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Cholecystokinin, Amphetamine and Diazepam and Feeding in Lean and Obese Zucker Rats[1]

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McLAUGHLIN, C. L. AND C. A. BAILE. *Cholecystokinin, amphetamine and diazepam and feeding in lean and obese Zucker rats.* PHARMAC. BIOCHEM. BEHAV. 10(1) 87–93, 1979.—The hyperphagia characteristic of some types of obesity may result from a deficiency in one or more components of the systems controlling satiety which in rats may include the gastrointestinal hormone cholecystokinin (CCK). Obesity may also influence responsivity to often used central nervous system (CNS)-acting drugs and combination of drugs. In these experiments it was shown that: (1) Zucker fatty rats were less sensitive than lean to intraperitoneal injections of 20 U/kg CCK after a 6-hr fast and when reduced were less sensitive than lean and less sensitive than when obese to injections of 5 U/kg CCK; (2) Although fatties were equally sensitive as leans to injections of 0.5 and 1.0 mg/kg d-amphetamine sulfate, when reduced, they were less sensitive; (3) Injections of 1.25 and 2.5 mg/kg diazepam produced smaller increases in food intake after a 6-hr fast in fatty and reduced fatty than lean rats; (4) Combination of diazepam with cholecystokinin in both fatty and lean rats produced feeding similar to that following injection of carrier; and (5) A similar additive effect was obtained in both fatty and lean rats when diazepam was combined with amphetamine; however, the fatty appeared to be more sensitive to the amphetamine than the diazepam effect. Thus the Zucker fatty rat appears to be less sensitive to these chemicals which affect food intake, which supports the contention that their CNS is generally less responsive.

Feeding behavior Zucker rats Cholecystokinin Amphetamine Diazepam Obesity

THE ZUCKER fatty rat inherits obesity as a Mendelian recessive trait [36]. The accumulation of fat is mainly the result of hyperphagia, i.e., the fatty voluntarily eats 50% more than normal littermates [26]. In addition, the fatty utilizes food eaten more efficiently, since more weight is gained in spite of pair feeding [6, 14, 34]. Although the fatty adjusts food intake in response to manipulations of environment and diet, he appears to be less sensitive to these changes [9]. Upon exposure to cold, fatties were less able than normals to compensate for the increased energy expenditure required to keep warm. When caloric density was greatly decreased with cellulose or increased with fat, caloric intake was insufficiently adjusted, although compensation was made for moderate changes. In addition fatties were less sensitive to the anorexia induced by amphetamine since intake by 5-hr deprived obese rats was reduced only 50% while that by lean rats was completely inhibited. These characteristics and abnormalities in metabolic and endocrine function are likely the result of differences in central nervous system regulation [7], since reduction of the fatty to his lean littermate weight neither produces normal body fat content nor eliminates other abnormalities [8, 13, 14, 27, 34].

Hyperphagia may result from deficiencies of one or more component of systems controlling satiety. One component for the rat proposed by Gibbs *et al.* [17] is cholecystokinin (CCK), a natural gastrointestinal hormone. When injected into 5.5-hr deprived rats, CCK decreased intake for 30 min following presentation of food. The injection of CCK does not produce an aversive effect [20], and after food has been consumed, rats exhibit the normal satiety behavior sequence, i.e., grooming, exploration and sleep [3]. This behavior is quite different from that following injections of the anorectic agent d-amphetamine sulfate as described by Blundell *et al.* [5]; this chemical delays the onset of feeding and produces hyperactivity (e.g., [1,21]). Sensitivity of obese patients or animals to various central nervous system-acting drugs has not been systematically studied. One of the most frequently prescribed classes of drugs, benzodiazepines, widely used as antianxiety drugs or tranquilizers, stimulate food intake. Administration of benzodiazepines has increased food intake in both short- and long-term studies with humans [19], rats [28], cats [25], dogs [28], horses [10], chickens, pigs, sheep and cattle [24]. Benzodiazepines have been used to attenuate the hyperactivity stimulated by amphetamine [1,21]. Although in rats and sheep [1,4] benzodiazepines have been shown to override both the anorexia and hyperactivity stimulated by amphetamine, Iorio *et al.* [21] in rats showed an effect only on the hyperactivity and not the anorexia.

In the following experiments to further study the control of food intake by the Zucker fatty rat, CCK was injected into lean and fatty, non-reduced and reduced littermates. To de-

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scribe the effect of obesity on sensitivity to central nervous system-acting drugs, the littermates were also injected with amphetamine and diazepam and combinations of the two drugs.

