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Site of Action of Anorectic Drugs: Glucoprivic- Versus Food Deprivation-Induced Feeding

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ANGEL, I., J. A. STIVERS, S. M. PAUL AND J. N. CRAWLEY. *Site of action of anorectic drugs: Glucoprivic- versus food deprivation-induced feeding.* PHARMACOL BIOCHEM BEHAV 27(2) 291–297, 1987.—Feeding induced by 2-deoxyglucose was compared with feeding induced by food deprivation in terms of antagonism by anorectic drugs and of anatomical site of action. Glucoprivic feeding was completely blocked by microinjection of amphetamine, fenfluramine, and mazindol into the paraventricular nucleus of the hypothalamus (PVN). Deprivation-induced feeding was not blocked by amphetamine, fenfluramine, or mazindol microinjected into the PVN. Neither the feeding induced by 2-deoxyglucose nor its reversal by amphetamine were blocked by pretreatment with the β-adrenergic antagonist, propranolol. Amphetamine and fenfluramine blocked both glucoprivic- and deprivation-induced feeding when microinjected into the perifornical region of the lateral hypothalamus. These data suggest that food consumption induced by 2-deoxyglucose treatment can be antagonized by anorectic drugs acting at recognition sites present in several hypothalamic nuclei, while deprivation-induced feeding acts through different receptor mechanisms which may be specific to the perifornical region of the lateral hypothalamus.

Hypothalamus Amphetamine 2-Deoxy-D-glucose Anorectic drugs Glucoprivic feeding Paraventricular hypothalamic nucleus

WE have previously demonstrated the presence of a low affinity, high capacity binding site for anorectic drugs, as assayed with either [^{3}H]amphetamine or [^{3}H]mazindol binding [2, 3, 13]. The potencies of a series of phenylethylamine derivatives in inhibiting the specific binding of [^{3}H]-ligand in rat hypothalamus correlated well with their anorectic potencies but not with their motor stimulatory actions, suggesting that this specific binding site may mediate the anorectic actions of phenylethylamines and mazindol [2, 3, 22]. Furthermore, a good correlation was obtained between their potencies in displacing [^{3}H]amphetamine and [^{3}H]mazindol from hypothalamic membranes, suggesting that both ligands may label the same anorectic site. Using [^{3}H]mazindol it was found that the hypothalamus is most enriched in this binding, particularly in the paraventricular area of the hypothalamus [4]. This binding site was found to be modulated both *in vivo* and *in vitro* by glucose levels and to be coupled to neuronal Na^+K^+ATPase activity, suggesting its involvement in the glucostatic regulation of food intake [1,14].

The PVN has been implicated as a regulatory site for several kinds of feeding behavior paradigms [12]. Lesions of the PVN induce an obesity syndrome similar to the ventromedial hypothalamus-lesioned rats [18]. Noradrenergic agonists [19,21], opioids [21,25] and peptides [10,25] stimulate feeding when microinjected into the PVN. Lesions of the PVN and NE microinjected into the PVN both block the satiety syndrome induced by peripherally administered cholecystokinin [8,10]. Other hypothalamic sites implicated in the regulation of feeding behaviors using electrolytic lesioning techniques include ventromedial, lateral and dorsomedial hypothalamic nuclei [5].

Anorectic agents, mainly phenylethylamines such as amphetamine, have been tested for their anatomic specificity in these hypothalamic nuclei primarily in feeding paradigms involving food deprivation. Using this method, it was found that the most responsive area for amphetamine anorexia was the perifornical area of the hypothalamus [17,20]. Furthermore, using this or similar methods for the study of anorexia, it was concluded that different anorectic drugs exert their action through distinct mechanisms [11]. For example, several drugs are believed to cause anorexia mainly through serotonergic mechanisms (e.g., fenfluramine, p-chloroamphetamine), and others through distinct noradrenergic mechanisms (e.g., amphetamine and mazindol through norepinephrine and dopamine). The alternative approach to induce feeding is the use of antimetabolites such as 2-deoxyglucose to induce cerebral cellular glucopenia. This stimulus induces carbohydrate specific feeding through

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mechanisms which may be neurochemically and/or anatomically different than mechanisms regulating feeding in the fasting paradigms [4,16]. One hypothesis of the mechanism of action of glucoprivic feeding focuses on the reduction in available metabolic resources to the cell, which is induced by 2-DG treatment [9,24].

