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The Effects of Amphetamine or Caffeine on the Response to Glucoprivation in Rats with Rostral Zona Incerta Lesions[1]

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McDERMOTT, L. J. AND S. P. GROSSMAN. *The effects of amphetamine or caffeine on the response to glucoprivation in rats with rostral zona incerta lesions.* PHARMAC. BIOCHEM. BEHAV. 12(6) 949–957, 1980.—Lesions in the rostral zona incerta (ZI) of male albino rats severly impaired feeding responses to 2-deoxy-D-glucose (2DG) and drinking responses to hypertonic saline during the first month after surgery. There was evidence of recovery 6 months after surgery but the magnitude of the improvement was small and severe impairments persisted in most subjects. A small but significant deficit in the feeding response to insulin persisted unabated after the 6-month recovery period. Caffeine or amphetamine pretreatment, but not apomorphine, increased ad lib feeding as well as the response to low doses of 2DG in rats with ZI lesions as well as in controls. The increased feeding response to 2DG after caffeine or amphetamine was larger than the sum of the effects of 2DG alone plus the effect of caffeine or amphetamine alone.

Zona incerta Recovery of function Psychomotor stimulants and 2-Deoxy-D-glucose Feeding 2- Deoxy-D-glucose Insulin Hypertonic saline Caffeine Amphetamine Apomorphine Lateral hypothalamic syndrome

ELECTROLYTIC lesions of the dorsolateral hypothalamic area (LH) produce profound effects on behavior including prolonged aphagia and adipsia and impaired responding to a variety of regulatory challenges (for review see [10,30]). The large lesions used in this research invariably damage the adjacent zone incerta (ZI) immediately dorsal to the LH. Lesions of the rostral zona incerta which do not infringe on the LH produce many of the effects on ingestive behavior which have been attributed to LH damage, including hypodipsia, attenuated response to a wide range of doses of glucoprivic and hydrational challenges, increased "finickiness," inefficient feeding, and alteration in circadian ingestive patterns [12, 23, 24, 25].

Several investigations have demonstrated that rats with LH lesions are extremely hypoactive, assume and maintain unusual "catatonic" postures, and do not respond readily to most sensory input [4, 22, 30, 31]. Although external stimuli produce autonomic discharge in these animals they seem insufficient to produce orienting responses or to maintain arousal [16].

It was demonstrated some years ago that microinjections of the neurotoxin 6-hydroxydopamine (6OHDA) into the substantia nigra produced similar sensory/motor dysfunctions, indicating that the LH syndrome might be due to an interruption of the dopaminergic nigrostriatal pathway which traverses the lateral-most aspects of the LH and adjacent portion of the internal capsule [32]. This interpretation has been supported by studies showing that intraventricular injections of 6OHDA that avoid nonspecific tissue damage also reproduce many of the deleterious effects of LH lesions on ingestive behavior [37,38]. Stricker and Zigmond [30] have proposed that the LH lesion syndrome may be due to the near total destruction of a dopaminergic "arousal" system. Partial recovery of ingestive behavior occurs, according to this hypothesis, because remaining portions of this system take over its function. Persisting deficits in responding to glucoprivic or hydrational challenges are thought to be due to an inability of the system to handle stressful stimuli. This hypothesis has received support from several studies suggesting that increased arousal can produce at least temporary remission of symptoms in LH-lesioned animals. Thus, feeding has been elicited in rats with LH lesions by handling or painful stimuli, which arouse the animals from their stupor [8, 20, 30]. Animals with electrolytic or 6OHDA lesions of the LH also recover ad lib food and water intake in parallel with sensory and motor capabilities [18, 21, 36]. Further, rats with LH lesions or intraventricular 6OHDA treatments exhibit improved responding to thirst stimuli when tested several months after surgery [31]. Although rats with ZI lesions do not exhibit overt sensory/motor or arousal

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dysfunctions similar to those observed in LH- or 6OHDA-lesioned subjects, it is of interest to determine whether regulatory deficits after ZI lesions (a) persist several months after surgery and (b) are reversed by the administration of central nervous system (CNS) stimulants.

Research indicates that catecholaminergic compounds (known for their excitatory effects on behavior) such as caffeine, amphetamine, or apomorphine stimulate feeding in animals with catecholamine-depleting LH or 6OHDA lesions or transection of the medial forebrain bundle [17, 26, 30, 36]. In an earlier study caffeine facilitated feeding responses to 2-deoxy-D-glucose (2DG) in rats that were aphagic or hypophagic after transections of the lateral components of the medial forebrain bundle, as well as in rats that overate after transection of medial components of the medial forebrain bundle [26]. Apomorphine has been reported to stimulate ad lib feeding and NaCl-induced drinking in 6OHDA-lesioned rats but not in animals with electrolytic lesions of the LH, although both groups exhibit similar impairments in ad lib ingestive behavior and arousal [17,19].

The purpose of the present experiments was to determine whether amphetamine, caffeine, or apomorphine facilitate feeding in response to 2DG in ZI-lesioned rats, which normally do not exhibit somnolence, akinesia, or aphagia and adipsia. The animals' response to regulatory challenges was retested 6 months after surgery to obtain information about possible recovery of function.

METHOD

Subjects

The subjects were 143 male albino rats weighing 250 to 300 g when obtained from Sprague Dawley, Madison, WI. They were housed individually in wire-mesh cages in a 12-hour light/12-hour dark (lights on at 7:00 a.m.) temperature-controlled colony (22°C ± 2°C). Except when noted, food pellets (Teklad 6% Fat Mouse and Rat Diet) and tap water were available ad lib.