METHOD

Animals

In these experiments 16 Zucker rats were used to form 8 pairs, each consisting of one normal and one "fatty" rat from the same litter. Of these pairs two were females, three were males and three were mixed (lean females and obese males). At the initiation of the experiment the mean weights ± standard error of the mean for the lean and obese females were 253 ± 21 and 547 ± 152 g, respectively, while those for the lean and obese males were 373 ± 29 and 580 ± 34 g, respectively. Each animal was individually caged in a room maintained at constant light and temperature. Purina Lab Chow pellets were available 18 hr day and water was available ad lib.

Experimental Design

In this experiment there were four sets of treatments: (1) Carrier (0.15 N NaCl), and 5 and 20 Ivy dog units (U) CCK/kg body weight, (2) Carrier (50% propylene glycol (PG), 50% water) and 1.25 and 2.50 mg diazepam/kg body weight, (3) Carrier (50% PG+saline), 50% PG+20 U CCK/kg, saline+diazepam, 20 U CCK/kg+diazepam, and (4) Carrier (50% PG+saline), 50% PG+0.5 mg amphetamine/kg, 50% PG+1.0 mg amphetamine/kg, diazepam−saline, diazepam+0.5 mg amphetamine/kg, diazepam+1.0 mg amphetamine/kg. For each set, the order of drug treatments was randomly assigned to pairs of Zucker rats so that each pair received all treatments and each treatment was represented at least once on each experimental day. Treatments within a set were administered at 24-hr intervals and at least three days elapsed between sets of treatments.

On experimental days rats were injected intraperitoneally with 1.0 ml/kg body weight of treatment solution(s) 6.0 hr after food had been removed. Fifteen min later food was placed in the cage and intake was measured following 30, 60 and 90 min and 18 hr.

The experiment was divided into two phases, during both of which the same sets of treatments were administered. After completion of Phase 1, the fatties were gradually reduced to two-thirds of their body weight and Phase 2 was begun. At the intitiation of Phase 2 body weights of the lean and reduced fatty males were 404 ± 22 and 419 ± 23 g; body weights of the lean and reduced fatty females were 254 ± 16 and 375 ± 60 g. When the reduced-fatty rats had reached two-thirds of their initial body weight, they were fed ad lib for 18 hr/day for two days before initiation of one of the four sets of treatments. After each set was completed the fatties were again reduced to two-thirds of their initial body weight.

Chemicals

Cholecystokinin (CCK) was obtained from the GIH Research Unit, Karolinska Institutet, Stockholm, Sweden. Its potency was 500 Ivy dog units of CCK activity/mg. Each 75 Ivy dog unit ampule contained 0.7 clinical units of secretin, and as stabilizers 0.4 mg cysteine and 0.1 mg cysteine hydrochloride. D-amphetamine sulfate was obtained from Smith, Kline and French Laboratories and diazepam was obtained from Hofmann LaRoche Laboratories.

Data Analysis

Food intake data for each time period of each treatment set for both lean and obese rats during both phases were subjected to an analysis of variance. When there were no differences between phases or groups, these were combined in the analysis. If there were differences in group or phase, these were analyzed separately. Differences between means were tested using Duncan's multiple range [15], and where noted, paired t-tests.

RESULTS

Cholecystokinin

The dose of 20 U CCK/kg decreased food intake in lean, fatty and fatty-reduced rats, Table 1. This dose decreased the cumulative food intake of lean rats in Phase 1 and 2 for the 30 min (Phase 1: $F(2,14)=8.99$, $p<0.01$; Phase 2: paired-$t=2.12$, $p<0.04$) period and in Phase 1 for the 60 min ($F(2,14)=8.52$, $p<0.05$) and 90 min ($F(2,14)=9.90$, $p<0.01$) periods. This dose also decreased intake of fatty rats in Phases 1 and 2 for the 60 min (paired-$t=2.86$, $p<0.02$; Phase 2: paired-$t=2.39$, $p<0.02$) and in Phase 1 for the 90 min (paired-$t=3.03$, $p<0.02$) periods. There was a trend toward increased 24-hr intake following 20 U CCK/kg for both phases and groups (lean, Phase 1, paired-$t=2.70$, $p<0.02$).

Fatty rats ate more than lean for all time periods (30 min: $F(1,84)=25.17$, $p<0.001$; 60 min: $F(1,84)=30.51$, $p<0.001$; 90 min: $F(1,84)=29.97$, $p<0.001$; and 18 hr: $F(1,84)=204.22$, $p<0.001$). Thus, to compare the responses of lean and fatty rats, percent of intake following injection of carrier was calculated for each rat for 5 and 20 U CCK/kg. Percent of carrier intakes in response to 20 U CCK/kg was less in lean than fatty rats for 30 min (58 ± 4 vs. 81 ± 8% paired-$t=2.72$, $p<0.01$) in Phase 1 and 5 U CCK/kg was less in lean rats than reduced fatty rats for 30 min (77 ± 13 vs. 108 ± 7% paired-$t=2.08$, $p<0.04$) in Phase 2. Lean rats responded similarly in both phases but percent of carrier intakes for fatty rats was less in response to 5 U CCK/kg when reduced than when obese.