We have recently found that the [^{3}H]mazindol binding site, similar to the previously studied [^{3}H]amphetamine binding site, responds differently to food deprivation and to 2-deoxyglucose glucoprivation [4,14]. Furthermore, this recognition site for anorectic drugs is known to be labeled similarly by the different groups of phenylethylamine anorectics and mazindol, suggesting a common mechanism of action for drugs such as fenfluramine, amphetamine and mazindol [3]. It was further found that the PVN contained the highest levels of binding in the brain, and that this area showed marked changes following food deprivation [4] and 2-deoxyglucose application (Angel *et al.*, in preparation). In order to test the involvement of the PVN in the physiological control of feeding, amphetamine, fenfluramine, and mazindol were microinjected into the PVN and tested for their ability to block both 2-DG- and food deprivation-induced feeding. Using the food deprivation paradigm, it was previously demonstrated that the PVN is non-responsive to amphetamine, and that amphetamine was mostly active in the perifornical region of the lateral hypothalamus, possibly by a beta-adrenergic mechanism [17]. In order to compare the two different feeding paradigms, the anorectic effects of these drugs in the perifornical region of the lateral hypothalamus were studied in comparison to their anorectic effects in the PVN, using both feeding paradigms.

METHOD

Male Sprague-Dawley rats, 200 grams starting weight, were individually housed in a temperature (23–25°C) and humidity controlled vivarium. The well-ventilated vivarium was maintained on an artificial day-night (12:12) cycle, lights on at 7 a.m. Indwelling guide cannulae were implanted stereotaxically under pentobarbital anesthesia, at a depth of one mm dorsal to the intended site of microinjection, as previously described [7]. For the medial PVN site, a unilateral guide cannula was implanted at stereotaxic coordinates according to Paxinos and Watson [23]: AP 1.8 mm posterior to bregma, LAT 0.2 mm to bregma, and 7.3 mm ventral to the surface of the skull. For the perifornical region of the lateral hypothalamus, bilateral guide cannulae were implanted at stereotaxic coordinates according to Paxinos and Watson [23]: AP 3.5 mm posterior to bregma, LAT ±1.0 mm to bregma, and 9.2 mm ventral to the surface of the skull ([17], and personal communication with Dr. S. F. Leibowitz). At the end of each experiment, fast green dye was microinjected identically to the drug injections, brains were removed and frozen sectioned at 50 microns, then stained with cresyl violet and examined for location of the cannula track and site of microinjection.

Experimental treatments were begun one week after surgery. Rats had free access to Purina-chow food pellets and water at all times except as described. Microinjections were performed using a 31 gauge injection tube, inserted into and 1 mm below the guide cannula. Anorectic drugs or saline were microinjected in a volume of 0.5 μl over a one minute period as previously described [7]. Each series of cannulated animals was used for both glucoprivic- and deprivation-induced feeding. Each animal received only a single drug, by a latin square design, allowing 7–8 days between experiments. Each drug test was repeated at least 3 times in different series. Each treatment group consisted of 3–10 rats. For the study of glucoprivic feeding, 10 min after the intracranial injection, rats were injected intraperitoneally with 2-deoxyglucose (Sigma, Chemical Co., St. Louis, MO), 300 mg/kg, and returned to their individual microinjection cages. Thirty minutes after this injection, Purina-chow pellets were introduced in a preweighted cup. Food intake was measured to the 0.1 gram by weighing the cup and the collected spillage ever 60 minutes for up to four hours. For the study of food deprivation-induced feeding, rats were food deprived for 18–24 hours, with free access to water. They then received microinjection of anorectic drug or saline and were returned to their individual microinjection cages. Food was present following the microinjection and was measured every 30–60 minutes in the same manner as described for the 2-DG treatment. Anorectic drugs were generously contributed by the following companies: d-fenfluramine (Servier, Paris) and mazindol (Sandoz, Nutley, NJ); d-amphetamine sulfate was purchased from (Sigma, St. Louis, MO). Statistical evaluations of the results were based on ANOVA with post hoc Newman-Keuls test for multiple comparisons.