Surgery

Surgery was performed under Nembutal anesthesia (50 mg/kg, intraperitoneal, IP) when the rats weighted 300 to 350 g. Bilateral electrolytic lesions were placed in the rostral zona incerta of 110 rats by passing a 1.0 mA anodal direct current for 12 sec through an electrode (no. 3 insect pin, insulated with Teflon except for the flattened tip) whose tip had been stereotaxically positioned at AP=5.4, L=1.5, and H=−1.6 (using the coordinates from the de Groot [7] atlas of the rat brain).

The electrode was stereotaxically lowered 5 mm into the brains of 13 rats at AP=5.4, L=1.5 and withdrawn without passing current; these 13 subjects served as operated controls. The 20 subjects which served as unoperated controls were given comparable doses of Nembutal at the time the control subjects underwent sham surgery.

Ad Lib Ingestive Behavior and Body Weight

For the first 15 days after surgery, as well as periodically throughout the experiment, the 24-hour ad lib intake of food pellets and tap water was recorded. The amount of food spillage found under each cage was also recorded and used to determine feeding efficiency and net food intake. The duration of postsurgical hypophagia was calculated for each rat by computing the number of consecutive days that the food intake for each animal remained below 20 g per 24 hours. The duration of the period of postsurgical hypodipsia was defined as the number of consecutive days that the intake of the lesioned rats was less than 75% of the mean of control intake.

Body weight was recorded daily at approximately 9:00 a.m. for 2 weeks, beginning with the preoperative weight on the day of surgery, and then weekly for the duration of the experiments. In addition, subjects were weighed daily during drug tests.

Animals that remained aphagic for more than 2 days or hypophagic for more than 10 days (by the criteria described above) were not included in any experiment, in order to ensure that group averages were not influenced by the effects of incidental damage to the lateral hypothalamic area. None of the animals was fed or watered intragastrically at any time during the course of the experiments. Animals that were unable to maintain stable or increasing body weight at any time were removed from the study. The data from 18 of the lesioned subjects were deleted due to aphagia or hypophagia and the data of eight lesioned rats and three controls were deleted because the animals contracted respiratory or urinary tract infections in the course of the experiments.

Long-term Regulatory Deficits

Beginning two weeks after surgery, 25 lesioned rats and 10 controls (Group A) were tested for their feeding or drinking responses to: (a) 2-deoxy-D-glucose (2DG) (Sigma Chemical Co., St. Louis, MO) (b) insulin (Iletin, Eli Lilly Co., Indianapolis, IN), and (c) hypertonic saline (1M NaCl) (Mallinckrodt, St. Louis, MO), during the light phase of the 24-hour cycle, using the following protocols:

Insulin or 2DG. At the beginning of each drug or control test the animals were weighed. Food present in their cages was removed and replaced with two fresh food pellets. One hour later these pellets were exchanged for four fresh, preweighed pellets (approximately 20 g), and papers to catch food spillage were placed under the cages. This 1-hour habituation period was designed to offset any stimulatory effects on feeding due to handling or the presentation of fresh food. On control tests the animals were injected IP with 1 ml of isotonic saline per rat. Six hours later food was removed from the cage, weighed, and returned to the cage. The accumulated spillage was also recorded. On the following day the same procedure was repeated, with the substitution of an insulin or 2DG injection.

The 2DG was dissolved in bacteriostatic water, in a 10% W/V solution, and injected IP in a dose based upon body weight. The insulin was injected IP as an isotonic solution of constant volume (e.g., 4 units/ml/rat). The order of drug tests was 450 mg/kg 2DG, 4 units (U) insulin, 250 mg/kg 2DG, and 650 mg/kg 2DG, with 5 days between each drug test. Water was available during these tests. Upon completion of these tests the subjects were tested with 1M NaCl.

1M NaCl. At the beginning of each drug or control test food and water were removed from the cage and fresh tap water was hung on the cage in a calibrated drinking tube. One hour later, the volume of water was recorded and the subjects were injected IP with 5 ml of isotonic saline. This hour served as a period of habituation to the fresh water and calibrated drinking tubes. Six hours after injection the remaining volume was recorded without disturbing the drinking tubes or subjects. Food was returned to the cage upon

completion of the experiment. Two days later (to allow for recovery from the period of food deprivation and resulting water deprivation), the same procedure was repeated except that 5 ml of 1M NaCl solution was injected. One-half milligram of procaine was dissolved in each 5-ml injection of 1M NaCl to alleviate pain at the injection site.

Eight lesioned rats that increased their food intake more than 2 g over a control test baseline in response to 450 mg/kg 2DG were deleted from subsequent tests and statistical analysis.

Beginning 6 months after surgery the 2DG, insulin, and hypertonic saline tests were repeated in a similar manner. Each test was preceded by a 6-hour saline baseline test on the preceding day.

