Brain adrenergic system in the feeding response induced by 2-deoxy-D-glucose

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MÜLLER, Eugenio E., Daniela Cocchi, and Paolo Mantegazza. Brain adrenergic system in the feeding response induced by 2-deoxy-D-glucose. Am. J. Physiol. 223(4): 945-930. 1972.—A reliable eating response was obtained in rats on injection of the glucose analogue, 2-deoxy-D-glucose (2-DG; 750 mg/kg, ip), which produces decreased intracellular glucose utilization of most tissues and particularly of brain. Intraventricular administration of an α-adrenergic blocking agent, phentolamine (50 and 10 μg), given concomitantly with 2-DG, greatly reduced the eating response to the latter, while propranolol (100 μg), a β-adrenergic drug, administered by the same route, was ineffective. At the doses used, neither drug affected basal food consumption. Similarly to phentolamine, central administration of another α-adrenergic blocker, azapetine (50 μg), reduced the 2-DG action, while MJ-1999 (100 μg), a β-adrenergic blocker, like propranolol had no effect. The increased food intake due to 2-DG was also inhibited by systemic administration of α-methyl-p-tyrosine (50 mg/kg), an inhibitor of catecholamine synthesis, and by the chemical sympathectomy due to central administration of 6-hydroxydopamine. The results provide evidence for the hypothesis that α-adrenergic modulation of postsynaptic activity by norepinephrine is involved in the neural control of the feeding response elicited by the insufficiency of metabolizable glucose.

Feeding behavior; central glucoprivation; blocking agents; α-methyl-p-tyrosine; 6-hydroxydopamine; glucoprivic control of feeding

A LARGE AND FAIRLY CONSISTENT body of evidence has been collected showing that in mammals the eating response is mediated by neuronal adrenergic receptors (15, 16, 25). Feeding is said to be facilitated by neurons ascending in the lateral hypothalamus via the medial forebrain bundle to form noradrenergic synapses in the diencephalon and forebrain (3, 13). Experimental proof has also been presented, however, suggesting that adrenergic synapses in the hypothalamus mediate satiety rather than feeding; for instance, from experiments involving the direct application of norepinephrine (NE) to the perifornical region of the medial forebrain bundle (23). Recently Smith and Epstein (29) have shown that the analogue of glucose, 2-deoxy-D-glucose (2-DG), a specific inhibitor of intracellular glucose utilization (7), induces increased food intake in monkeys and rats as a result of cerebral glucoprivation. In the light of the mentioned controversy on the role played by brain catecholamines in the eating response, it seemed of interest to investigate whether or not 2-DG-induced hyperphagia required the integrity of the adrenergic system. Therefore, in the work to be reported, the effect of the pharmacological interruption of cerebral noradrenergic transmission on the feeding response elicited by the administration of 2-DG was studied in the rat.

MATERIALS AND METHODS

Animals. Sprague-Dawley female rats, 140–180 g body wt, were used. They were fed a standard laboratory diet and given tap water. Standard vivarium conditions included a room temperature of 22 ± 2 C and 14 hr/day artificial light (0600-2000).

Experimental procedure. In most of the experiments (see results) a cannula was implanted in the right lateral ventricle of the brain while the animal was under light barbiturate anesthesia (Nembutal, 20 mg/kg, ip) and at least 2 or 3 days were allowed for recovery. The cannula was made from polyethylene tubing (PE-10), cut to a length of 4 cm. The method involved forming a small bulb in the cannula by momentarily heating a small annular section of the tubing. A drill was used to make a hole in the skull which exactly fitted the outer dimensions of the cannula. The latter was then inserted by hand directly into the hole until the bulb rested against the surface of the skull. Further details of this method were described by Altaffer et al. (1). Animals implanted with this type of cannula have been used, when required, for several months with minimal loss due to blockage or damage to the cannulas. In our experiments, the implanted rats were then placed in individual cages, and for 2 consecutive days their spontaneous food intake was measured hourly, beginning at 10 AM, for the next 6 hr. For each group treatment, at each hour of the experiment, the ingested food was calculated as the difference between the food intake on the experimental day and the mean food intake during the same time on the 2 days prior to the experiment. Tap water was available during the experiment, but water intake was not measured. Analysis of variance (two-way classification) was performed on the obtained data with the aim of evaluating the significance of the following sources of variation: experimental times, treatments, times x treatments interaction, and the residual error. To induce an eating response, 2-deoxy-D-glucose (Nutritional Biochemicals Corporation) was injected dissolved in saline (0.5 ml/100 g ip), in a dose of 750 mg/kg on the day of the experiment at 10 AM. For intraventricular administration of chemicals, the sealed end of the polyethylene cannula was cut off and the injection was made by inserting a Hamilton syringe into the cut can-
nula tip. Drugs were delivered by hand through the cannula dissolved in a volume of pyrogen-free saline (22.5 μl), which accounted also for the dead space of the cannula (2.5 μl). The accuracy of the cannula position had been ascertained in previous experiments by checking the diffusion of a trypan blue solution injected into the ventricles of control animals. Animals were not used more than once.