RESULTS

Figure 1 presents the effects of d-amphetamine sulfate (20 μg) microinjected into the PVN on feeding induced by 2-DG and by 18 hour food deprivation. The magnitude of food intake induced by 2-DG and by food deprivation was comparable in the two parallel experiments. Amphetamine significantly inhibited 2-DG-induced feeding at all time points observed (for 1 hr: ANOVA, $F(2,30)=4.54$, $p<0.05$; $p<0.05$ for 2-DG vs. 2-DG + amphetamine by Newman-Keuls test; similar results were observed at other time points). Amphetamine had no significant effect on food deprivation-induced feeding (for 1 hr: ANOVA, $F(2,31)=9.97$, $p<0.01$; n.s. for food-deprived vs. food-deprived + amphetamine). Using a similar time-scale (e.g., 10 min delay between PVN injection and intraperitoneal injection and 30 min delay in the introduction of food) for the food deprivation-induced feeding, identical results were obtained. Further application of double doses of amphetamine into this site also failed to inhibit food deprivation-induced feeding (data not shown).

Figure 2 presents the effects of d-fenfluramine HCl (20 μg) microinjected into the PVN on feeding induced by 2-DG and by 18 hour deprivation. The magnitude of food intake induced by 2-DG and by food deprivation was comparable in the two parallel experiments, at both the one hour and the four hour time point of access to food. Similar results were also obtained at other time points tested (data not shown). Fenfluramine significantly inhibited 2-DG-induced feeding, at both the one hour (ANOVA, $F(2,19)=13.35$, $p<0.01$; $p<0.05$ for 2-DG vs. 2-DG + fenfluramine by Newman-Keuls test) and the four hour time point (ANOVA, $F(2,21)=12.01$, $p<0.01$; $p<0.05$ by Newman-Keuls test). Fenfluramine had no effect on feeding induced by food deprivation at the time point of one hour access to food (ANOVA, $F(3,16)=14.7$, $p<0.01$; n.s. for food deprivation vs. food deprivation + fenfluramine). Fenfluramine significantly increased food intake induced by food deprivation at the time point of four hours access to food (ANOVA, $F(3,16)=18.1$, $p<0.01$; $p<0.05$ by Newman-Keuls test).

Figure 3 presents the effects of mazindol (20 μg) microinjected into the PN on feeding induced by 2-DG and by 24

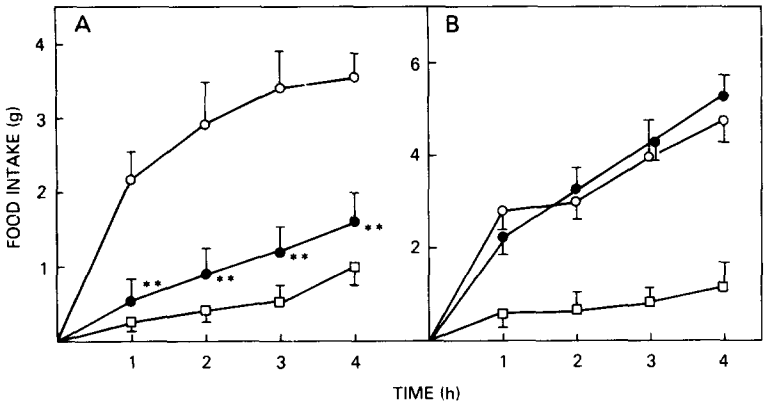

FIG. 1. Time course of food intake following 2-deoxyglucose (A) or 24 hr food deprivation (B) and the effect of amphetamine sulphate (20 μg) injected into the PVN. (A) Rats were injected with 0.2 μl of either saline (○) or amphetamine (●) into the PVN as described. Ten min later they were injected intraperitoneally with either saline (□) or 300 mg/kg 2-deoxyglucose (○). Food was introduced 30 min later and monitored every hour. (B) After 24 hour food deprivation, the rats were injected with 0.2 μl of either saline (○) or amphetamine sulphate (20 μg) (●) into the PVN and food intake monitored immediately. Control, fed rats (□) were injected with 0.2 μl saline into the PVN.