Diazepam

In Phase 1 injection of 1.25 and 2.50 mg diazepam/kg increased food intake in lean rats for the 60 min (paired-$t=1.94$ and 1.91, respectively, $p<0.05$) and 90 min ($F(2,14)=6.21$, $p<0.01$) periods; 2.5 mg diazepam/kg increased intake for the 90 min period in fatty rats (paired-$t=2.60$, $p<0.02$), Table 2. In Phase 2, 2.5 mg diazepam/kg increased intake for 30 min in lean rats, $F(2,14)=7.81$, $p<0.005$, and for 30, 60 and 90 min periods in fatty rats (30 min: paired-$t=2.18$, $p<0.05$; 60 min: $F(2,14)=6.02$, $p<0.01$; 90 min: $F(2,14)=8.63$, $p<0.004$). Fatty rats ate more than lean in Phase 2 during the 30 min period, $F(1,37)=8.13$, $p<0.01$, and in both phases during the 60 min, $F(1,84)=8.50$, $p<0.005$; 90 min, $F(1,84)=10.34$, $p<0.005$, and 18 hr $F(1,84)=5.26$, $p<0.05$, periods. Thus to compare the responses of lean and fatty rats percent of intake following injection of carrier was calculated for each rat for 1.25 and 2.50 mg diazepam/kg. The percent increase in response to diazepam was greater for lean than fatty rats for 2.50 mg diazepam/kg in Phase 1 for the 60 min period (55 ± 24 vs.

TABLE 1

FOOD INTAKE (G) FOLLOWING INTRAPERITONEAL INJECTION OF 1.0 ML/KG BODY WEIGHT CONTAINING 0, 5 AND 20 IVY DOG UNITS OF CHOLECYSTOKININ/KG BODY WEIGHT INTO ZUCKER LEAN (298 ± 27 G, PHASE 1; 318 ± 30 G, PHASE 2) AND FATTY LITTERMATES (584 ± 36 G, PHASE 1; 405 ± 21 G, PHASE 2) (N=8)

	Phase 1			Phase 2 (Fatty Reduced)		
	Treatment (Ivy dog units/kg body weight)					
Period	0	5	20	0	5	20
+30 min						
Lean	3.4 ± 0.5[b]	3.1 ± 0.6[b]	2.0 ± 0.3[a]	4.0 ± 0.6	3.0 ± 0.6	2.4 ± 0.3*
Fatty	5.1 ± 0.4	4.4 ± 0.4	4.1 ± 0.4	4.2 ± 0.7	4.5 ± 0.7	3.4 ± 0.5
+60 min						
Lean	3.5 ± 0.6[b]	3.7 ± 0.6[b]	2.3 ± 0.3[a]	4.5 ± 0.7	3.8 ± 0.4	3.5 ± 0.4
Fatty	5.5 ± 0.6	4.8 ± 0.4	4.5 ± 0.4*	6.2 ± 0.6	4.9 ± 0.7	4.6 ± 0.5*
+90 min						
Lean	3.8 ± 0.5[b]	4.0 ± 0.6[b]	2.6 ± 0.4[a]	5.6 ± 0.4	5.5 ± 1.1	5.0 ± 0.3
Fatty	5.7 ± 0.4	5.0 ± 0.6	4.8 ± 0.4*	7.5 ± 0.7	6.7 ± 0.5	5.9 ± 0.3
+18 hr						
Lean	17.9 ± 1.5	19.2 ± 2.1	19.5 ± 1.5*	18.9 ± 1.1	19.2 ± 2.1	21.1 ± 2.0
Fatty	28.3 ± 0.8	29.4 ± 1.7	29.4 ± 1.4	29.6 ± 2.2	29.8 ± 1.2	30.8 ± 1.4

[ab]Means with different superscripts for a group, time period and phase are different, $p<0.05$, ANOVA.
*Different from 0 treatment of the group, time period and phase, $p<0.05$, paired-t test.

3 ± 17, $F(1,22)=10.42$, $p<0.01$) and in Phase 2 for 30 min period (37 ± 19 vs. 121 ± 10, $F(1,22)=7.94$, $p<0.03$. Percent of carrier intake was not different for fatty whether or not reduced. Leans responded similarly in both phases, although for the 90 min period percent of carrier intake was greater in Phase 2 than Phase 1, $F(1,37)=6.97$, $p<0.01$.