Pharmacological treatments—adrenergic α- and β-blockers. The following adrenergic α- and β-blockers were used: phenolamine methanesulphonate (Regitin, Ciba), azapetine hydrochloride (Iliard, Roche), propranolol (Inderal, I.C.I.), and MJ-1999 (4’-(2-isopropylamino-1-hydroxyethyl)methanesulphonanilid; Mead Johnsson). Phenolamine was injected through the implanted cannula (50 or 10 μg/20 μl pyrogen-free saline) or given intraperitoneally (50 μg) on the day of the experiment at 10 AM; azapetine, propranolol, and MJ-1999 were given centrally in a dose of 50 or 100 μg, respectively. When used in combination with 2-DG, drugs were given immediately before the systematic administration of the latter. Drug doses are expressed as free base.

Blockade of catecholamine (CA) synthesis by α-methyltyrosine (α-MT). Blockade of CA synthesis was accomplished by dl-α-methyl-p-tyrosine methyl ester (Kistner Labtjanst AB, Göteborg) (31), with a dose of the substance (50 mg/kg) which has been shown to decrease brain catecholamine levels by about 40 % for at least 12 hr (8). After 2 days of basal measurements of food intake, at 6 AM of the experimental day, three groups of rats were injected with saline (0.5 ml/100 g body wt, ip—first group) or α-MT methyl ester (50 mg/kg, ip in 0.5 ml saline—second and third groups). At 10 AM the first and second groups received intraperitoneally the usual dose of 2-DG, while the third group was injected intraperitoneally with saline. Food intake was then determined for the next 6 hr at hourly intervals.

6-Hydroxydopamine experiments. In experiments designed to test the effect of 2-DG on the food intake of rats whose central adrenergic terminals had been chemically destroyed, Sprague Dawley female rats, 130-140 g body wt, received through the implanted cannula 20 μl of a solution of 6-hydroxydopamine hydrobromide or of the vehicle. The 6-hydroxydopamine (6-OHDA) was dissolved in pyrogen-free saline containing ascorbic acid (1 mg/ml). The solution was prepared just before use and was kept on ice (34). Doses of 6-OHIDA are expressed in terms of the salt. Two injections were given, 200 and 100 μg (1 week apart); all control animals were injected with an equal volume of the vehicle solution. After an interval of 12 days from the first administration of the drug, to allow the reestablishment of the preinjection food intake, controls and 6-OHDA treated rats were given 2-DG (750 mg/kg ip) at 10 AM of the day of the experiment, and food intake was then determined at hourly intervals for 6 hr, as previously described. At the completion of the experiment, animals were killed by decapitation, brains were removed and weighed, the hypothalamus was removed, dissected at 4 C according to the procedure of Glowinski and Iversen (14), weighed, and brain and hypothalamic noradrenaline and dopamine levels (DA) were estimated fluorometrically according to Neff et al. (26). Significance of differences between groups was calculated by the Student’s t test.

RESULTS

2-DG hyperphagia. Feeding was markedly stimulated by 2-DG. From Fig. 1, which shows the pooled results of seven separate experiments, it appears that the administration of 750 mg/kg ip of 2-DG increased food intake about threefold starting from the 2nd hr after the injection and for the rest of the test period. From the analysis of variance, it appears that the 2-DG-treated groups differ significantly from controls (F = 290.14, P < 0.01) and that two different trends in food intake are present during the experimental times (F = 18.10, P < 0.01). As a result of 2-DG administration, drowsiness, stupor, and ataxia were noticed; these were especially prominent in the first 2 hr.