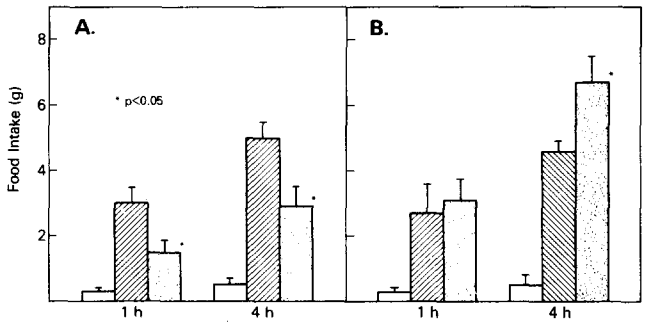

FIG. 2. Effect of fenfluramine on 2-deoxyglucose- (A) and food deprivation- (B) induced feeding upon PVN application. Experiments were performed as described in Fig. 1. (A) Open bars represent saline controls; dashed bars represent 2-deoxyglucose + saline; dotted bars represent 2-deoxyglucose + fenfluramine. (B) Open bars represent fed saline controls; dashed bars represent food deprived + saline; dotted bars represent food deprived + fenfluramine.

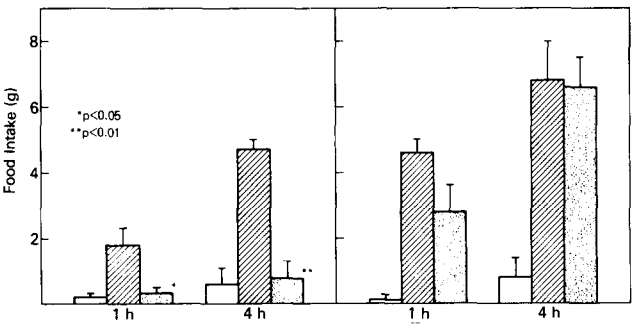

FIG. 3. Effect of mazindol on 2-deoxyglucose- (A) and food deprivation- (B) induced feeding upon PVN application. Experiments were performed as described in Figs. 1 and 2.

hour deprivation. The magnitude of food intake induced by food deprivation was somewhat greater than the food intake induced by 2-DG in these two parallel experiments, at both the one hour and the four hour time points of access to food. Mazindol significantly inhibited feeding induced by 2-DG at both the one and the four hour time points of access to food (1 hr, ANOVA, $F(2,7)=8.08$, $p<0.05$; $p<0.05$ by Newman-Keuls test; 4 hr, ANOVA, $F(2,7)=30.3$, $p<0.01$; $p<0.01$ by Newman-Keuls test). Similar results were also obtained at all other time points investigated (data not shown). Mazindol had no significant effect on food intake induced by food deprivation at either the one or the four hour time point of access to food, although some reduction was obtained in the first hour time point (1 hr, ANOVA, $F(2,8)=13.5$, $p<0.01$; n.s. by Newman-Keuls test; 4 hr, ANOVA, $F(2,8)=20$, $p<0.01$; n.s. by Newman-Keuls test).

Table 1 presents the effects of dl-propranolol either upon microinjection into the PVN or following intraperitoneal application on amphetamine antagonism of 2-DG-induced feeding. No significant differences were detected between food intake of rats receiving 2-DG + amphetamine and rats receiving propranolol (35 μg into the PVN) + 2-DG + amphetamine (ANOVA, $F(3,12)=3.53$, $p<0.05$; n.s. by Newman-Keuls test for 1 hr). In addition, propranolol had no significant effect on 2-DG-induced feeding at one, two, or three hours of access to food. Food intake for rats receiving 2-DG + propranolol + amphetamine was significantly lower than food intake for rats receiving 2-DG + propranolol (ANOVA, $F(3,16)=3.5$, $p<0.05$; $p<0.05$ by Newman-Keuls test). Similarly, food intake for rats receiving 2-DG + amphetamine was significantly lower than food intake for rats receiving 2-DG + vehicle ($p<0.05$), in these parallel experiments. Following IP injection of propranolol (10 mg/kg), there was no significant effect on 2-DG induced feeding (ANOVA, $F(3,15)=14.4$, $p<0.01$; n.s. by Newman-Keuls