2DG Given in Conjunction with Caffeine, Amphetamine, or Apomorphine

Caffeine or amphetamine. Five weeks after surgery the animals of Group B (59 rats with zona incerta lesions and 20 controls) were tested for their feeding response to intraperitoneal injections of three doses of 2DG (450, 650, and 250 mg/kg) using the methods described above. (Twenty-three of the lesioned subjects and 10 of the controls had been tested previously for their 24-hour drinking response to 1M NaCl—see [25] for details). Twelve lesioned rats that increased their intake in response to 450 mg/kg of 2DG by more than 2 g in 6 hours were deleted from the subsequent testing. After the completion of 2DG tests the animals were divided into two groups (B1 and B2) consisting of equal numbers of lesioned and control subjects. The rats were then tested for their feeding response to caffeine or amphetamine (Sigma Chemical Co., St. Louis, MO) given alone and in conjunction with injections of each of the three doses of 2DG. Each 6-hour drug test was preceded by a 6-hour saline control test on the previous day. Group B1 was tested with 0.1 mg/kg amphetamine (AMPH01) and 10 mg/kg caffeine (CAFF10) alone and in conjunction with 450, 250, and 650 mg/kg 2DG. Group B2 was tested with 0.5 mg/kg amphetamine (AMPH05) and 20 mg/kg caffeine (CAFF20) alone and in conjunction with the same doses of 2DG. The drug solutions used were 0.1 or 0.5 mg AMPH/ml isotonic saline and 10 or 20 mg CAFF/ml isotonic saline.

Apomorphine. Upon completion of the above series of drug tests, 20 lesioned subjects of Group B were tested at 3-day intervals for their feeding response to the following combinations of 2DG and apomorphine (APOMOR). Half of the rats were tested with 2 DG (250 mg/kg) alone, 2DG (250 mg/kg) plus APOMOR (0.1 mg/kg), APOMOR (0.1 mg/kg) alone, and isotonic saline alone. The other half of the animals were tested with the same series of drugs except that the dose of 2DG was 450 mg/kg instead of 250 mg/kg. Apomorphine hydrochloride (Merck, Rahway, NJ) was dissolved in saline containing 0.2 mg/kg of ascorbic acid and was injected IP 30 and 60 min after the injections of 2DG. The drug tests were conducted in the manner described above. Food intake was recorded 1 and 2 hours after the 2DG and/or 30 min after the first injection of APOMOR and 60 min after the second injection of APOMOR. These time intervals and doses of apomorphine were chosen on the basis of studies reporting significant effects on ad lib feeding and on drinking in response to an osmotic challenge in brain-damaged subjects [17,19].

Summary Statistics

The following statistics designed to summarize the deficits of rats with ZI lesions were computed for the subjects of Group A: (1) HYPOPHAGIA—The number of consecutive days that food intake remained below 20 g per 24 hours. (2) HYPODIPSIA—The number of consecutive days that water intake remained below 75% of the mean control intake. (3) 2DG(1MON) and (4) 2DG(6MON)—The average change from baseline in response to three doses of 2DG tested 1 or 6 months after surgery, respectively. (5) 2DG (IMPROV)—The difference between 2DG (1MON) and 2DG (6MON). (6) NACL(IMPROV)—The difference between the change from baseline in response to 1M NACl when tested 1 and 6 months after surgery.

Correlations

Coefficients of covariance were computed between HYPOPHAGIA and 2DG(6MON) and 2DG(IMPROV), and between HYPODIPSIA and NACL(IMPROV) and the response to 1M NaCl when tested 6 months after surgery.

Data Analysis

A variety of statistical procedures was used to determine the significance levels of the behavioral effects of zona incerta lesions presented below. They include Student's *t*-test for independent measures, Sandler's A for correlated measures, and analysis of variance for multifactor designs with repeated measures [28,35]. Most of the data were analyzed with the aid of the DATA-TEXT computerized analysis [3]. Data from animals that did not complete all behavioral tests were omitted from this analysis.

Histological Procedure

Upon completion of the experiments, the lesioned rats were sacrificed with an overdose of Nembutal and perfused transcardially with isotonic saline followed by a 10% formal saline solution. Fifty-μm sections cut through the area of the lesions were stained with cresyl violet. The sections were projected onto pages of the Pellegrino and Cushman [27] atlas of the rat brain. The data from animals which did not have bilateral damage in the area denoted "zona incerta" between AP=5.8 and AP=4.6 were deleted from the statistical analyses.

RESULTS

Histology

The data from seven of the lesioned subjects (two from Group A and five from Group B) were deleted from the statistical analysis because the lesions did not damage the zona incerta bilaterally. In the remaining 57 rats (15 of Group A and 42 of Group B) whose data are presented below, bilateral damage was observed in the zona incerta between AP=5.8 and 4.6.

The zona incerta lesions typically extended 1.7 mm in the anterior-posterior dimension from the anterior hypothalamic nucleus (AP=6.0) to the mammillary bodies (AP=4.3). The lesions extended 1.3 mm in the medial-lateral dimension and damaged tissue from the mammillothalamic tract to the internal capsule (L=0.9 to 2.2). The dorsal-ventral extent of the lesions was 1.2 mm from the ventral thalamus to the dorsal hypothalamus (H=−1.2 to −2.4). Figure 1 presents a