Cholecystokinin + Diazepam

Since the intakes for fatties were greater than for lean for all time periods for the combined phases (30 min: $F(1,116)=7.88$, $p<0.01$; 60 min: $F(1,108)=25.65$, $p<0.001$; 90 min: $F(1,108)=23.08$, $p<0.001$; 18 hr: $F(1,115)=275.75$, $p<0.001$), data for each group were analyzed separately. Since the intakes for Phase 1 were not different from those in Phase 2 for either lean or fatty rats for any time period, the data were combined for both lean and fatty rats. As illustrated in Fig. 1, for the 30 min period, in lean rats 20 U CCK/kg decreased intake 41%, diazepam increased intake 55% and the combination resulted in intakes not different from carrier but greater than 20 U CCK/kg and less than diazepam, $F(3,53)=12.98$, $p<0.001$. For the 60 min period the responses were similar (Carrier=3.7 ± 0.5, 20 U CCK/kg=2.2 ± 0.3, diazepam=5.3 ± 0.7 and 20 U CCK/kg+diazepam=3.6 ± 0.5, $F(3,53)=11.11$, $p<0.001$), but for the 90 min period no intakes were different from

TABLE 2

FOOD INTAKE (G) FOLLOWING INTRAPERITONEAL INJECTIONS OF 1.0 ML/KG BODY WEIGHT CONTAINING 0, 1.25 AND 2.50 MG DIAZEPAM/KG BODY WEIGHT INTO ZUCKER LEAN (300 ± 28 G, PHASE 1,305 ± 30 G, PHASE 2) AND FATTY (593 ± 34 G, PHASE 1; 397 ± 22 G, PHASE 2) LITTERMATES

	Phase 1			Phase 2 (Fatty Reduced)		
	Treatment (mg/kg body weight)					
Period	0	1.25	2.5	0	1.25	2.5
+30 min						
Lean	2.6 ± 0.4	4.0 ± 0.7	3.8 ± 0.4	3.4 ± 0.6[a]	4.2 ± 0.5[a]	5.1 ± 0.6[b]
Fatty	2.8 ± 0.4	3.8 ± 0.6	2.8 ± 0.7	4.7 ± 0.3	5.1 ± 0.3	5.6 ± 0.4*
+60 min						
Lean	3.2 ± 0.5	4.7 ± 0.5*	4.3 ± 0.5*	4.5 ± 0.8	4.7 ± 0.4	5.6 ± 0.7
Fatty	4.1 ± 0.3	4.8 ± 0.9	4.2 ± 0.7*	5.9 ± 0.4[a]	5.7 ± 0.3[a]	7.6 ± 0.6[b]
+90 min						
Lean	3.4 ± 0.5[a]	5.0 ± 0.5[b]	5.5 ± 0.7[b]	5.1 ± 0.8	5.1 ± 0.4	6.8 ± 1.0
Fatty	4.6 ± 0.5	5.2 ± 0.7	6.0 ± 0.5*	6.2 ± 0.4[a]	6.6 ± 0.3[a]	8.8 ± 0.6[b]
+18 hr						
Lean	16.8 ± 1.5	17.2 ± 1.3	19.3 ± 1.7	20.7 ± 1.8	19.5 ± 1.4	20.4 ± 1.3
Fatty	25.8 ± 1.8	24.8 ± 1.9	26.5 ± 2.5	28.2 ± 1.8	28.2 ± 1.4	26.8 ± 1.4

[ab]Mean with different superscripts for a group, time period and phase are different; $p<0.05$; ANOVA.
*Different from 0 treatment of the group, time period and phase, $p<0.05$, paired-t test.

