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Behavioural Changes on Diet Selection and Serotonin (5-HT) Turnover in Rats Under Pizotifen Treatment[1]

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GALANOPOULOU, P., G. GIANNACOPOULOS, C. THEOPHANOPOULOS, M. K. COUVARIS AND D. VARONOS. *Behavioural changes on diet selection and serotonin (5-HT) turnover in rats under pizotifen treatment.* PHARMACOL BIOCHEM BEHAV **37**(3) 461–464, 1990.—The effects of pizotifen on protein and carbohydrate self-selection in rats over a seven-day period, and on 5-HT turnover was studied. Four groups of male Wistar rats were individually caged and ad lib fed with a standard (SD) and (50%) carbohydrate-enriched diet (CED), sweet (diet group I) or not (diet group II). Food intake was measured daily 4 hr after IP injection of pizotifen (2.5 mg/kg) or vehicle. 5-HT and 5-HIAA in the hypothalamus (Hy), striatum (St) and hippocampus (Hi) were assayed on the 8th day of the experiment. Pizotifen increased the consumption of SD. The absolute intake of CED remained totally and daily unchanged, while the percentage proportion was reduced. Total food intake was increased by the drug which seemed to affect the proportion rather than the absolute amounts of carbohydrate and protein consumed. This effect was independent of the carbohydrate taste. There was a decrease of 5-HT levels in the Hi, while 5-HIAA/5-HT ratio was increased in the Hy and in the Hi of animals that consumed sweet carbohydrate. The above data suggest a role of pizotifen on 5-HT central metabolism and diet selection and support the view that changes of 5-HT metabolism in the Hy and Hi are responsible for protein selection and the regulation of SD/CED ratio, but they cannot explain drug's effect on total food intake.

Pizotifen 5-HT 5-HIAA 5-HT turnover Brain regions Food intake Diet selection Carbohydrate intake Protein intake

CLINICAL studies have shown that patients on pizotifen or other serotonin antagonist treatment develop an excessive craving for carbohydrates which is not a reflection of any underlying change in glucose tolerance or insulin level (6, 13, 18). It has also been reported that these drugs increase appetite which results in gain in weight (19,20). It should be emphasised that increase on food intake is more difficult to detect in animals (1).

In addition to a postulated action on total food intake and the structure of feeding behavior, it has been suggested that 5-HT plays a role in the selection of nutrients, particularly protein and carbohydrate (1). Thus, drugs that enhance central serotonergic transmission selectively decrease rat consumption of carbohydrates without affecting protein intake (22).

The purpose of this study was two-fold: first, to investigate the effect of pizotifen on food intake as well as to examine any tendency to additional carbohydrate consumption under drug's treatment. For this reason animals were simultaneously given two diets: standard laboratory diet with sufficient supplies to nutrients and a carbohydrate-enriched diet. Secondly, to find the relationships between these behavioural patterns on food intake and the 5-HT turnover changes in different regions of the brain.

METHOD

Animals

Four groups of 12 male Wistar strain rats each 70 ± 10 days old, weighing 170 ± 10 g, were individually housed in metabolic cages each supported by two feeders in a quiet environment, with a 12-hr light/dark cycle and allowed free access to water.

Procedure

Five days prior to the start of the experimental treatment, the rats were acclimatized and were trained to consume their food

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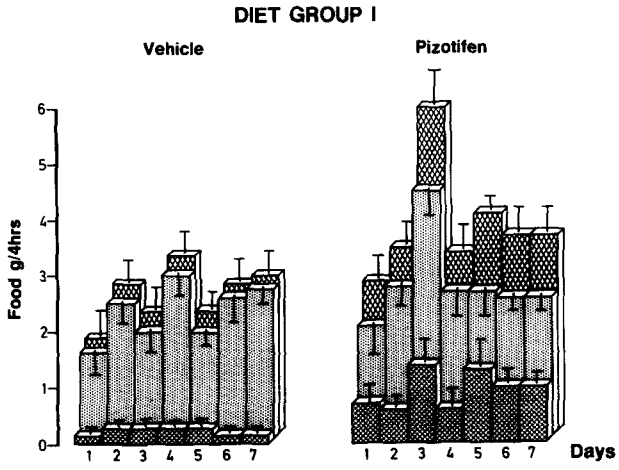

FIG. 1. Daily selective consumption of SD (heavily dotted bars), CED (lightly dotted bars), and total food intake (cross-hatched bars) by rats given choice of diet group I. Each value is the mean ± SEM from 12 rats. Diet group I: SD, $p<0.01$; total food, $p<0.05$.

