cortical synaptosomes (10,19). In addition, tianeptine pretreatment increases 5-HT metabolism and amplifies the effect of 5-HT-depleting drugs (10) while Whitton et al. (24) reported that tianeptine attenuates the K^+-evoked rise of extracellular 5-HT in the hippocampus. Lastly, recent behavioral studies have indicated that acute tianeptine treatment reduces the frequency of wet-dog shakes and forepaw treading elicited by L-5-hydroxytryptophan (5-HTP, the precursor of 5-HT) while leaving unaffected behaviors elicited by postsynaptic 5-HT receptor stimulation (17,24). In this context, it is noteworthy that tianeptine prevents also the overnight loss in body weight elicited by combined administration of 5-HTP and benserazide (a peripheral aromatic amino acid decarboxylase inhibitor) in free-feeding rats (3). Although feeding behavior was not measured, this latter study suggests that tianeptine may also oppose the anorexigenic effect of increased central serotonergic activity (1,18).

In view of the aforementioned data, the purpose of this work was to analyze (in more appropriate conditions) the influence of tianeptine upon the hypophagia that results from increased 5-HT release. Thus, food-deprived rats were pretreated either with saline or tianeptine and then administered 1 h later with saline, 5-HTP (2), or dextrofenfluramine (d-FEN, a selective 5-HT reuptake blocker endowed with 5-HT-releasing properties) (12–14). Diets were presented 30 min later and individual food intakes measured for the first 4 h that followed food presentation.

METHOD

Animals

Ninety-two male Wistar rats (IFFA CREDO, Les Oncins, France), weighing 180–200 g, were used in this study. They were housed in an animal quarter maintained at 21 ± 2°C (under a 12 L : 12 D cycle with light on at 0800 h) with free access to water and standard food pellets (A04 type, UAR, Epinay, France). Rats were individually housed at least 3 days before the onset of the experiments.

Procedures

The procedure was similar to that described by Kennett and Curzon (16). Between 1800 and 1815 h the day before experimentation, food was removed from the cages. Between 1300 and 1315 h of the following day, animals were pretreated either with saline or tianeptine (10 mg/kg, IP) and treated 30 min later either with saline, 5-HTP (20 or 40 mg/kg, IP), or d-FEN (1.5 or 3 mg/kg, IP). Another 30 min later (i.e., between 1400 and 1415 h), a weighed amount of food was placed in the cage and subsequent food intakes measured 1, 2, 3, and 4 h after food presentation.

In rats treated with the highest dose of d-FEN (3 mg/kg, IP), a similar paradigm to that described above was used to assess the influence of tianeptine upon brain levels of d-FEN and of its active metabolite, namely *d*-norfenfluramine (d-NORFEN; a 5-HT releaser endowed with potent anorexigenic properties) (12–14). Thus, at the end of another 4-h test saline- and tianeptine-pretreated rats were sacrificed by decapitation and the brains, rapidly dissected out, were stored at −40°C until analysis (3 weeks later). d-FEN and d-NORFEN were extracted with benzene from aliquots (1 ml) of brain homogenates (10 ml/g) after the addition of an internal standard and analyzed by electron capture gas liquid chromatography (22). Standard curves ranging in concentrations from 25–500 ng/ml (as free bases) of brain homogenates were linear and were used in the analysis of unknown brain samples. The coefficient of variation was less than 15% in all cases.

Drugs

5-HTP (Sigma-Coger, Paris, France), tianeptine sodium salt, and d-FEN HCl (both from I.R.I. Servier, Courbevoie, France) were dissolved in physiological saline and injected IP at a volume of 1 ml/kg (tianeptine) or 2 ml/kg (5-HTP and d-FEN). All solutions were prepared 2 h before injection. 5-HTP and d-FEN were injected in the respective dosages of 20 or 40 mg/kg and 1.5 or 3 mg/kg 30 min before food presentation. To maintain comparability with other studies (3,7, 10,19), tianeptine was injected at the dose of 10 mg/kg 1 h before saline, 5-HTP, or d-FEN administration.

Statistics

Values are given as mean ± SEM. Individual food intakes were analyzed by means of an analysis of variance (with pretreatment and final treatment as main factors) with repeated measures (time), followed by Tukey's multiple-comparison test. The influence of tianeptine upon brain d-FEN and d-NORFEN levels was analyzed by Student's *t*-test.