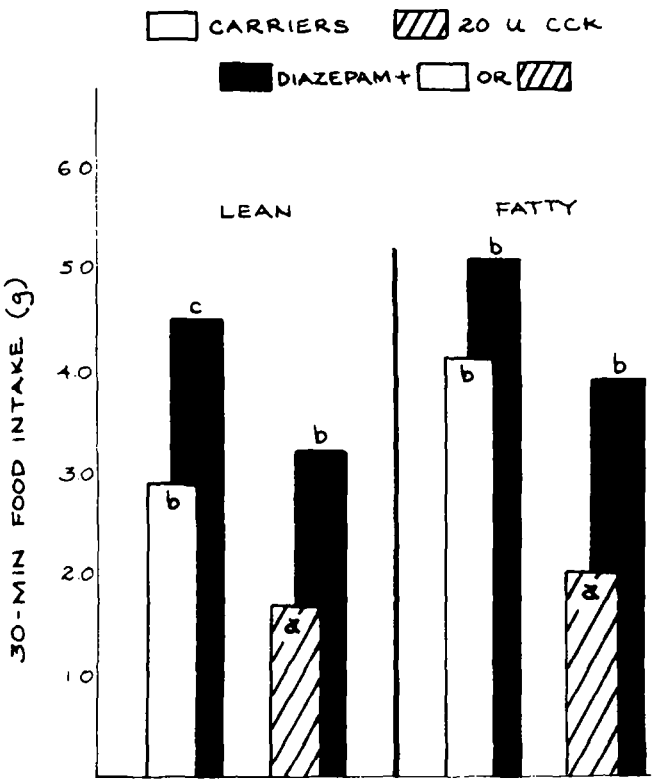

FIG. 1. Thirty-min food intake following intraperitoneal injection of cholecystokinin and diazepam separately and in combination into Zucker lean and fatty littermates. The open bar is the response to the carrier. The adjacent closed bar is the response to diazepam. The hatched bar is the response to 20 U CCK/kg and the adjacent bar is the response to the 20 U CKK/kg + diazepam. Values are means of Phases 1 and 2. [a,b,c]Bars with the same letter are not different, $p<0.05$.

carrier (Carrier=4.7 ± 0.5, 20 U CCK/kg=3.4 ± 0.6, diazepam=5.9 ± 0.7 and 20 U CCK/kg+diazepam=4.7 ± 0.5). In fatty rats 20 U CCK/kg decreased intake at 30 min 44%; and, when combined with diazepam, resulted in intakes not different from control, $F(3,50)=8.28$, $p<0.001$. For the 60 min period intake in response to CCK was still decreased (3.4 ± 0.3 g, 33%), to diazepam was increased (6.3 ± 0.5, 24%), and to the combination (5.4 ± 0.4) was not different from that to the carrier (5.1 ± 0.4) or to diazepam alone, $F(3,53)=10.01$, $p<0.001$. For the 90 min period diazepam alone (4.6 ± 0.3) and with CCK (6.8 ± 0.5) increased intake compared to carrier (5.6 ± 0.4) and CCK alone (4.6 ± 0.3, $F(3,53)=9.22$, $p<0.001$).

Amphetamine

To compare responses of fatty to lean rats to amphetamine food intake responses to amphetamine treatments were analyzed separately from the combination with diazepam treatments. In lean rats in Phase 1 both 0.5 and 1.0 mg amphetamine/kg decreased food intake for the 30, 60 and 90 min periods and the latter decreased 18 hr intake (30 min: $F(2,12)=36.49$, $p<0.001$; 60 min: $F(2,12)=11.16$, $p<0.01$; 90 min: $F(2,12)=13.62$, $p<0.001$; 18 hr: $F(2,12)=4.80$, $p<0.05$). In Phase 2 both doses decreased intake for the 30 and 60 min periods (30 min: $F(2,12)=8.21$, $p<0.006$; 60 min: $F(2,12)=5.21$, $p<0.05$). In the fatty rats 0.5 and 1.0 mg/kg amphetamine/kg decreased food intake during Phase 1 only. Both doses decreased intake for the 30 and 60 min periods (30 min: $F(2,12)=32.66$, $p<0.001$; 60 min: $F(2,12)=21.95$, $p<0.001$) and the higher dose also decreased intake for the 90 min period, $F(2,12)=9.57$, $p<0.01$, and 18 hr periods, $F(2,12)=5.53$, $p<0.05$. Fatty rats ate more than lean for all time periods (30 min: $F(1,71)=11.78$, $p<0.005$; 60 min: $F(1,68)=14.65$, $p<0.001$; 90 min: $F(1,74)=6.56$, $p<0.03$; 18 hr: $F(1,70)=178.41$, $p<0.001$). To compare responses of lean and fatty pairs, and to measure the effect of weight reduction of obese, percent of carrier intakes were calculated for both 0.5 and 1.0 mg amphetamine/kg. In Phase 1 groups did not respond differently; in Phase 2 percent of carrier intakes were less for lean than reduced obese at 30 min (47 ± 15 vs. 98 ± 32%, $F(1,19)=4.85$, $p<0.05$) and 60 min (59 ± 12 vs. 97 ± 28, $F(1,19)=5.59$, $p<0.05$). For the lean rats percent of carrier intakes were less in Phase 1 than Phase 2 only for the 30 min, $F(1,19)=4.52$, $p<0.05$, period; but for fatties it was less for the 30 min, $F(1,19)=13.65$, $p<0.001$, 60 min, $F(1,19)=8.04$, $p<0.01$, and 90 min periods, $F(1,19)=5.71$, $p<0.03$; indicating decreased sensitivity to amphetamine in Phase 2 when obese were reduced.