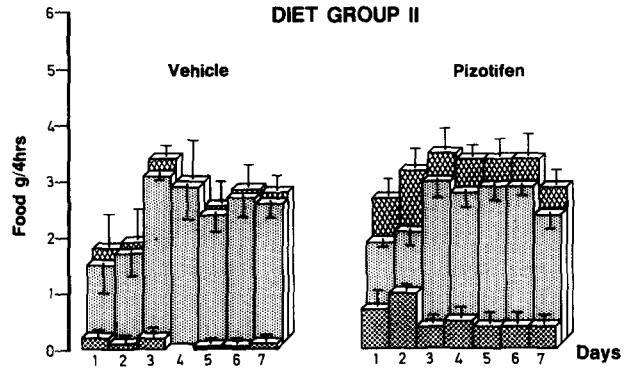

FIG. 2. Daily selective consumption of SD (heavily dotted bars), CED (lightly dotted bars), and total food intake (cross-hatched bars) by rats given choice of diet group II. Each value is the mean ± SEM from 12 rats. Diet group II: SD, $p<0.01$; total food, $p<0.05$.

from two different diet groups for 24 hr after treatment of sham IP injections at times corresponding to subsequent drug administration (9 a.m.).

At the start of the experiment the animals were randomly sorted into 4 groups, two of which were used to examine the effects of the vehicle (control) or pizotifen (provided by Sandoz Pharma Co.) on diet group I, whilst the other two were used to examine the effects of the same treatment on diet group II.

Each diet group was supplied with the following pair of pulverized and well-mixed food compositions: Diet group I: Standard rat diet (SD), surcose-enriched diet (SED) (50% SD + 50% sucrose). Diet group II: Standard rat diet (SD), dextrin-enriched diet (DED) (50% SD + 50% dextrin). The standard laboratory rat diet had the following composition: proteins 20%, fat 2–5%, cellulose 6%, ash 18%, calcium 1–1.8%, phosphorus 0.8–1% and humidity 13%.

Animals were given simultaneous access to each pair of diets 30 min after IP injection of the vehicle or pizotifen (2.5 mg/kg). Feeders with preweighed food were removed after 4 hr of food consumption selected by animals and weighed every successive day of the experiment. Following that, animals were allowed ad lib access to the same pair of diets until the next day when the experiment was repeated. The position of the feeders was alternated daily.

Animals were decapitated between 1–2 p.m. on the eighth day and 2 hr after treatment with the vehicle or drug. The brains were rapidly removed and hypothalamic, hippocampal and striatal regions were taken. Brain 5-HT and 5-HIAA were determined fluorometrically following acid-butanol extraction according to the Curzon and Green method (5), and the 5-HIAA/5-HT ratio (as an indirect index of 5-HT turnover) was calculated. The results were analysed by analysis of variance and the Student's *t*-test.

RESULTS

Food Intake

There was a significant increase of food consumption in the pizotifen treatment groups versus controls in both dietary groups (diet group I: $F=8.5$, $p<0.01$; diet group II: $F=5.33$, $p<0.05$) (Figs. 1 and 2 and Table 1). Under both experimental conditions animals of each diet group preferred the carbohydrate-enriched (sweet or not) diet (diet group I: $F=109.4$, $p<0.01$; diet group II: $F=64.6$, $p<0.01$).

Despite the fact that the absolute intake of carbohydrate-enriched food was unchanged both totally and daily during all the 7 days of treatment, the percentage proportion was reduced

TABLE 1

EFFECT OF PIZOTIFEN OR VEHICLE ON STANDARD DIET (SD), CARBOHYDRATE-ENRICHED DIET (CED) AND TOTAL FOOD INTAKE

		Food Intake (9/4 hr)			% of Total Food Intake		
Diet Group	Treatment	SD	CED	Total Intake	SD	CED	SD/CED
I	Vehicle	0.19 ± 0.02	2.40 ± 0.21	2.60 ± 0.21	7.30 ± 1.05	92.3 ± 1.0	0.08
	Pizotifen (2.5 mg/kg)	0.98 ± 0.13†	2.70 ± 0.30	3.68 ± 0.36‡	26.60 ± 3.5*	73.40 ± 3.5*	0.36
II	Vehicle	0.11 ± 0.02	2.32 ± 0.2	2.44 ± 0.21	4.50 ± 1.0	95.08 ± 1.0	0.05
	Pizotifen (2.5 mg/kg)	0.54 ± 0.10†	2.50 ± 0.17	3.02 ± 0.11‡	17.80 ± 3.3†	82.5 ± 3.3*	0.22

*$p<0.001$; †$p<0.01$; ‡$p<0.05$.
Percentage contribution of SD and CED on total food intake. Values are means ± SEM from 12 rats and 7-day measurements.