Action of adrenergic α- and β-blockers on 2-DG-induced hyperphagia. Before evaluating the ability of the drugs used to influence 2-DG hyperphagia, their effect on food intake was assessed in the absence of the glucose inhibitor. It is apparent from Fig. 2 that phenolamine or propranolol given intraventricularly (50 or 100 μg, respectively) did not affect per se the unstimulated intake of food (saline vs. phenolamine: F = 0.88, NS; saline vs. propranolol: F = 0.26, NS).

When 2-DG was given intraperitoneally at 10 AM, the simultaneous administration of phenolamine through the implanted cannula resulted in a dramatic reduction of food

### Figure 1

![Mean values of food intake in 7 experiments during the 6 hr following ip injection of 2-DG. Number of rats (n) = 40. Brackets stand for ±SE.](http://ajplegacy.physiology.org/)

### Figure 2

![Effect of intraventricular administration of phenolamine or propranolol on basal food intake. Saline, phenolamine, or propranolol were given intraventricularly at 10 AM and food intake was measured during following 6 hr. n = 6 for saline and phenolamine; n = 10 for saline and propranolol. This same description also applies to Figs. 3-9.](http://ajplegacy.physiology.org/)
intake (saline vs. phentolamine: $F = 47.12, P < 0.01$) (Fig. 3—pooled results of four experiments). In contrast to phentolamine, the administration of propranolol did not influence the effect of 2-DG given concurrently (saline vs. propranolol: $F = 2.91, NS$) (Fig. 4—pooled results of three experiments). In subsequent experiments phentolamine was administered centrally at two different doses (50 and 10 µg). It can be seen from Fig. 5 (pooled results of two experiments) that, while at the higher dose used the drug elicited a striking blocking effect on 2-DG hyperphagia (saline vs. phentolamine 50 µg: $F = 39.39, P < 0.01$), at the lower dose the blockade appeared reduced (saline vs. phentolamine 10 µg: $F = 22.08, P < 0.01$), even though analysis of variance did not show a statistical significance between the two groups (phentolamine 50 µg vs. phentolamine 10 µg: $F = 2.71, NS$ (significance for $P = 0.05$)).

To exclude the possibility that this effect of phentolamine might be due to a peripheral action of the blocker after its absorption from the cerebrospinal fluid, in a separate experiment phentolamine (50 µg) was administered intraperitoneally, simultaneously with 2-DG. This dose of the drug administered peripherally had no inhibitory effect on 2-DG-induced hyperphagia (saline vs. phentolamine 50 µg: $F = 2.67, NS$). Results similar to those given by phentolamine were obtained with another $\alpha$-adrenergic blocker, azapetine 50 µg (saline vs. azapetine: $F = 5.96, P < 0.05$), whereas, like propranolol, MJ-1999, a $\beta$-blocking agent, did not affect the hyperphagic action of 2-DG (saline vs. MJ-1999: $F = 0.54, NS$) (Fig. 6). The behavioral effect of these drugs, noticed after central administration, consisted of a slight depression, which was present only in the animals treated with the higher dose of phentolamine or with propranolol.

**Blockade of CA synthesis by $\alpha$-MT.** After having established in a preliminary experiment that $\alpha$-MT (50 mg/kg ip) given at 6 AM on the day of the experiment did not modify the basal intake of food (saline vs. $\alpha$-MT: $F = 0.03, NS$), the drug was administered according to the same schedule to animals treated with 2-DG. It is evident that in this instance the CA-synthesis blocker reduced markedly the stimulant effect of 2-DG (saline vs. $\alpha$-MT: $F = 5.95, P < 0.05$) (Fig. 7). No gross changes in behavior were noticed in the $\alpha$-MT treated animals.

