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The Effect of Metoprine on Glucoprivic Feeding Induced by 2-Deoxy-D-Glucose

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LECKLIN, A., M. JÄRVIKYLÄ AND L. TUOMISTO. *The effect of metoprine on glucoprivic feeding induced by 2-deoxy-D-glucose.* PHARMACOL BIOCHEM BEHAV **49**(4) 853–857, 1994.—Metoprine is a histamine *N*-methyltransferase inhibitor that elevates endogenous histamine (HA) levels. Because the histaminergic mechanism may be involved in the regulation of feeding behavior as well as in body glucose homeostasis, the effect of metoprine on glucoprivic feeding was studied in Wistar rats. Although metoprine treatment (10 and 20 mg/kg, IP) decreased feeding, the rats still responded to the administration of 400 mg/kg of 2-deoxy-D-glucose (2-DG) by increasing their feed intake. No difference was seen in the 6-h cumulative feed intake after administration of 2-DG between the metoprine- and solvent-treated rats. However, the response was delayed, and with 20 mg/kg metoprine the feed intake was significantly reduced during 2 h after 2-DG application. Both 2-DG and metoprine elevated plasma glucose concentration despite their opposite effects on feeding. Hypothalamic HA or its metabolite levels were not affected by 2-DG. The results suggest that the effects of metoprine and 2-DG are largely independent of each other, and that the feeding modulating function of HA is on such a level that it does not prevent the glucoprivic emergency response.

Histamine 2-Deoxy-D-glucose Feeding Glucoprivation Methylhistamine

TREATMENT of eating disorders, such as obesity and anorexia nervosa, is problematic and often a favorable outcome is not reached because the understanding of the basic physiological mechanisms regulating food intake is incomplete. Among other neurotransmitters, the brain histaminergic systems have been suggested to be involved in the regulation of feeding behavior. The administration of histamine (HA) (3,9,33) or its precursor histidine (20,28) suppresses feeding. Inhibition of HA catabolism by metoprine with a subsequent accumulation of endogenous HA also decreases feed intake (13). Furthermore, α-fluoromethylhistidine, a compound that blocks histamine synthesis and agents with antihistaminic activity have been shown to facilitate feeding (6,20–22,24, 25,34).

Although the manipulation of histaminergic systems has been shown to alter feeding behavior, and histaminergic neurons in the hypothalamus have been suggested to play a role in maintaining glucose homeostasis (17,18), the involvement of HA in glucoprivic feeding control has not been studied. 2-Deoxy-D-glucose (2-DG) is an antimetabolic glucose analogue that produces a rapid reduction in cerebral glucose utilization. Rats respond to 2-DG treatment by increasing their short-term feed consumption (23). The aim of the present study was to examine whether the inhibition of HA catabolism by metoprine (4) affects 2-DG-induced feeding. Because eating is physiologically connected to periprandial drinking (5,11), effects on body fluid balance, drinking behavior, and urine excretion were also measured.

METHOD

Animal Care

Male Wistar rats (270 ± 29 g) were adapted for 4 days to plastic metabolic cages (Tecniplast® 1700) in a temperature-controlled room (20 ± 1°C) with a light–dark cycle of 14 L : 10 D with lights switched on at 0700 h. Relative air humidity was 40–60%. Before and throughout the experiments all the rats had free access to tap water and R3 feed (Ewos, Södertälje, Sweden) that was given in powdered form in the metabolic cages.

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Chemicals

Metoprine (Wellcome Research Laboratories, USA) was dissolved in 1% lactic acid (Sigma Chemical Co., St. Louis, MO) and 2-DG (Sigma Chemical Co.) was dissolved in saline.

Procedure

On the test day, at 0700 h, 10 or 20 mg/kg of metoprine or solvent was administered IP to the rats (n = 86). Two hours later (at 0900 h) the rats were given 400 mg/kg 2-DG or saline IP. Individual cumulative feed consumptions were measured over time periods of 1, 2, 3, 4, and 6 h. Water consumption and urine output over the 6-h period were measured, and urine samples were taken for the measurement of osmolality.

A separate group of rats (n = 24) was administered either 20 mg/kg metoprine or solvent, and 2 h later they were given 2-DG (400 mg/kg) or saline. Three hours after that the rats were decapitated and trunk blood was collected. The heads were cooled in liquid nitrogen and the hypothalami were dissected on ice and then stored at −70°C.