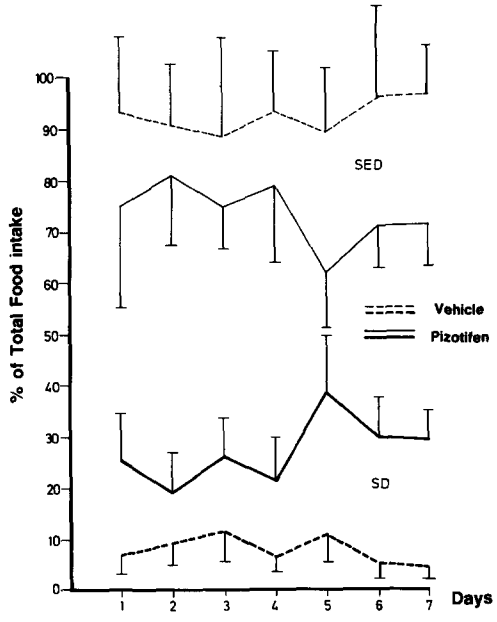

FIG. 3. Pattern of selective consumption as percent of total food intake by rats given diet group I. Each value is the mean ± SEM from 12 rats.

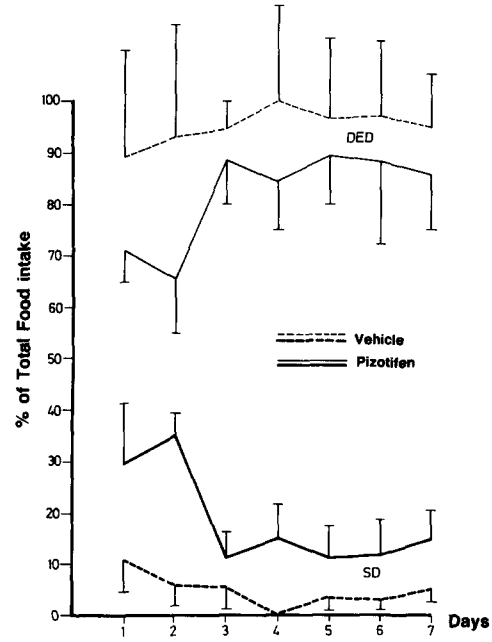

FIG. 4. Pattern of selective consumption as percent of total food intake by rats given diet group II. Each value is the mean ± SEM from 12 rats.

($p<0.001$). Additionally, an inverse relationship between the percentage proportion of the standard (SD) and the carbohydrate-enriched diet (CED), respectively, was observed. The effect was independent of the carbohydrate taste (Figs. 3 and 4).

Since the drug increased the consumption of SD in both experimental conditions (diet group I: $F=35.4$, $p<0.01$; diet group II: $F=17.7$, $p<0.01$), while the absolute intake of CED remained constant, there was a subsequent increase in total food intake in both treatment groups (diet group I: $F=6.61$, $p<0.05$; diet group II: $F=5.6$, $p<0.05$). It should be stressed that animals which received larger amounts of SD consumed more protein since in the CED the protein content is halved.

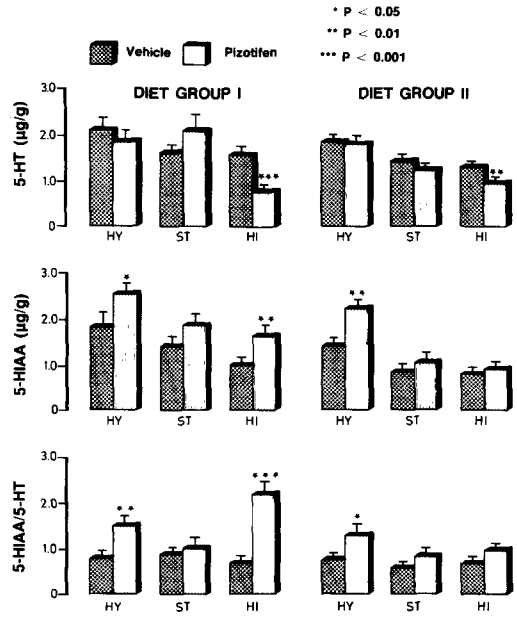

FIG. 5. Levels of 5-HT, 5-HIAA and ratio of 5-HIAA/5-HT in hypothalamus (HY), striatum (ST) and hippocampus (HI) of rats given diet group I or II. The height of each column represents the mean ± SEM, as determined from 12 rats.