TABLE 1

EFFECTS OF PROPRANOLOL AND AMPHETAMINE ON 2-DEOXYGLUCOSE-INDUCED FEEDING

Drugs	Food-Intake (g)
Experiment 1—Propranolol Injected IP	
2-deoxyglucose	3.2 ± 0.1 (3)
propranolol + 2-deoxyglucose	2.2 ± 0.5 (3)*
amphetamine + 2-deoxyglucose	0.28 ± 0.17 (4)†
propranolol + amphetamine + 2-deoxyglucose	0.49 ± 0.4 (4)†‡
Experiment 2—Propranolol Injected Into the PVN	
2-deoxyglucose	2.3 ± 0.5 (6)
propranolol + 2-deoxyglucose	2.8 ± 0.8 (4)*
amphetamine + 2-deoxyglucose	0.6 ± 0.3 (5)†
Propranolol + amphetamine + 2-deoxyglucose	1.2 ± 0.2 (3)†‡

In experiment 1: rats were injected IP with either saline or propranolol (10 mg/kg). Fifteen min later they were injected into the PVN with 0.2 μl of either saline or amphetamine (20 μg). Ten min later they were injected IP with 2-deoxyglucose (300 mg/kg) and 30 min following this injection food was introduced.

In experiment 2: rats were microinjected into the PVN with 0.5 μl of either distilled water or propranolol (35 μg). Ten min later they were injected into the PVN with either saline or amphetamine sulfate (20 μg). Ten min later they were injected IP with 2-deoxyglucose and food was introduced 30 min following the last injection. Data represent the one-hour food intake as mean ± SEM of the indicated number of determinations.

*Not significant compared to 2-deoxyglucose.

†$p<0.05$ compared to 2-deoxyglucose.

‡Not significant compared to amphetamine + 2-deoxyglucose.

n.s. by Newman-Keuls test). Furthermore, no significant differences were obtained between rats receiving 2-DG + amphetamine and 2-DG + amphetamine + propranolol.

Figure 4 presents the effects of amphetamine (20 μg) microinjected bilaterally into the perifornical region of the lateral hypothalamus on feeding induced by 2-DG and by 24 hour food deprivation. Amphetamine significantly inhibited food intake induced by 2-DG at the one, two, three and four hour time points of access to food (for 1 hr, ANOVA, $F(3,45)=18.7$, $p<0.01$; $p<0.01$ by Newman-Keuls test; for 4 hr, ANOVA, $F(3,45)=9.99$, $p<0.01$; $p<0.05$ Newman-Keuls test). Comparison of food intake induced by 24 hour deprivation in rats treated with vehicle or amphetamine also revealed a significant effect of amphetamine on deprivation-induced feeding at the 0.5, one, two, and three hour time points of access to food (for 30 min, ANOVA, $F(3,16)=9.75$, $p<0.01$; $p<0.05$ by Newman-Keuls test). Amphetamine did not significantly block the effects of food deprivation measured at the fourth hour of cumulative access to food [ANOVA, $F(3,45)=9.99$, $p<0.01$; n.s. by Newman-Keuls test).

Figure 5 presents the effects of fenfluramine (2 μg) microinjected into the perifornical region of the lateral hypothalamus region on feeding induced by 2-DG and by 24 hour food deprivation. Fenfluramine significantly inhibited food intake induced by 2-DG at the one, two, three and four hour time points of access to food (for 1 hr, ANOVA, $F(3,16)=10.65$, $p<0.01$; $p<0.05$ by Newman-Keuls test).