RESULTS

The respective effects of 5-HTP (20 or 40 mg/kg, IP) and d-FEN (1.5 or 3 mg/kg, IP) on hourly rates of food intake by saline- and tianeptine-pretreated rats are shown in Table 1. Food intakes were significantly affected either by the final treatments or by the time of analysis ($p < 0.0001$ for both factors). In addition, pretreatment × treatment and treatment × time interactions could be noted ($p = 0.0161$ and $p < 0.0001$, respectively). It is noteworthy that in saline-injected rats tianeptine pretreatment (10 mg/kg, IP, 30 min before final treatments) did not significantly affect the amount of food eaten during the first, second, third, or fourth hour of analysis (Table 1) but decreased that totally consumed over the 4 h of analysis (8.6 ± 0.4 g/4 h and 6.0 ± 0.6 g/4 h in saline- and tianeptine-pretreated animals, respectively: $p < 0.01$). Moreover, the first hour of analysis was the period during which saline- and tianeptine-pretreated rats consumed most of their food, compared to the other periods (Table 1).

TABLE 1

EFFECTS OF TIANEPTINE PRETREATMENT (10 mg/kg) ON FOOD INTAKE IN 20-h FOOD-DEPRIVED RATS INJECTED WITH EITHER 5-HTP OR d-FEN

	Food Intake (g)			
Treatments	1 h	2 h	3 h	4 h
Saline				
+ Saline	4.9 ± 0.4	0.8 ± 0.4	0.7 ± 0.4	2.2 ± 0.4
+ 5-HTP (20)	3.6 ± 0.4*	0.6 ± 0.4	0.9 ± 0.4	1.3 ± 0.4
+ 5-HTP (40)	1.9 ± 0.3†	1.8 ± 0.5	0.1 ± 0.1	0.8 ± 0.5†
+ d-FEN (1.5)	3.2 ± 0.3†	2.0 ± 0.4*	0.8 ± 0.4	1.1 ± 0.4*
+ d-FEN (3)	0.4 ± 0.2†	0.5 ± 0.3	0.6 ± 0.3	0.7 ± 0.3†
Tianeptine				
+ Saline	4.6 ± 0.4	0.1 ± 0.1	0.1 ± 0.1	1.4 ± 0.6
+ 5-HTP (20)	2.9 ± 0.5†	0.3 ± 0.2	1.3 ± 0.5*	1.0 ± 0.4
+ 5-HTP (40)	2.0 ± 0.3†	1.2 ± 0.4*	0.1 ± 0.1	1.0 ± 0.5
+ d-FEN (1.5)	2.1 ± 0.2†	1.9 ± 0.2†	0.5 ± 0.4	1.3 ± 0.5
+ d-FEN (3)	1.1 ± 0.3†	0.8 ± 0.2	1.1 ± 0.3*	1.0 ± 0.4

Saline or tianeptine was administered IP 30 min before saline, 5-HTP or d-FEN, and food restored another 30 min later. Values are the means ± SEM of 8 rats. Doses of 5-HTP and d-FEN (in mg/kg IP) are indicated in brackets. *$p < 0.05$, †$p < 0.01$ for the effects of 5-HTP and d-FEN against saline.

Acute administration of 5-HTP or d-FEN triggered immediate dose-dependent reductions in food intakes: In both cases, the first hour of analysis was that during which these drugs exerted their hypophagic effects (Table 1). As shown in Table 1, tianeptine pretreatment proved ineffective against either 5-HTP- or d-FEN-elicited immediate hypophagia. Besides, 5-HTP (40 mg/kg) and d-FEN (1.5 or 3 mg/kg) decreased the amount of food consumed by saline- but not tianeptine-pretreated rats throughout the fourth hour. However, this lack of hypophagic effect of 5-HTP or d-FEN in tianeptine-pretreated rats was solely due to a decrease in food consumed by saline-treated rats (Table 1).

In keeping with the observations that a) the highest dose of d-FEN diminished the amount of food eaten by saline-pretreated rats during the fourth hour and b) brain d-NORFEN levels are at their maximal levels 4 h after d-FEN administration (12), brain d-FEN and d-NORFEN levels were measured 4 h after d-FEN injection into saline- and tianeptine-pretreated rats. Actually, Table 2 shows that tianeptine pretreatment did not affect brain d-FEN and d-NORFEN levels, compared to saline pretreatment, thereby indicating that the inability of tianeptine to counteract d-FEN-induced hypophagia was not due to changes in d-FEN pharmacokinetics.