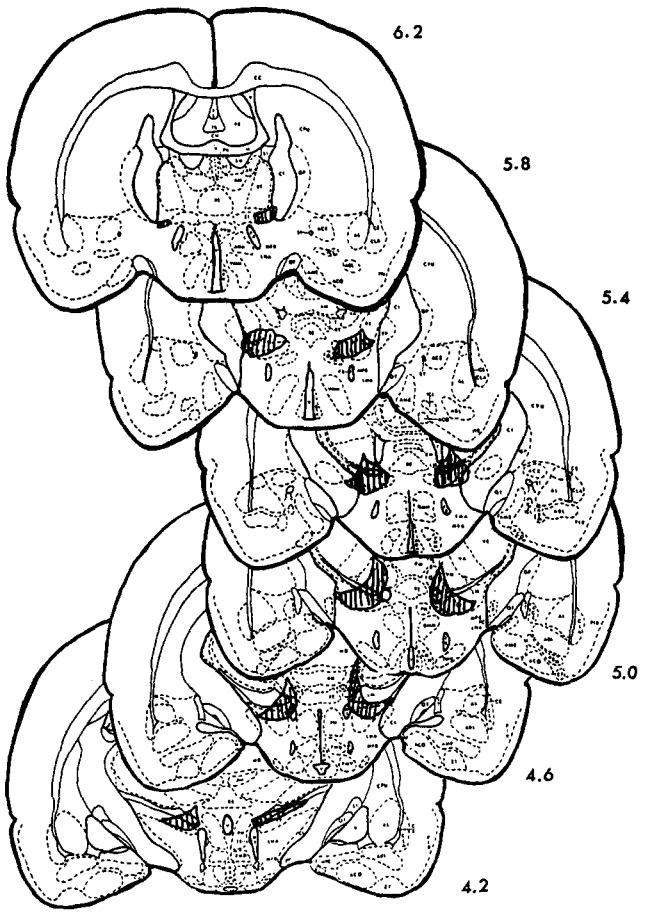

FIG. 1. Schematic representation (after Pellegrino and Cushman [27]) of the rat brain with bilateral destruction of the rostral zona incerta.

diagram of a representative lesion. The present lesions were somewhat larger than those reported earlier by these authors (see [23, 24, 25] for details).

Ad Lib Intake and Body Weight

Zona incerta lesions decreased ad lib food intake significantly ($p<0.001$) during the first week after surgery, with non-significant differences thereafter (GROUP×DAYS interaction, $p<0.001$) (see Fig. 2). The mean duration of hypophagia was 7.2 ± 1.2 days. Water intake decreased markedly after surgery and remained significantly below control levels throughout the experiment ($p<0.001$), as did body weight ($p<0.01$).

Long-term Regulatory Deficits

2DG. The control animals significantly ($p<0.01$) increased their food intake over the saline control baseline after each of the three doses of 2DG both 1 and 6 months after surgery (Fig. 3). Rats with ZI lesions did not reliably increase their intake after any of the three doses of 2DG when tested 1 month after surgery but did show a small but statistically significant ($p<0.01$) response to all three doses when retested 6 months after surgery. However, only six of

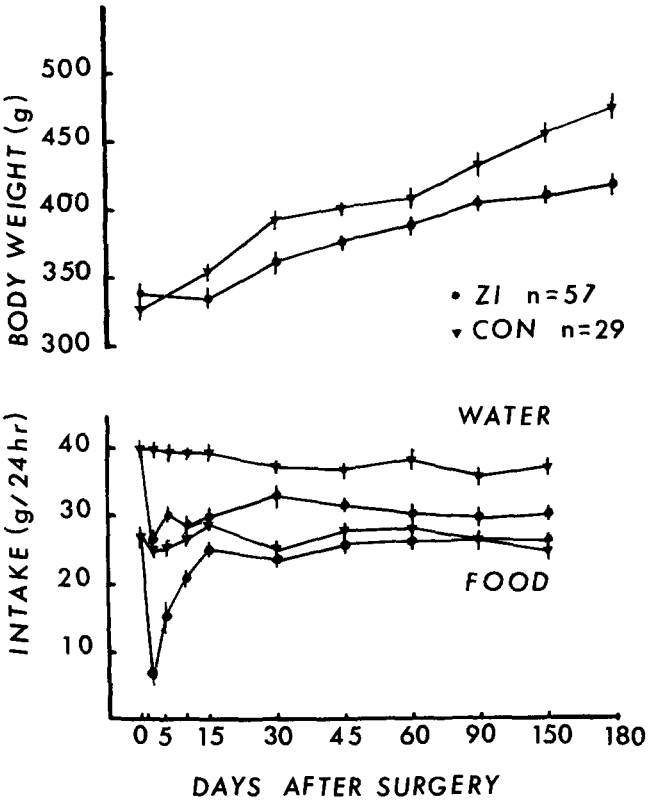

FIG. 2. Average 24-hour ad lib food and water intake and body weight of lesioned (ZI) and control (CON) rats as a function of time since surgery. Measurements on DAY 0 represent mean preoperative level.

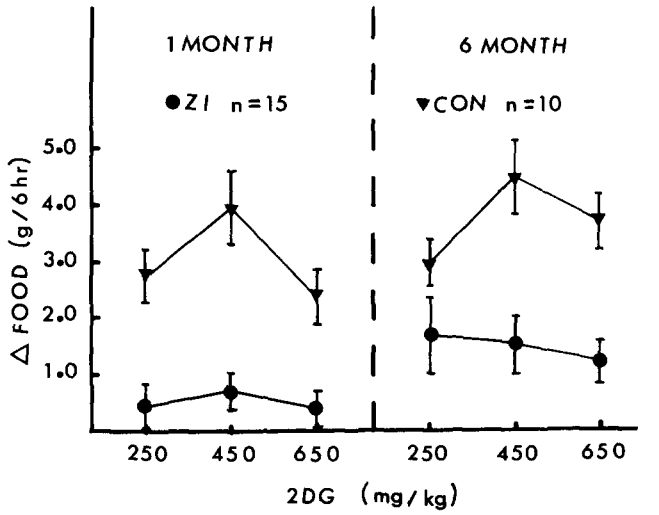

FIG. 3. Mean food intake of lesioned (ZI) and control (CON) rats expressed as change from intake on baseline tests during 6 hours after the injection of three different doses of 2-deoxy-D-glucose (2DG) that occurred 1 (left) or 6 (right) months after surgery, during the light phase of the cycle.