Amphetamine + Diazepam

There was no difference in intake between Phase 1 and 2 for both lean and fatty rats for any time period, thus the data were combined and means were tested for differences. Thirty-minute food intakes in response to amphetamine and diazepam are illustrated in Fig. 2. In lean rats diazepam increased intake (61%) compared to carrier and the combination of diazepam with both doses of amphetamine which decreased intake (61 and 81% for 0.5 and 1.0 mg amphetamine/kg), resulted in intakes not different from carrier, $F(5,137)=31.02$, $p<0.001$. Results were similar for 60 and 90 min periods. In fatty rats during the 30 min period diazepam when combined with 0.5 mg amphetamine/kg resulted in intakes similar to carrier, but when combined with the higher dose of amphetamine resulted in intakes less than carrier (34%, $F(5,72)=11.52$, $p<0.001$). For the 60 and 90 min periods intakes in response to diazepam alone were greater than for carrier (36 and 36%, respectively) and intakes in response to the combination of diazepam with the higher dose of amphetamine were not different from carrier intakes (60 min: $F(5,79)=10.97$, $p<0.001$; 90 min: $F(5,72)=7.16$, $p<0.001$).

DISCUSSION

In these experiments fatty and normal rats were injected with the same dose/kg body weight. As a result, the fatty non-reduced received almost twice the dose as the lean; however, their metabolic mass is also approximately twice and it would be expected that drugs and their actions would be affected by metabolic mass. An alternative would have been to base doses on lean body mass, which in the obese is a smaller percent of body weight because of increased fat content. Adjustment of dose by body weight was selected because this method was used in similar experiments with CCK, Antin *et al.* [3], Gibbs *et al.* [17] and Smith *et al.* [30]. Amphetamine also has been injected per kg body weight by Blundell *et al.* [5], Iorio *et al.* [21], and Sanghvi *et al.* [29], and in particular in obese rats by Bray and York [9] and

TABLE 3

FOOD INTAKE (G) FOLLOWING INTRAPERITONEAL INJECTIONS OF D-AMPHETAMINE SULFATE (A) INTO ZUCKER LEAN (301 ± 28 G, PHASE 1; 323 ± 33 G, PHASE 2) AND FATTY RATS (593 ± 34 G, PHASE 1; 403 ± 18 G, PHASE 2)

	Lean			Fatty		
	Treatment					
	Carriers	0.5 mg/kg A	1.0 mg/kg A	Carriers	0.5 mg/kg A	1.0 mg/kg A
+30 min						
Phase 1	2.9 ± 0.5^{z}	0.7 ± 0.2^{y}	0.3 ± 0.2^{y}	4.2 ± 0.2^{z}	1.7 ± 0.5^{y}	0.5 ± 0.2^{y}
Phase 2	3.2 ± 0.7^{z}	1.7 ± 0.3^{y}	0.9 ± 0.4^{y}	3.4 ± 0.5	3.5 ± 0.7	2.0 ± 0.8
+60 min						
Phase 1	3.2 ± 0.5^{b}	1.6 ± 0.4^{a}	0.9 ± 0.3^{a}	4.9 ± 0.3^{c}	3.3 ± 0.8^{b}	1.1 ± 0.4^{a}
Phase 2	4.0 ± 0.5^{b}	2.4 ± 0.6^{a}	2.3 ± 0.5^{a}	4.6 ± 0.6	4.0 ± 0.6	3.0 ± 0.6
+90 min						
Phase 1	4.5 ± 0.7^{y}	3.2 ± 0.4^{y}	1.1 ± 0.4^{y}	5.3 ± 0.5^{b}	4.1 ± 0.6^{b}	2.0 ± 0.7^{a}
Phase 2	4.9 ± 0.6	4.7 ± 0.6	3.7 ± 0.6	6.3 ± 1.0	5.1 ± 0.6	4.9 ± 0.7
+18 hr						
Phase 1	19.5 ± 1.7^{b}	19.6 ± 1.6^{b}	17.6 ± 1.6^{a}	30.5 ± 2.0^{b}	31.3 ± 1.8^{b}	27.6 ± 0.8^{a}
Phase 2	19.8 ± 1.7	19.1 ± 1.8	18.4 ± 0.9	28.2 ± 1.9	27.7 ± 2.8	29.1 ± 1.5

^{abcd}Means with different superscripts for a group, time period and phase are different; $p<0.05$, ANOVA.
^{xyz}Mean with different superscripts for a group, time period and phase are different; $p<0.01$, ANOVA.