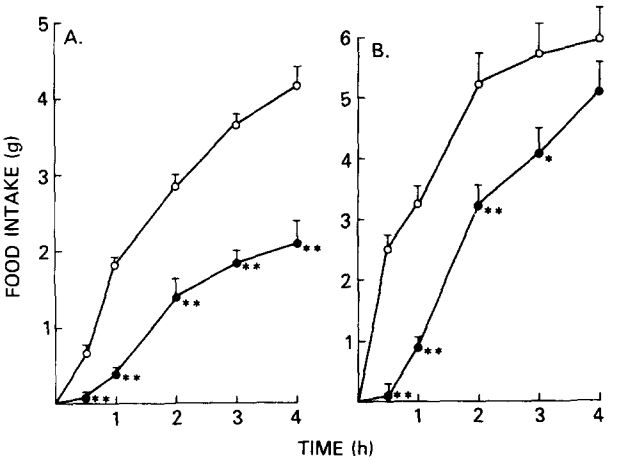

FIG. 4. Effect of amphetamine, applied bilaterally into the perifornical region of the lateral hypothalamus on 2-deoxyglucose- (A) and food deprivation- (B) induced feeding. Experiments were essentially performed as described in Fig. 1, injection 0.5 μl of amphetamine sulphate (20 μg).

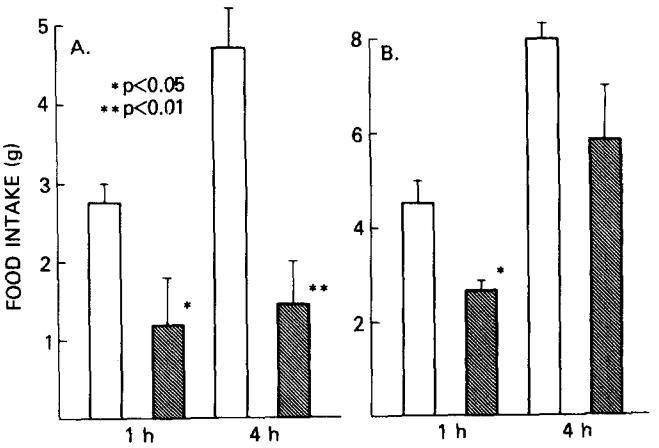

FIG. 5. Effect of fenfluramine application into the perifornical region of the lateral hypothalamus on 2-deoxyglucose- (A) and food deprivation- (B) induced feeding. Experiments as described in Fig. 4.

Comparison of food intake induced by 24 hour food deprivation in rats treated with vehicle or fenfluramine also revealed a significant effect of fenfluramine on deprivation-induced feeding, at the first two hours of cumulative access to food (for 1 hr, ANOVA, $F(3,16)=10.6$, $p<0.01$; $p<0.05$ by Newman-Keuls test). Similar results were also obtained using mazindol (20 μg) on both 2-DG and deprivation induced feeding (data not shown).

DISCUSSION

The mechanism of action of anorectic drugs has been extensively studied. In most experiments, either a 4-hour daily feeding schedule has been performed, or food deprivation-induced feeding has been monitored. Food intake could also be stimulated in sated animals by glucoprivation, using either glucose antimetabolites such as 2-deoxyglucose or 5-thioglucose or by using insulin. Using these methods, it was concluded that different groups of anorectic drugs may