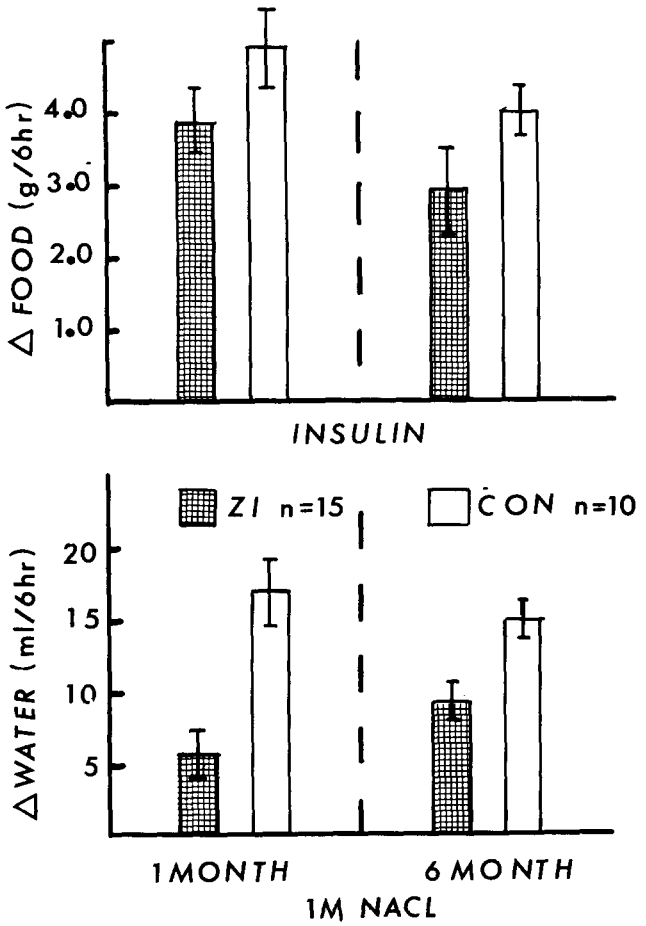

FIG. 4. Mean intake of lesioned (ZI) and control (CON) rats measured 6 hours after injections of insulin (4 units) or hypertonic saline (1M NaCl) expressed as change from 6-hour baseline intake. Tests were conducted 1 (left) and 6 (right) months after surgery during the light phase of the cycle.

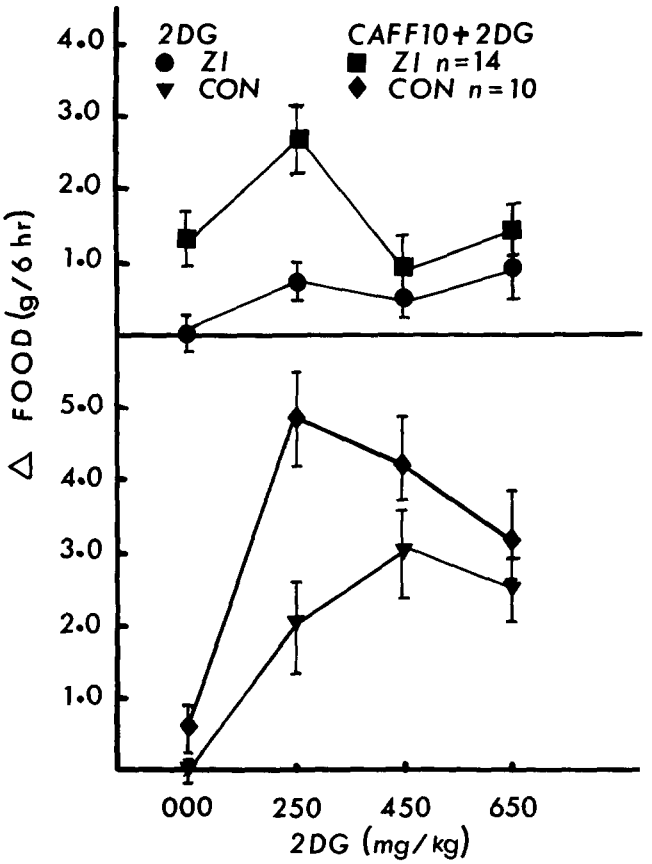

FIG. 5. Change from baseline food intake during 6-hour tests of the response of lesioned (ZI) and control (CON) rats to 2-deoxy-D-glucose (2DG) given alone or in conjunction with caffeine (10 mg/kg) (CAFF10+2DG). 000 indicates the change from saline baseline in response to 0 mg/kg 2DG (i.e., vehicle only). CAFF10+000 2DG indicates change from baseline in response to 2DG vehicle plus CAFF10 (i.e., CAFF10 given alone).

the 15 rats with ZI lesions increased their intake by 2 g or more during the 6-month test, and the difference between the response of the experimental and control groups was highly reliable ($p<0.001$) both 1 and 6 months after surgery. There was no significant correlation between HYPOPHAGIA and 2DG(IMPROV) or 2DG(6MON).

Insulin. Both the experimental and control groups increased their food intake significantly ($p<0.001$) after insulin (Fig. 4) both 1 and 6 months after surgery. Rats with ZI lesions ate 20 to 25% less than the controls on both series of tests ($p<0.01$). There was no indication of recovery 6 months after surgery. The response of both lesioned and control animals was significantly ($p<0.01$) smaller 6 months as opposed to 1 month after surgery.