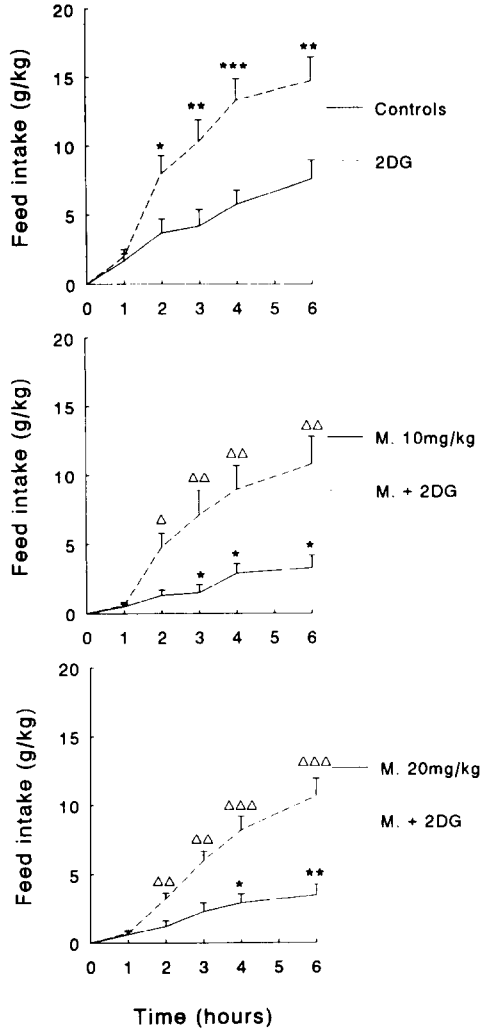

FIG. 1. Cumulative feed consumption after the treatment with 400 mg/kg of 2-DG or saline in metoprine (10 or 20 mg/kg)- and solvent-treated rats. Mean ± SEM of 13-16 animals. Statistical evaluation was carried out by the Kruskal-Wallis test: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. the controls; $\Delta p < 0.05$, $\Delta\Delta p < 0.01$, $\Delta\Delta\Delta p < 0.001$ vs. respective metoprine group.

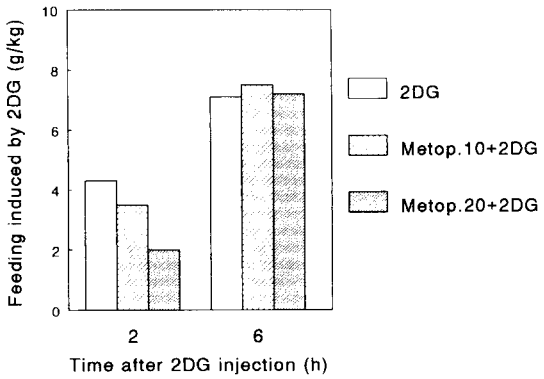

FIG. 2. Glucoprivic feeding response to 2-DG in solvent- and metoprine-treated rats 2 and 6 h after 2-DG injection. The values are group means. They were calculated as increases in the cumulative feed consumption by using the level after pretreatment (solvent or metorine) as the baseline. The feed intake of the group pretreated with metoprine 20 mg/kg differed statistically significantly from that of the controls 2 h after 2-DG injection ($p < 0.01$).

Analyses

The hypothalamus was homogenized by sonication (soniprep 150 ultrasonic disintegrator) in 0.4 N perchloric acid containing 5 mM EDTA. The homogenate was centrifuged at 24,000 × g for 15 min at +4°C. HA was assayed by HPLC (37). *tele*-Methylhistamine (Me-HA), the main metabolite of HA catabolism in the brain, was estimated by gas chromatography-mass spectrometry by the method described by Hough and coworkers (7) with some modifications as adapted to brain samples (35).

Proteins were assayed by the method of Lowry (15). Plasma glucose was determined with glucose dehydrogenase method (Granutest® 100). Urine osmolality was measured with an Osmostat OM-6020 osmometer based on the freezing-point depression method.

Statistics

The group means ± SEM were calculated. The Kruskal-Wallis test was used to assess differences between groups.

TABLE 1

GLUCOSE CONCENTRATION IN THE PLASMA 3 h AFTER ADMINISTRATION OF 2 DG IN METOPRINE- AND SOLVENT-TREATED RATS HAVING FREE ACCESS TO FEED DURING THE EXPERIMENT

Treatment	Glucose (mM)
Controls	5.3 ± 0.3
2 DG	10.4 ± 0.5*
Metoprine 20 mg/kg	6.9 ± 0.3*
Metoprine 20 mg/kg + 2 DG	10.0 ± 0.7*

Values are mean ± SEM (n = 6). Statistical significance vs. the controls is determined by the Kruskal-Wallis test: *$p < 0.01$.