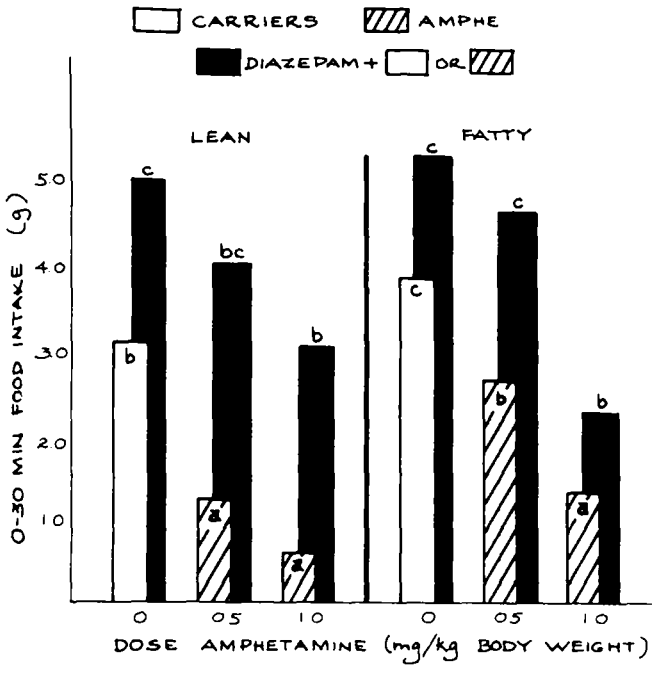

FIG. 2. Thirty-min food intake following intraperitoneal injection of amphetamine sulfate and diazepam separately and in combination into Zucker lean and fatty littermates. The open bar is the response to carrier. The adjacent bar is the response to diazepam alone. The hatched bars are the response to the indicated dose of amphetamine and the adjacent closed bars to each hatched bar are the responses to the indicated dose of amphetamine plus diazepam. Values are means of Phases 1 and 2. ^{abx}Bars with the same letter are not different, $p<0.05$.

Epstein [16]. Benzodiazepines are also injected per kg body weight [21], but there is no example of injection into obese rats. It may be that the drugs, injected intraperitoneally, might be absorbed less rapidly in the obese because of the mass of fatty tissue in the area; however, the fact that the fatty, when reduced, remained less sensitive, is evidence that the extra fat is not the only factor causing different responses in the two types of rat.

It is hypothesized that the Zucker fatty rat, eating more than its littermate, and less sensitive to satiety signals, would be less sensitive to the satiating effect of CCK, and when reduced, with increased drive to replete energy stores, may be even less sensitive. In these experiments, since the fatty ate more than its lean littermate, when percent of carrier intakes in response to 20 U CCK/kg was compared, it was smaller for the lean than fatty during the 30 min period in Phase 1; in response to 5 U CCK/kg percent of carrier intake was smaller for lean than fatty reduced in Phase 2. This means food intake was decreased significantly less in the obese than lean by 20 U CCK/kg. However, when CCK was injected with diazepam, percent of carrier intake was similar (41 vs 44% for obese and lean, respectively). It must be remembered that almost twice the CCK was injected into the fatty to elicit the above responses. Essentially weight reduction did not alter the response of the fatty to CCK.

Injections of 0.5 and 1.0 mg d-amphetamine sulfate/kg in the fatty when not reduced elicited similar responses as injections in lean littermates. Under different conditions Bray and York [9] injected 3 mg amphetamine/kg into lean and fatty rats adapted to a 5-hr feeding schedule. This dose, administered before the daily feeding, completely inhibited the lean rats from eating, but only suppressed eating by the fatty rats 50%, showing decreased sensitivity by the fatty to the anorectic drug. In our experiments, the fatties when obese were as sensitive as the lean, but when reduced were less sensitive than lean to amphetamine. Also when the fatties were reduced they responded less to the food intake depressing effect of 1.0 mg amphetamine/kg than when non-reduced.

Injections of CCK and amphetamine, both of which decreased food intake, elicited different feeding behaviors. After injection of CCK, the rats commenced eating as soon as food was placed in the cage, and, since intake was decreased, eating terminated sooner or the rats ate more slowly, and displayed the normal satiety sequence [3]. However, when food was placed in the cage after amphetamine injections, the 6-hr deprived rats did not eat immediately, but displayed hyperreactivity and motility. Blundell *et al.* [5] have measured feeding behavior following injections of the anorectic agents amphetamine and fenfluramine and have shown that while amphetamine results in delayed initiation of feeding, fenfluramine results in early termination of feeding.