1M NaCl. Both the experimental and control groups increased their water intake significantly ($p<0.001$) after 1M NaCl (Fig. 4) both 1 and 6 months after surgery. Rats with ZI lesions drank less than controls ($p<0.001$) both 1 and 6 months after surgery but showed significant ($p<0.02$) recovery 6 months after surgery. One month after surgery nine of 10 controls increased their water intake by more than 6 ml after hypotonic saline. Only five of 15 rats with ZI lesions did so. Three of the 10 lesioned subjects that did not drink more than 6 ml at 1 month exhibited marked improvement at 6 months. The mean of the remaining seven experimental subjects was 3.1 ml as opposed to 3.0 ml, 1 month after surgery. There was no reliable correlation between HYPODIPSIA and NACL(IMPROV) or NACL(6MON).

2DG Given with Caffeine, Amphetamine, or Apomorphine

Caffeine. Both doses of caffeine (10 or 20 mg/kg) significantly increased the average feeding response to 2DG of both lesioned and control subjects ($p<0.001$) (see Figs. 5 and 6). The overall feeding response of controls to 2DG alone or in conjunction with both doses of caffeine was significantly larger than that of rats with ZI lesions ($p<0.001$). The TREATMENT×DOSE OF 2DG interaction for both doses of caffeine was significant ($p<0.05$ and $p<0.001$, respectively), indicating that caffeine (10 or 20 mg/kg) given in conjunction with the low dose of 2DG was more effective ($p<0.01$) than when given with moderate or high doses of 2DG. The high dose of caffeine was more effective on the average than the low dose of caffeine in stimulating the feeding response to 2DG in lesioned subjects but not in control subjects, as indicated by a significant DOSE OF CAFFEINE×GROUP interaction ($p<0.01$).

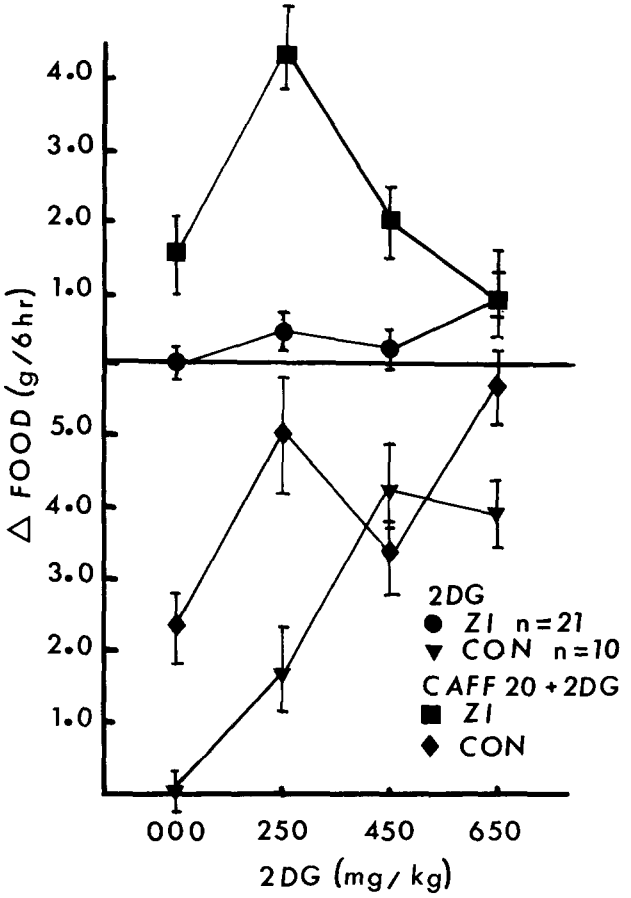

FIG. 6. Change from baseline food intake during 6-hour tests of the response of lesioned (ZI) and control (CON) rats to 2-deoxy-D-glucose (2DG) given alone or in conjunction with caffeine (20 mg/kg) (CAFF20+2DG). 000 indicates the change from saline baseline in response to 0 mg/kg 2DG (i.e., vehicle only). CAFF20+000 2DG indicates change from baseline in response to the 2DG vehicle plus CAFF20 (i.e., CAFF20 given alone).