TABLE 2

WATER CONSUMPTION, URINE OUTPUT, AND OSMOLALITY PER 6 h AFTER ADMINISTRATION OF 2 DG (400 mg/kg) OR SALINE IN METOPRINE- AND SOLVENT-TREATED RATS

	Water Consumption (ml/kg b.wt.)	Urine Output (ml/kg b.wt.)	Urine Osmolality (mosmol/kg H_2O)
Controls	14.0 ± 1.4	12.5 ± 1.0	1476 ± 117
2 DG	28.0 ± 3.9*	22.2 ± 3.0*	1638 ± 105
Metoprine 10 mg/kg	29.3 ± 4.7*	28.6 ± 5.0†	751 ± 137†
Metoprine 10 mg/kg + 2 DG	34.3 ± 6.0*	30.9 ± 4.5†	1096 ± 100‡
Metoprine 20 mg/kg	64.8 ± 11.1†	63.3 ± 11.8†	412 ± 67†
Metoprine 20 mg/kg + 2 DG	42.6 ± 6.1†	36.9 ± 5.7†	1079 ± 133

Values are mean ± SEM (n = 8–16) calculated per rat body weight (b.wt.) and those marked with symbols differ significantly from the controls (Kruskal–Wallis): *$p < 0.01$, †$p < 0.001$, ‡$p < 0.05$.

RESULTS

Figure 1 illustrates the changes in feeding behavior after injections of 2-DG or saline in metoprine- and solvent-treated rats. Glucoprivation induced by 2-DG increased feed consumption. Compared with the feed intake of the controls, the Kruskal–Wallis test for each time point revealed significant increases in feed consumption at 2 h ($p < 0.05$), 3 h ($p < 0.01$), 4 h ($p < 0.001$), and 6 h ($p < 0.01$) after the administration of 2-DG. On the other hand, metoprine treatment suppressed feeding. Both doses of metoprine caused statistically significant decreases in feed consumption [metoprine 10 mg/kg: 3 h, 4 h, 6 h ($p < 0.05$) and metoprine 20 mg/kg: 4 h ($p < 0.05$) and 6 h ($p < 0.01$)].

During the 6-h test, 2-DG-treated rats ate about 7.2 g/kg more than the controls ($p < 0.01$). Also, after administration of 2-DG the metoprine pretreated groups ate 7.2–7.5 g/kg more than the rats receiving only metoprine (10 mg/kg, $p < 0.01$, and 20 mg/kg, $p < 0.001$). However, there was a dose-related delayed feeding response to 2-DG. During the first 2 h, the 2-DG-induced feed intake was significantly less ($p < 0.01$) in the group pretreated with 20 mg/kg of metoprine than in that one treated with solvent (Fig. 2).

2-DG increased plasma glucose levels ($p < 0.01$) (Table 1), as did metoprine ($p < 0.01$), although it decreased feeding, which shows that the rise in the plasma glucose concentration was due to the treatment and not due to the amount of feed eaten. After 2-DG the glucose levels were almost twofold but were not affected by metoprine pretreatment.

2-DG slightly increased water consumption ($p < 0.01$) and urine volume ($p < 0.01$), but it did not affect urine osmolality. Metoprine alone or together with 2-DG increased 6-h water consumption and urine volume dose dependently. Also, a dose-dependent decrease in urine osmolality was seen in these groups (Table 2).

Hypothalamic HA and Me-HA concentrations were not markedly affected by 2-DG 3 h after its application, but 20 mg/kg of metoprine significantly increased HA ($p < 0.05$) and decreased Me-HA ($p < 0.01$) concentrations in the hypothalamus (Table 3).

DISCUSSION

The present results indicate that the inhibition of HA catabolism by metoprine in the rat postpones the glucoprivic feeding response to 2-DG administration. Still, the net increases in 2-DG-induced feed intake during 6 h were not different in the metoprine- and solvent-pretreated animals. It is interesting that area postrema lesions have also been demonstrated to inhibit 2-DG response at 2 h but not at 6 h (23). The marked suppression of feed consumption by metoprine (13) was confirmed by the present study, which supports the suggestion of an inverse relationship between brain histaminergic activity and feeding behavior (3,6,8,20,22,24,25). H_1 receptors, particularly those in the ventromedial hypothalamus and the paraventricular nucleus, are thought to regulate feed intake (24), whereas stimulation of central H_2 receptors causes no change in feeding behavior (6,8,12). In the present study, metoprine injected IP inhibited HA catabolism both in the brain and in the periphery. Although peripheral HA is effectively deaminated by diamine oxidase, participation of peripheral mechanisms cannot be ruled out. However, one would expect the activation of peripheral H_2 receptors to stimulate rather than suppress feeding by increasing gastric acid secretion.