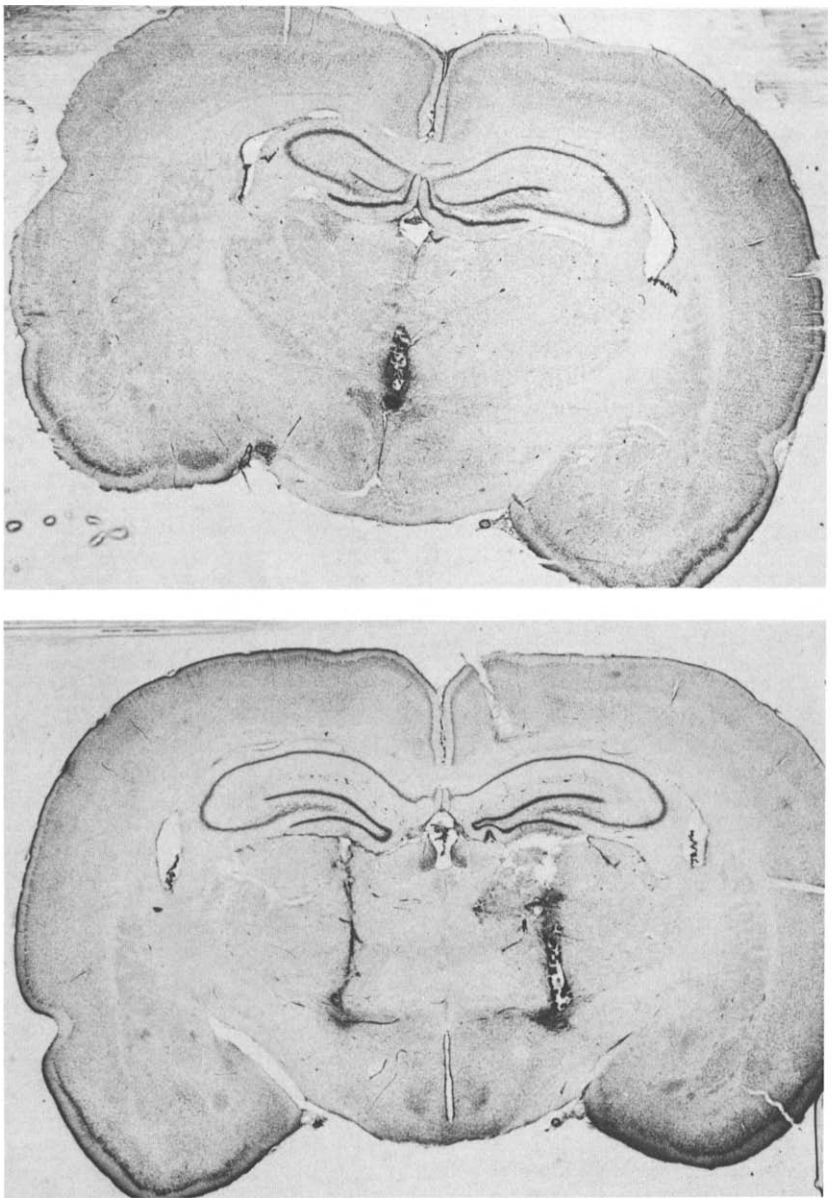

FIG. 6. Illustrations of typical cannula placements, as seen in frontal sections of rat brain, sectioned at 50 microns and stained with cresyl violet. Top: Paraventricular nucleus of the hypothalamus (unilateral); Bottom: Perifornical region of the lateral hypothalamus (bilateral).

mediate anorexia through distinct mechanism, e.g., through norepinephrine, dopamine or serotonin mediated processes [11]. It has been shown that the glucoprivic feeding elicited by 2-deoxyglucose is specific for carbohydrate [16], and is believed to be stimulated by a reduction in cerebral cellular energy utilization [9,24]. Recently, it was demonstrated that anorectic drugs from distinct groups similarly inhibit 2-deoxyglucose-induced glucoprivation, but only the "catecholaminergic" drugs were able to block insulin-induced feeding [6]. These, and other data, suggest that anorectic drugs interact with different systems, depending on the test paradigm used.

We have previously described a binding site for anorectic drugs, which could be labeled either by [^{3}H]amphetamine [14,22], [^{3}H]mazindol [2,3] or by [^{3}H]p-chloroamphetamine (Angel *et al.*, in preparation). The potencies of phenylethylamine anorectic drugs and mazindol to displace these binding sites were found to be in good correlation to their anorectic potencies, suggeeting that this binding site may mediate the anorectic action of these drugs.

Interestingly, both amphetamine derivatives, fenfluramine and mazindol, were all on the same correlation line indicating that these drugs interact through a single recognition site. Other studies on the anorectic binding site have revealed that this site is directly regulated, both *in vivo* and *in vitro*, by glucose and by glucoprivic signals [1,4]. It is thus suggested that this binding site may specifically mediate glucoprivic feeding and the effect of anorectic drugs on

glucoprivic feeding. Furthermore, this binding was modulated both by food deprivation and by 2-deoxyglucose application in opposite directions, e.g., the binding was reduced after food deprivation and elevated after 2-deoxyglucose. The present study extends these findings to the effects of three anorectic drugs, injected directly into the PVN on food deprivation- versus 2-deoxyglucose-induced feeding.