**2-DG hyperphagia in animals treated with 6-OHDA.** Intraventricular administration of the first dose of 6-OHDA (200 µg) resulted in a prompt decrease of food intake and body weight which lasted 1 day. Some animals developed convulsions and two animals died. When the treatment was...
repeated (100 µg 6-OHDA), the effect on food intake and body weight was less evident and was present also in the animals that received the diluent intraventricularly (Fig. 8). Twelve days after the first injection of 6-OHDA, when food intake was back to normal, the animals underwent the experiments with 2-DG. As with α-MT, chemical sympathectomy by 6-OHDA resulted in a profound reduction and delay of the hyperphagic response (saline vs. 6-OHDA: F = 8.12, P < 0.01) (Fig. 9). Determination of brain CA levels showed that 6-OHDA treatment resulted in 70.5 and 32.8% reduction of NE and DA, respectively, in the whole brain, while in the hypothalamus the percent reduction of NE was 36.6%. The method used for brain CA determination did not allow the measurement of DA in a single hypothalamus (Table 1).

**DISCUSSION**

The results of these studies are compatible with the view that adrenergic mediation plays a role in the 2-DG-induced eating response. Minute amounts of the α-noradrenergic antagonists phentolamine and azapetine, when administered by the intraventricular route concomitantly with 2-DG, reduced the latter drug’s stimulant effect on food intake. Administration of a lower dose (10 µg) of phentolamine did not appear to diminish the amount of food ingested so drastically. The similar effect exhibited by azapetine, the most active adrenergic blocking agent of the dibenzazepine series (27), is difficult to reconcile with the possibility that the action of phentolamine on 2-DG hyperphagia was unrelated to α-adrenergic blockade. Specificity of α-receptor blockade is suggested by the failure to obtain suppressive effects with the highly potent β-adrenolytic agent, propranolol. Indeed, in three separate experiments this drug did not affect the stimulant action of 2-DG, and similar results were obtained with MJ-1999, another highly active and selective β-blocker (19). The present findings agree with the reported ineffectiveness of propranolol in preventing the eating response due to intrahypothalamic noradrenaline administration (19). Similarly to the α-noradrenergic antagonists, α-methyltyrosine, a specific inhibitor of CA biosynthesis (31), in a dose that lowers brain levels of noradrenaline and dopamine (8) but is unable to affect the basal consumption of food, reduced significantly the hyperphagic effect of 2-DG.

Intraventricular 6-OHDA produces a selective degeneration of catecholaminergic nerve terminals with consequent loss of noradrenaline and dopamine (34). In rats pretreated with 6-OHDA, in which the basal food intake had returned to normal after a transient hypophagia, the eating response elicited by 2-DG was markedly reduced, even though not

![Fig. 7](image-url)  
**Fig. 7.** Action of α-methyl-p-tyrosine (α-MT) on 2-DG hyperphagia. α-MT was given ip at 6 AM on day of experiment. n = 6.

![Fig. 8](image-url)  
**Fig. 8.** Food intake and body weight changes of rats after intraventricular administration of 6-hydroxydopamine (6-OHDA). On 12th day after 1st injection of 6-OHDA, animals underwent 2-DG experiment (see Fig. 9).

![Fig. 9](image-url)  
**Fig. 9.** Effect of chemical sympathectomy by 6-hydroxydopamine (6-OHDA) on 2-DG hyperphagia. n = 6.

**TABLE 1. Effect of 6-hydroxydopamine (6-OHDA) on brain levels of noradrenaline (NE) and dopamine (DA) in the rat**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hypothalamus</th>
<th>Residual Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE, µg/g</td>
<td>% Decrease</td>
</tr>
<tr>
<td>Controls</td>
<td>1.17 ± 0.11(5)</td>
<td>36.6</td>
</tr>
<tr>
<td>6-OHDA treated*</td>
<td>0.74 ± 0.009(3)</td>
<td></td>
</tr>
</tbody>
</table>

NE and DA values are means ± se, number of animals given in parentheses. * Rats were injected with 2 doses of 6-OHDA (200 µg/20 µl and 100 µg/20 µl) and were killed 12 days after the last injection. †P < 0.02 vs. controls. ‡P < 0.001 vs. controls.
completely suppressed. In contrast to a more pronounced reduction of noradrenaline and dopamine levels in the whole brain, the percentage fall in hypothalamic NE levels was only about 40%, and this might be, besides the possible presence of a "disuse" supersensitivity to the transmitter due to chronic interruption of transmitter function (28), the reason for the incomplete blockade effected by the chemical sympathectomy. In agreement with the present findings on basal intake of food after 6-OHDA treatment, it has been reported recently that stereotactic injections of this chemical along the medial forebrain bundle into the lateral hypothalamic area induces only transient aphagia and adipsia (30). The pronounced effect of 6-OHDA on the response to 2-DG at a time when there is no effect on ad libitum feeding would imply a participation of the brain catecholaminergic system only in the initiation of feeding by glucoprivic receptors, a mechanism that is not essential for commonplace feeding or body-weight regulation in the overall multifactor scheme (17).

