5-hydroxytryptophan-stimulated prolactin levels in cafeteria diet fed rats: An in vivo evaluation of the central serotonergic tonus


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ABSTRACT. Hyperphagia in rats fed a cafeteria diet might be related to the palatability of the diet or to diet-induced changes in central neurotransmitters regulating the feeding behavior. In this study the central serotonergic tonus in adult male Wistar rats was evaluated in vivo after 6 weeks of feeding a cafeteria diet by the prolactin response to the administration of 5-hydroxytryptophan (5HTP), the immediate serotonin precursor. Blood was taken just before, 30, 60 and 90 min after the ip injection of 50 mg/kg 5HTP for the determination of prolactin by a standard RIA. Means (±SE) basal prolactin concentrations were comparable between cafeteria fed rats and control rats, fed normal laboratory chow (12.7±5.4 vs 7.7±4.5 ng/ml). The 5HTP-stimulated prolactin secretion in the cafeteria diet fed rats, determined by the peak value (95.8±17.2 vs 119.1±27.0 ng/ml) as well as by the integrated area under the curve (5478±774 vs 5916±2275 ng/ml, 90 min) was not significantly lower than in the control rats. In conclusion, our results did not show a significantly decreased 5HTP-induced prolactin release in cafeteria-fed rats, suggesting that a low hypothalamic serotonergic tonus is probably not involved in the overeating of this dietary-induced obesity model.

INTRODUCTION

Obesity can be produced in laboratory animals by a variety of dietary manipulations. Cafeteria food (food marketed for human consumption such as cookies, cheese, salami, peanut butter, etc.) induce in rats a greater weight gain than standard high fat or high sugar diets. The variety and the palatability of such a cafeteria diet have been put forward as major factors contributing to the overeating in this obesity model (1). The exaggerated response seen in animals with hypothalamic lesions compared to neurologically intact rats and the differences observed in weight gain between rats of the same and of different strains suggest that other factors, such as central or hypothalamic factors regulating the eating behavior, might be involved (1-3). A diet-induced change in central serotonergic tonus might play a role in the hyperphagia of cafeteria diet fed rats: low levels of 5-hydroxyindolacetic acid (5HIAA), a metabolite of serotonin, have been found in the ventromedial nucleus of the brain in rats after 2 months feeding a cafeteria diet, while fasting was shown to increase the central serotonergic tonus (4, 5). In the present study, we wanted to test whether a lower central serotonergic tonus existed in cafeteria diet fed rats compared to rats fed normal laboratory chow. The prolactin response to the administration of 5-hydroxytryptophan (5HTP), the direct precursor of serotonin, was used to assess the functional activity of the serotonergic neurons: peripheral injection of 5-hydroxytryptophan is known to increase the brain serotonin concentration and to induce the release of prolactin in the blood (6, 7).

MATERIALS AND METHODS

Animals

Thirteen male Wistar rats (Proefdierencentrum KUL, Leuven, Belgium), weighing 140-150 g were housed five or four per cage at a constant temperature (22 C) under a 12L:12D light regime (lights on at 07:00 h). A standard laboratory rat chow (Sourifarat, maintenance rats and mice diets, France) and water was available ad libitum for all rats, while 5 rats were offered supplementary different kinds of palatable foods every 2 days, e.g. cheese, ham, bacon, salami, crackers, cookies, muffins, etc. All rats were weighed weekly. One week before the in vivo experiment rats were manipulated daily.
Drugs
L-5-hydroxytryptophan (5HTP, Sigma, St. Louis, Mo, USA) was dissolved in 0.9% NaCl immediately before use. The drug and corresponding control vehicle (0.9% NaCl) were injected ip in a volume of 0.5 ml.

In vivo experiments
After six weeks of feeding, the five animals fed the cafeteria foods and four animals receiving only the lab chow were injected intraperitoneally with 50 mg/kg dissolved 5HTP, while the four other laboratory chow fed rats were injected an equal volume of NaCl 0.9%. Just before the injection and 30, 60 and 90 min later 2 ml of blood was taken in plastic centrifuge tubes by tail puncture. The experiment was started at 14:00 h in each group.

The prolactin assay
The prolactin concentration in serum and culture medium was measured by double anti-body radioimmunoassay (RIA) according to the procedures outlined by the NIAMDD (Bethesda, USA). The results are expressed as ng/ml of the Niamdd standard. The interassay variation of the assay in our hands was lower than 10%. All samples from the same experiment were analyzed together.

Statistical analysis
The Mann-Whitney U test and Wilcoxon Rank test were used for comparison and the Kendall Rank test for correlation analysis. A 0.05 level of probability was used as the criterion of significance for all data. Results were expressed as mean±SE.

RESULTS
Despite the cafeteria fed rats were clearly hyperphagic and gaining more weight on average, their mean body weight was not yet significantly higher than that of the others (373±15 vs 344±10 g) at the moment of the experiment. No significant difference in mean basal serum prolactin concentration between the cafeteria diet fed group and the lab chow fed group was found (12.7±5.0 ng/ml vs 7.7±4.5 ng/ml). As shown in Figure 1, 5HTP induced in the cafeteria fed rats (n = 5) as well as in the laboratory chow fed rats (n = 4) a significant increase in mean circulating prolactin concentration at 30, 60 and 90 min, compared to the prolactin response seen in the saline injected laboratory chow fed animals (n = 4). The calculated area under the prolactin response curve (5478±774 vs 5916±2275 ng/ml. 90 min) and the peak PRL concentration (95.8±17.2 vs 119.1±27.0 ng/ml) after 5HTP injection were not significantly different between the cafeteria diet fed rat and normal chow fed rats. The two lowest prolactin responses occurred in the cafeteria fed rats. In both groups a high interindividual variation in peak prolactin response was observed (CV between 40% and 46%). The amplitude of the prolactin response was neither related to basal prolactin concentration nor to the weight gain of the animal.

DISCUSSION
The ip injection of 5HTP induced a dramatic increase in serum PRL in rats fed a normal laboratory chow as well as in those offered cafeteria foods. This increase in prolactin cannot only be described to the stressful procedure of blood sampling since the response to 5HTP was at least 14 times higher than in vehicle injected animals. Previous studies in rats using 5HTP in a higher dose have shown a 4 up to 20 times increase of prolactin in the blood (7, 8). The finding of an enhanced response to 5HTP in fluoxetine (a compound that blocks the amine pump of serotonin neurons) -treated animals and the restoration of prolactin release to suckling by 5HTP in by p-chlorophenylalanine (an inhibitor of serotonin synthesis) serotonin-depleted animals increases the evidence that 5HTP is exerting a selective response to serotonin neurons (9, 10). Since 5HT1, 5HT2, as well as 5HT3 receptors appear to be involved in the mediation of the prolactin response to 5HTP, the relative insensitivity of a specific 5HT receptor subtype cannot be assessed pharmacologically by the 5HTP challenge test (11, 12). Anatomically the 5HTP-stimulated prolactin release acts through the paraventricular nucleus, which is through its serotonergic and α2-adrenergic inputs.