Anatomical specificity of the actions of anorectic drugs also appears to depend on the test paradigm. Amphetamine, fenfluramine, and mazindol effectively blocked feeding induced by 2-deoxyglucose, but not by 18–24 hour food deprivation, when microinjected into the paraventricular nucleus of the hypothalamus. The PVN has the highest concentration of [^{3}H]mazindol binding in the rat brain [4], whereas the lateral hypothalamus has comparatively low concentrations of binding. All drugs were applied in one dose of approximately 100 nmols, and further compared using half or double the dose. No effect on food deprivation-induced feeding was obtained using up to 250 nmols of amphetamine (data not shown). These data confirm prior results [17] in which amphetamine was unable to block food deprivation-induced feeding. However, all drugs tested were very potent in inhibiting 2-deoxyglucose-induced feeding. The order of potency of this effect was mazindol > fenfluramine > amphetamine, similar to known potencies when injected intraperitoneally, both on deprivation- and on 2-deoxyglucose-induced feeding [6]. In the perifornical region of the lateral hypothalamus, amphetamine was a potent inhibitor of food deprivation-induced feeding (Fig. 4), similar to the previously published results [17]. However, this inhibition was of shorter duration than the inhibition of glucoprivic feeding, abating after 2–3 hour of food intake measurements. Furthermore, fenfluramine, applied to this area, was also a potent inhibitor in both feeding paradigms, with similar kinetics of anorexia as amphetamine (Fig. 5). In all experiments with food deprivation-produced feeding, the food was introduced immediately after the intracerebral injections of drugs, similar to previously published methods [17]. However, in experiments where the food deprivation-induced feeding was conducted in delayed time-scale, similar to the schedule used for 2-deoxyglucose, no significant reduction of food intake by amphetamine was observed. These data suggest that also in the perifornical region of the lateral hypothalamus, different mechanisms of anorexia may act when different feeding paradigms are used. Studies to further differentiate these phenomena in the perifornical region of the lateral hypothalamus are currently in progress.

It has been postulated that amphetamine anorexia in the perifornical region of the lateral hypothalamus may be mediated through the β-adrenergic receptors [17]. In order to test the role of β-adrenergic receptors in mediating amphetamine anorexia we have thus investigated the direct effect of dl-propranolol, a beta-antagonist, microinjected into the PVN, on both 2-deoxyglucose-induced feeding and on the anorectic effect of amphetamine. Propranolol, microinjected into the PVN, did not block the glucoprivic feeding induced by 2-deoxyglucose, indicating that the feeding signal is not mediated through the beta-receptor, but may instead have a direct metabolic effect. The conclusion that β-adrenergic mechanisms are not directly involved in 2-deoxyglucose-induced feeding was supported by other studies that used direct measurements of norepinephrine turnover, showing the lack of additivity between feeding induced by 2-deoxyglucose and by clonidine [24]. Furthermore, propranolol, in a dose that completely blocked beta-receptors, failed to block amphetamine anorexia in the PVN, indicating that this anorexia is not mediated through a β-adrenergic mechanism. Taken together, both the potency of anorectic drugs in inhibiting glucoprivic feeding in the PVN and the lack of an effect of propranolol differentiate this anorexia from the previously known effects of amphetamine in the lateral hypothalamus [17].

In conclusion, food consumption induced by 2-deoxyglucose treatment was antagonized by anorectic drugs microinjected into the paraventricular nucleus of the hypothalamus and into the perifornical region of the lateral hypothalamus, while food consumption induced by starvation was antagonized by anoretic drugs microinjected into the perifornical region of the lateral hypothalamus but not the paraventricular nucleus of the hypothalamus. Our previously described binding site for anoretic drugs is most concentrated in the PVN [4], and appears to be more closely related to 2-DG-induced feeding than to starvation-induced feeding. These results suggest that glucoprivic feeding acts through specific receptor mechanisms which may be localized in the paraventricular nucleus of the hypothalamus.

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