Our experimental results provide support for the thesis that catecholaminergic neurons are essential components in the central neural networks subserving feeding behavior in response to the insufficiency of metabolizable glucose. However, they do not define where and how in the brain the activation of α-adrenergic receptor sites intervene in the elicitation of feeding. Glucoprivation induced by 2-DG given systemically is not confined to the periphery, but very likely is also central, as suggested by the drowsiness, stupor, and ataxia which characterized the first 2 hr following 2-DG administration, and by the possibility of its counteraction by centrally given glucose (unpublished results). Moreover, glucoreceptors sensitive to the inhibition of glucose metabolism by 2-DG have been localized in the lateral hypothalamus (9, 10, 24). Bilateral destruction or electrical stimulation of this area stops feeding and even causes death by starvation (33) or induces feeding, respectively (2). Histochemical work has demonstrated that most of the noradrenergic terminals in the hypothalamus and forebrain originate from axons whose cell bodies are in the lower brainstem; the axons ascend in the lateral hypothalamic area via the medial forebrain bundle to form noradrenergic synapses in the diencephalon and forebrain (3, 13). When CA are placed at certain sites in the lateral hypothalamus of the rat, eating is elicited (5, 11, 15, 16), whereas α-adrenergic blockers prevent the eating response to noradrenaline (6).

Quite recently, the neurochemical studies of Leibowitz (20, 21) have proposed and tested the hypothesis that in the hypothalamus α- and β-receptors function antagonistically to regulate hunger in the rat. More specifically, it has been suggested that the α-"hunger" system elicits feeding and the β "satiety" system suppresses it. According to the postulated neurochemical hunger-regulating circuit, α-adrenergic "hunger" receptors located in the ventromedial area stimulate hunger by inhibiting ventromedial β-adrenergic "satiety" cells, whose β-satiety terminations, in turn, would inhibit feeding neurons in the lateral hypothalamus, on which the β-satiety receptors are located, and thereby induce anorexia. In the present experiments, since the α blockers were injected into the lateral ventricle, they could have acted almost anywhere in the brain adjacent to the ventricular system to produce their action. The recent observations of Berger et al. (4), however, that intraventricular administration of phentolamine suppressed feeding in rats that had recovered from lateral hypothalamic lesions, while in keeping with the present findings, strongly favor the lateral hypothalamus as the site of action of the α adrenergic drugs. It appears of interest in this context that the lateral hypothalamus and the immediately adjacent structures are a major focus for some essential portion of the system that mobilizes food intake during glucoprivation; rats that are eating and regulating food intake after hypophysis are not eating more than insulin- or hypoglycemic induced hypoglycemia (12) and start eating after intraventricular administration of noradrenaline (4). Their analogy with the phentolamine-hypophagic rats after the glucoprivation by 2 DG seems to be more than coincidental and strongly suggests the participation of the adrenergic system in the glucoprivic control of feeding in the lateral hypothalamus. On recalling that brain CA systems in the infant rat are far from being competent (18, 22) and Teitelbaum's concept (32) that, with respect to feeding, the rats with lateral hypothalamic lesions is more like an infant than an adult, one is tempted to speculate that in both situations, the critical common denominator resides in an inadequacy of the central adrenergic circuits. However, since Leibowitz's studies have shown that the α-hunger receptors are predominantly located in and act to inhibit the ventromedial satiety area, one cannot dismiss the possibility that the reported effects of the α blockers might be a consequence of the inhibition of an adrenergic inhibiting tone at the level of the ventromedial nucleus. Thus, the possible participation of the ventromedial satiety device to the feeding response induced by acute glucoprivation will be the subject of further investigations.

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