The intake by the lean Zucker rat in response to injections of diazepam was increased at least 26% in 30 min. In all cases increases in food intake by the fatty rat both when obese and reduced were less than those by the leans, and percent of carrier intake was less for obese than lean for some time periods indicating decreased sensitivity of the obese to the food intake stimulant. Food intakes would likely be more significantly different if diazepam had been injected into satiated rats; however, the 6 hr deprivation schedule was maintained since injections were to be combined with food intake depressants for which the feeding regime is better suited to measure differences. The generally lower responses of the Zucker fatty rat may have been complicated by the greater susceptibility of the non-reduced rat to the tranquilization caused by diazepam. Since the dosage was administered/kg body weight, twice as much diazepam was injected into the non-reduced fatty as into the lean. Response to diazepam by the fatty was not affected by body condition.

Diazepam is thought to act via the inhibitory neurotransmitter gamma aminobutyric acid (GABA) in the central nervous system [11,12]. CCK's mechanism and site of action for decreasing food intake is unknown, but caerulein, a decapeptide similar to CCK [2], when injected intraperitoneally was found selectively bound to the ventromedial hypothalamus [31]. In addition, microinjections of caerulein into the ventromedial hypothalamus but not lateral hypothalamus depressed intake. Systemic injection into rats with ventromedial hypothalamic lesions resulted in decreased sensitivity while injections into rats with lateral hypothalamic lesions resulted in increased sensitivity [31]. However, Kulkosky *et al.* [22] found that rats with ventromedial lesions responded the same as lean rats. Thus the action of CCK also may be mediated by the central nervous system. The question in these experiments was whether the response to diazepam or to CCK would be dominant or whether the response to the combination would be a balance of the two separate effects. Clearly there is an integration, perhaps by the central nervous system, of the effects of each which results in control level feeding in both fatty and lean rats.

Other investigators have shown that diazepam attenuates the increased motor activity stimulated by amphetamine [1,21]. It was anticipated that the food intake response might be also attenuated, depending partially on doses used. The interaction observed indicates a possible blocking of amphetamine by diazepam and might be explained by the mechanisms of action postulated for each. The facilitation of the GABA system by diazepam may decrease turnover rate of catecholamines [12]. There is evidence that amphetamine increases activity of norepinephrine systems by increasing turnover rate and release and blocking catecholamine reuptake activation [18]. Anorexia induced by amphetamine is inhibited by blockers of norepinephrine synthesis. Thus, if diazepam were decreasing turnover rate of norepinephrine and blockers of norepinephrine synthesis inhibit amphetamine-induced anorexia, the food intake response observed may be the result of blocking norepinephrine synthesis by diazepam. When diazepam was combined with the higher dose of amphetamine, the lean rats had control level intakes while the non-reduced fatty ate less than control. This may be because the higher levels injected/kg body weight in the obese may have released and depleted norepinephrine and altered its storage and metabolism.

Diazepam may interact differently with CCK than with amphetamine. While amphetamine in the lean rats decreased intake 70% and diazepam increased it about 60%, the combination resulted in intakes 30% above control. On the other hand, CCK decreased intake 50% and the combination with diazepam resulted in control level intakes. It seems possible that injections of the dose of amphetamine which resulted in a 50% depression, as with 20 U/kg CCK, when combined with diazepam, would result in intakes similar to those following injection of diazepam alone. This is evidence the two drugs may be decreasing intake by different mechanisms or they interact differently with diazepam. Injection of amphetamine directly into the lateral hypothalamus has been shown to decrease food intake more than injection into other areas of the central nervous system [23]; in this area amphetamine may decrease hunger signals. On the other hand, caerulein is selectively bound to the ventromedial hypothalamus [31] where it may increase satiety signals which inhibit the lateral area. Diazepam may be postulated to interact with both the decreased hunger signals elicited by amphetamine and the increased satiety signals caused by CCK, resulting in intake similar to that following injection of carriers.

Thus the Zucker fatty rat, which eats more than its lean littermate, responds to agents which affect feed intake in lean rats. However, there is evidence that they may be less sensitive to the effect of these agents on intake. Obese appear to be equally sensitive as leans to the doses of amphetamine used, which almost completely inhibited intake. Although weight reduction of the obese has no effect on their response to CCK and diazepam, it decreases the food intake depressing effect of amphetamine. Obese, when both obese and reduced, respond to combinations of diazepam with CCK and amphetamine similarly to lean rats. The controllers for increased food intake in the fatty rat appear to be less regulated by body fat content and perhaps more by basic differences in neurotransmitter levels of metabolism. Considerable differences in metabolism between lean and fatty Zucker rats have been reported [7, 8, 26, 34] and these may play a role in accounting for differences in response to agents which affect food intake. Bray [7] noted that norepinephrine levels are increased in the median eminence and decreased in the periventricular nucleus of the obese Zucker rat compared to lean. Since these agents are known to affect neurotransmitter levels, a difference in baseline levels may well account for differences in food intake responses.

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