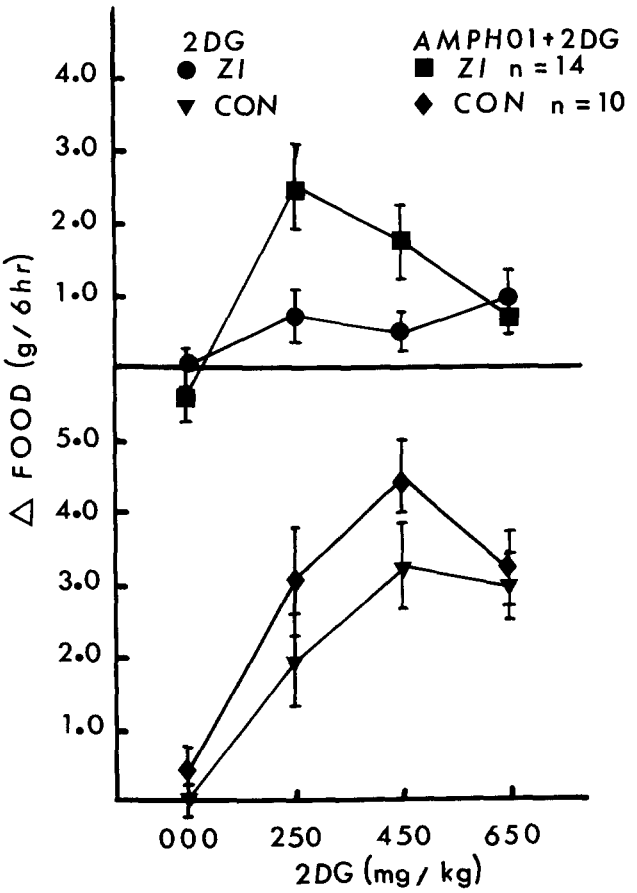

FIG. 7. Change from baseline food intake during 6-hour tests of the response of lesioned (ZI) and control (CON) rats to 2-deoxy-D-glucose (2DG) given alone or in conjunction with amphetamine (0.1 mg/kg) (AMPH01+2DG). 000 indicates the change from saline baseline in response to 0 mg/kg 2DG (i.e., vehicle only). AMPH01+000 2DG indicates change from baseline in response to the 2DG vehicle plus AMPH01 (i.e., AMPH01 given alone).

Both doses of caffeine given alone (CAFF10+000 2DG and CAFF20+000 2DG) produced significant increases in feeding over saline baseline in rats with ZI lesions. A further analysis of the data indicates that the feeding response to the combination of 250 mg/kg of 2DG and caffeine (CAFF10) or (CAFF20) is not simply due to the additive effect of each of these drugs. The sum of the net feeding response to CAFF20 alone (i.e., the amount eaten after CAFF20 minus the amount eaten on the saline control test) plus the net feeding to 250 mg/kg 2DG alone is significantly less ($p<0.01$) than the net feeding to the combined stimulus (CAFF20+250 2DG) for lesioned but not control subjects. The controls (but not the lesioned rats) increased their intake significantly more ($p<0.01$) in response to the combination of 250 mg/kg of 2DG plus the 10 mg/kg of caffeine (CAFF10+250 2DG) than the sum of their net response to 250 mg/kg 2DG alone and 10 mg/kg caffeine alone (CAFF10+000 2DG).

Amphetamine. The low dose of amphetamine (AMPH01) did not affect feeding when given alone but produced a significant increase in the feeding response to 2DG in both lesioned and control rats ($p<0.006$) (Fig. 7). The facilitative effect of the low dose of amphetamine was due entirely to its interaction with low or moderate doses of 2DG ($p<0.05$). The high doses of amphetamine (AMPH05) also had no significant effects on feeding when given alone but reliably *decreased* the feeding response to 2DG of controls ($p<0.01$) (Fig. 8). The rats with ZI lesions did not respond reliably to 2DG given alone or in conjunction with the higher dose of amphetamine. The feeding response of controls to 2DG given alone or in conjunction with AMPH01 or AMPH05 was significantly larger than that of lesioned subjects ($p<0.001$).

Apomorphine. Apomorphine did not reliably ($p>0.05$) stimulate feeding when given alone and did not increase 2DG-induced feeding in lesioned subjects. Neither the response to 2DG alone, 2DG+APOMOR, or APOMOR alone was significantly different from the saline baseline of lesioned rats on either the 1- or 2-hour test for either dose of 2DG. There was no significant difference between the response to 2DG alone and to 2DG+APOMOR.

DISCUSSION

It was the purpose of the present experiments to examine the long-term effects of ZI lesions on the rat's ability to

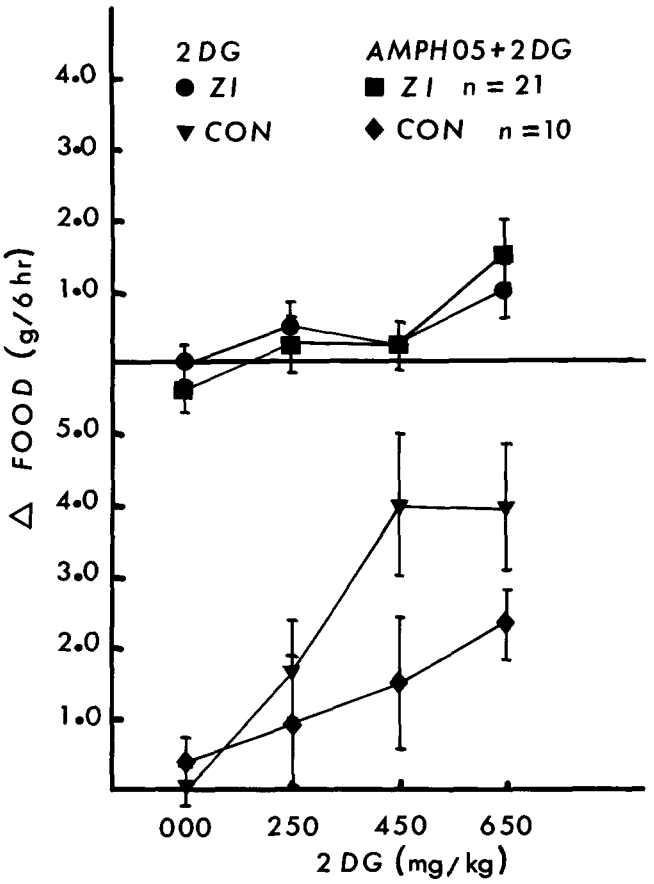

FIG. 8. Change from baseline food intake during 6-hour tests of the response of lesioned (ZI) and control (CON) rats to 2-deoxy-D-glucose (2DG) given alone or in conjunction with amphetamine (0.5 mg/kg) (AMPH05+2DG). 000 indicates the change from saline baseline in response to 0 mg/kg 2DG (i.e., vehicle only). AMPH05+000 2DG indicates change from baseline in response to the 2DG vehicle plus AMPH05 (i.e., AMPH05 given alone).

respond to glucoprivic and hydrational challenges and to investigate the efficacy of central nervous system stimulants in reversing the pronounced impairments in responding to 2DG that are characteristic of the ZI-lesioned rat.