DISCUSSION

It is now well accepted that enhanced 5-HT release triggers satiation in rats and humans (1,18). Such evidence has been first brought about by studies that involved the use of 5-HT precursors, reuptake inhibitors, and/or releasers (2,12–14) and confirmed by the use of selective 5-HT receptor agonists (9,16,21). In the present study, both 5-HTP and d-FEN were found to decrease (in a dose-dependent manner) food consumption, thus extending previous results (2,12–14). It is noteworthy that both drugs a) act mainly through central mechanisms (2,12–14), b) elicit 5-HT release (at least at the doses used herein) (8,11), and c) decrease selectively the size and duration of feeding bouts, thereby confirming that increased release of 5-HT from central serotonergic neurons leads to precocious satiety.

TABLE 2

INFLUENCE OF TIANEPTINE PRETREATMENT (10 mg/kg) ON BRAIN d-FEN AND d-NORFEN CONCENTRATIONS IN 20-h FOOD-DEPRIVED RATS INJECTED WITH d-FEN (3 mg/kg)

	Brain Concentrations (μg/g)	
Pretreatment	d-FEN	d-NORFEN
Saline	0.63 ± 0.09	3.07 ± 0.21
Tianeptine	0.71 ± 0.07	2.88 ± 0.12

Saline or tianeptine was administered IP 30 min before d-FEN, and food restored another 30 min later. Rats were sacrificed 4 h after food presentation. Values are the means ± SEM of 6 rats.

The results presented herein revealed that tianeptine pretreatment did not significantly affect the hourly rate of food consumption, although it decreased cumulative 4-h food intake. However, compared with 5-HTP and d-FEN (which both decreased the amount of food eaten throughout the first hour) analysis of food intakes in tianeptine-pretreated rats confirmed that tianeptine is devoid of any 5-HT reuptake-inhibitory and/or 5-HT-releasing properties (at least immediately after administration) (10,19). On the other hand, the mechanisms underlying the effect of tianeptine pretreatment on 4-h food consumption in saline-injected rats are at present unknown as this study did not include a microstructural analysis of feeding. Indeed, the paradigm used herein, that is, food deprivation, could account for the cumulative hypophagic effect of tianeptine as tianeptine does not affect feeding in free-feeding animals [(23); Codegoni and Mennini, unpublished observations]. Another hypothesis would be a side effect of tianeptine, for example, upon the gastrointestinal tract [where 5-HT is present and plays numerous roles: (20)], thereby affecting the amount of food ingested following a first large meal. To my knowledge, this hypothesis has never been addressed before.

As pointed out in the introductory section, the main issue of this study was to investigate whether tianeptine, by virtue of its ability to stimulate 5-HT reuptake opposed 5-HT-induced satiety. Thus, past reports indicated that tianeptine pretreatment (at doses similar to that used herein) reduces both the frequency of wet-dog shakes and the amplitude of forepaw treading elicited by 5-HTP (17,23,24). Moreover, a recent study has shown that tianeptine opposes the loss in body weight elicited by increased central serotonergic activity (as suggested by the combined administration of 5-HTP and a peripheral amino acid decarboxylase inhibitor) (3), while De Simoni et al. (7) reported that the increased amount of 5-HT released after d-NORFEN (or 5-HTP) administration is actively taken up and metabolized into 5-hydroxyindoleacetic acid (5-HIAA) in tianeptine-pretreated rats. Actually, the present study proved unable to confirm these data as tianeptine did not alleviate the immediate hypophagic effects of 5-HT release, thereby extending the observation that tianeptine administration to fed rats trained to eat their daily ration in 4 h (21) does not affect d-FEN-elicited anorexia (Codegoni and Mennini, unpublished results). In the present study, it is noteworthy that the lack of effect of tianeptine pretreatment cannot be accounted for by an interaction between tianeptine and the access of d-FEN molecules to the brain and/or d-FEN metabolism. Then, the possibility that tianeptine opposes the consequences of 5-HT release in some paradigms, but not in others, remains open.

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