5-HT Turnover

The effects of pizotifen on regional levels of 5-HT, 5-HIAA and the 5-HIAA/5-HT ratio are shown in Fig. 5. It seems that pizotifen in both experimental conditions (diet group I and II) increased 5-HT turnover in the Hy ($p<0.01$, $p<0.05$) as evidenced by the increased 5-HIAA levels ($p<0.05$, $p<0.01$), while the 5-HT concentrations remained relatively unchanged.

No changes of 5-HT turnover in the St were observed. A decrease of 5-HT levels was observed in the Hi under both experimental conditions ($p<0.01$, $p<0.001$) with 5-HT turnover increased significantly ($p<0.001$) only in diet group I as evidenced by the increased 5-HIAA levels ($p<0.01$).

DISCUSSION

Earlier pharmacological studies have indicated that pizotifen has strong antiserotonergic properties (20). Pizotifen (BC 105) belongs to the benzocycloheptathiophene group and is therapeutically used against migraine (6, 15, 20). Its action against migraine has also been proposed to be based upon the antiserotonergic properties of the drug (10,15).

The gain in weight due to an increase in appetite is a common occurrence during pizotifen therapy in humans. Thus, some patients (especially the first few weeks of treatment) describe their appetite as voracious and there is often a specific craving for sweets. Indeed, some patients stop treatment due to unacceptable weight gain despite the fact that their migraine attacks cease significantly (6,20).

In our present experiment the drug led to an increase of total food intake, a finding which is consistent with clinical results (14,21). Surprisingly, the drug did not lead the animals to more carbohydrate consumption. On the contrary, animals consumed larger amounts of SD (with higher protein content) and the same amount of carbohydrate-enriched diet as the control group. Additionally, there was a reduction of the percentage proportion of CED on behalf of SD on a day-to-day basis, and the effect is independent of the carbohydrate's taste. Based on the percentage proportions of SD and CED we have observed a regulatory role of pizotifen on SD/CED ratio which favours the SD portion.

There was also an inverse relationship between CED and SD on a day-to-day basis. This finding is supported by other investigators as well who claim that this is observed on a meal-to-meal basis too, and is explained as a procedure which may allow animals to achieve daily as well as weekly constancy in macronutrient consumption (3, 11, 12). In our experiments, this observation was evident for the first 6 days in diet group I and the first 5 days in diet group II.

The above behavioural patterns on food intake and diet selection can partially be explained with the observed changes of 5-HT metabolism. There was an increase of 5-HIAA and 5-HIAA/5-HT ratio in Hy in both diet groups and in Hi in group I with 5-HT levels decreased in Hi of both diet groups.

These results show an increased serotonergic activity under drug treatment. It is known that the serotonergic type of action on food intake is a protein-sparing and carbohydrate-suppressing effect, and is achieved by drugs that enhance serotonergic transmission (1,22). According to our results, pizotifen's effect on serotonergic system seems to be mainly focused in Hi.

It seems logical that pizotifen, by blocking certain 5-HT receptor(s), may cause a reflex stimulation on serotonergic activity, but the heterogeneity of 5-HT receptors makes it difficult for the time being to estimate the central mode of pizotifen action on serotonin receptors. There are authors who claim that the drug acts as a selective 5-HT_{1A} antagonist (17) or a selective 5-HT_2 antagonist as well (4). On the other hand, the increased serotonergic activity cannot by itself explain the increased food intake observed under drug treatment. Additionally, if the drug's effect is focused exclusively on serotonergic system, a reduction in carbohydrate consumption should have been expected too (22). So it is possible that the drug affects appetite by a dual mode of action, i.e., by a simultaneous interference to another neurotransmitter or by a peripheral action as well.

Previous studies have shown that the drug has strong antihistaminic, mild anticholinergic and adrenolytic properties (20). Other studies have shown that pizotifen possesses mild hypoglycaemic properties (since blood glucose fasting levels were significantly reduced in humans after 2 weeks of treatment) which might be due to either inhibition of glucogenolysis or glyconeogenesis in the liver since no effect on insulin levels was observed (8,9).

On the other hand, Drash and Sicuteri suggest that a mild hypoglycaemic effect is a property of all antiserotonin agents (7,16). Although such an action hasn't been established in experimental animals, it is possible to occur and probably plays a role on the appetite-stimulating properties of the drug.

In conclusion, the above data suggest a role of pizotifen on appetite stimulation and 5-HT metabolism and support the view that changes of 5-HT metabolism in Hy and Hi mainly are involved in nutrient (particularly protein and carbohydrate) selection, but cannot explain the drug's effect on total food intake.

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