Glucoreceptors mediating feeding responses to 2-DG have been found in the lateral hypothalamic area (1,36) and in the caudal hindbrain, possibly in the area postrema and the nucleus of the solitary tract (23). At least the latter nuclei seem to be important for the integration of peripheral inputs and contain neural machinery for the reflexive glucoprivic control (23). Without involvement of higher brain areas, decerebrate animals can maintain even sophisticated feeding behaviors (16). Such independence in the most basic and immediate responses might explain the fact that changes in brain neuro-

TABLE 3

HYPOTHALAMIC HISTAMINE (HA) AND METHYLHISTAMINE (Me-HA) CONCENTRATION 3 h AFTER ADMINISTRATION OF 2 DG IN METOPRINE- AND SOLVENT-TREATED RATS HAVING FREE ACCESS TO FEED DURING THE EXPERIMENT

Treatment	HA (ng/mg prot.)	Me-HA (ng/mg prot.)
Controls	4.69 ± 0.27	1.57 ± 0.25
2 DG	5.64 ± 0.47	1.52 ± 0.28
Metoprine 20 mg/kg	6.10 ± 0.56*	0.45 ± 0.03†
Metoprine 20 mg/kg + 2 DG	5.98 ± 0.30*	0.46 ± 0.08†

Values are mean ± SEM (n = 6). Statistical significance vs. the controls is determined by the Kruskal–Wallis test: *$p < 0.05$, †$p < 0.01$.

transmitters have not been found to correlate very closely with the glucoprivic responses. Even changes in the turnover of monoamines, which are well known to participate in the regulation of appetite (14,26), are minor after 2-DG treatment (29). Similarly, no changes in HA turnover were seen in the present experiment.

Modest effects on brain neurotransmitters could be understandable if these transmitters were involved in long-term modulation of feeding rather than in immediate alarm responses, such as the glucoprivic response. This kind of explanation would be plausible also with metoprine, because it could alter the level of feed intake, but had little effect on the size of the response to 2-DG. This implies that HA has an inhibitory modulatory effect on feed intake, but it is not involved in the glucoprivic feeding response itself. The effect at 2 h could be simply explained by the tonic histaminergic inhibition that would take some time to be overcome by the glucoprivic response. Another possibility is that the rise in blood glucose levels induced by metoprine could delay the initiation of glucoprivic response.

Feeding has been shown to increase the release of HA as well as serotonin into the extracellular space of rat hypothalamus by using microdialysis technique (8,27). This is in agreement with a modulatory inhibitory function of histaminergic neurons when satiety is reached. The present results suggest that no major changes in HA release on the level of the whole hypothalamus occurred shortly after 2-DG application. This does not exclude changes in individual nuclei or minor changes in HA turnover that cannot be picked up by measuring basal values of Me-HA. Neither it does exclude possible long-term changes in neuronal HA, which have been suggested to lead to a delayed suppression of feeding found after initial hyperphagia (10,30,32).

HA has also been suggested to participate directly in the regulation of glucose homeostasis (17,18). Increased plasma glucose levels, accompanied with polydipsia and polyuria, occurred in the present study as well as in that with dogs (2) during histaminergic stimulation. Interestingly, a marked enhancement of HA turnover occurs in the brains of mice with streptozotocin-induced diabetes if they are starved, but no change is seen as long as the animals are allowed free access to feed (19). Part of these responses could be exlained by increased sympathetic activity (31) and stimulated release of catecholamines from the adrenal medulla (17), but the exact role of HA in the regulation of energy balance remains to be determined.

In conclusion, the elevation of endogenous HA levels by metoprine decreased feed intake, but it did not prevent the rats from responding to the glucoprivic stimulus of 2-DG administration, though the response was delayed. The hypothalamic concentrations of HA and its metabolite, Me-HA, were not influenced by 2-DG. The results suggest that feeding modulating function of HA is on such a level that it does not prevent the response to glucoprivic emergency.

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