The principal observations relevant to the former question are the findings that (a) there is recovery of the ZI-lesioned rat's ability to respond to 2DG and hypertonic NaCl between the first and sixth months after surgery but (b) the magnitude of the improvement is small and severe impairments persist in most subjects. The insulin tests revealed a small but statistically reliable impairment relative to controls similar to that observed previously [25] both 1 and 6 months after surgery, and no evidence of recovery. (The response of both experimental and control groups 6 months after surgery was smaller than that observed 1 month after the lesion.)

The small but statistically reliable impairment in insulin feeding is worthy of comment. The dose-response analysis of insulin feeding reported earlier [24] failed to reveal significant overall effects of ZI lesions on the feeding response to most doses of insulin ranging from 0.5 to 8 units (the response to 4 units was significantly smaller than control, whereas the response to 0.5 and 1 units was significantly larger although the latter effect appeared to be due to abnormally high baseline intake of controls). A similar variability in the ZI-lesioned rat's response to 8 U/kg (about 3 U/rat) has been reported recently [29]. Rats with ZI lesions ate significantly less than controls (by about the same margin as in the present study) on two tests but slightly more on an intervening third test [29]. An earlier report [33] failed to observe significant impairments of insulin feeding. It is not clear at this time what variable(s) may be responsible for the variability in these results, but the overall pattern indicates conclusively that feeding responses to insulin are far less affected, if at all, than feeding responses to 2DG, which often are abolished entirely.

The second question addressed by the present experiments, concerning the efficacy of several CNS stimulants to facilitate feeding responses to 2DG, can be answered in the affirmative although an interpretation of the effects is complicated by several factors. Both 10 and 20 mg/kg of caffeine and 0.1 mg/kg of amphetamine facilitated the feeding response to low doses of 2DG in rats with zona incerta lesions. At first glance, this effect would seem to be compatible with an arousal-impairment interpretation of the ZI-lesion effect. However, neurologically intact controls showed similar facilitative effects on 2DG eating, and both experimental and control groups increased food intake over control baseline when caffeine was administered alone. This pattern of effects suggests that caffeine and low doses of amphetamine may facilitate feeding responses to 2DG because these compounds counteract the stupor that is such a prominent component of the body's response to 2DG. It should be noted, however, that the facilitative effects of caffeine and amphetamine were most pronounced at the lowest dose of 2DG, where stupor and other "side effects" are relatively mild, and all but absent at the highest dose, where severe cellular glucopenia occurs accompanied by severely debilitating stupor.

It is interesting to note in this connection that the response to the caffeine/2DG combination appeared to be far greater at the lowest dose of 2DG than a simple addition of the caffeine-alone and 2DG-alone effects would lead one to expect. The interaction effect is even clearer in the case of the lower dose of amphetamine which had essentially no effect by itself but markedly facilitated the response to low and intermediate doses of 2DG. In both cases, the efficacy of the combination was more pronounced in the experimental animals but it is difficult to discount the influence of markedly different baselines (2DG alone).

The higher dose of amphetamine (which is still among the lowest doses commonly used in related research) produced no reliable effects on food intake when given alone, but severely depressed the feeding response to 2DG in controls. Rats with ZI lesions did not show this interactive effect. This is a potentially interesting observation in view of earlier reports [6] that rats with large LH lesions appear to be insensitive to the anorexigenic effects of amphetamine. However, one must again recall that the baseline intakes were not comparable in this test. Whereas 2DG alone markedly elevated food consumption in controls, it had essentially no effect on rats with ZI lesions. The effects of amphetamine thus may have been obscured by a "bottom" influence in the experimental animals.

The facilitative effects of caffeine (which increases the sensitivity of catecholamine receptors) and amphetamine (which increases catecholamine release) on 2DG eating may be due to a direct action on noradrenergic (NE) feeding-

related pathways. Both compounds affect dopaminergic neurons as well, but the apparent failure of apomorphine to affect feeding in this experiment (a dopamine receptor agonist) suggests that NE pathways may be involved. Lesions of the zona incerta (which lies in the projection of ascending catecholaminergic pathways) [14] abolished 2DG-induced feeding and produced significant depletions of forebrain norepinephrine but not striatal dopamine [34].

Noradrenergic pathways have been implicated in the control of feeding by numerous reports of the effects of microinjections of NE and related compounds into the upper brainstem [9,15], and the pronounced effects of NE-depleting surgical and neurotoxin-produced lesions in lower portions of the brainstem [1,11]. Noradrenergic pathways have been specifically implicated in 2DG-induced feeding by reports of impaired responding to 2DG after knife cuts in the tegmentum, lateral or medial components of the medial forebrain bundle or striatum that depleted forebrain NE (as well as 5HT) [2, 13, 26]. In some but not all of these investigations there was evidence of a significant correlation between the deficit in 2DG feeding and the magnitude of forebrain NE depletion.

In one report [26] caffeine was found to improve 2DG feeding, the magnitude of the effect being significantly correlated with forebrain serotonin depletion. It is not clear now how this result fits into the general picture of a feeding-related NE system, but it is consistent with the observation that caffeine elevates brain serotonin and increases serotonin turnover [5].

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