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Insulin, 2-Deoxy-D-Glucose, and Food Deprivation as Discriminative Stimuli in Rats

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SCHUH, K. J., D. W. SCHAAL, T. THOMPSON, J. P. CLEARY, C. J. BILLINGTON AND A. S. LEVINE. *Insulin, 2-deoxy-D-glucose, and food deprivation as discriminative stimuli in rats.* PHARMACOL BIOCHEM BEHAV 47(2) 317-324, 1994.—Using a two-lever drug discrimination procedure, two groups of four rats each were trained to discriminate the stimulus effects of 1.0 U/kg insulin or 125 mg/kg 2-deoxy-D-glucose (2-DG) from saline. A third group was trained to discriminate food deprivation produced by feeding 23 h prior to sessions from satiation produced by feeding 2 h prior to sessions. Differential responding was a direct function of dose or deprivation level in each group. Rats trained to discriminate insulin responded as if they had received insulin when they received 2-DG and vice versa. Insulin and 2-DG produced deprivation-appropriate responding in two of four rats trained to discriminate food deprivation. Low insulin and 2-DG doses produced drug-appropriate responding in rats deprived 47 h, but not in rats deprived 23 h. Blood glucose level was altered by the training doses of insulin and 2-DG, but not by 23-h deprivation. These results indicate that operations that induce feeding produce discriminable stimuli, and that these effects overlap or interact. Thus, drug discrimination procedures can be useful in the analysis of ingestive behavior.

2-Deoxy-D-glucose Discrimination Insulin Food deprivation Glucoprivic feeding Rats

A DISCRIMINATION procedure commonly used to study the discriminative stimulus effects of drugs was used to teach rats to discriminate the effects of administering insulin or 2-deoxy-D-glucose (2-DG) from saline. A separate group of rats was taught to discriminate the conditions produced by feeding 23 h prior to sessions (food deprivation) from the conditions produced by feeding 2 h prior to sessions (satiation). Insulin and 2-DG produce glucoprivation and increase feeding in satiated rats (11,12). Levels of circulating glucose are known to play an important and, as some have suggested, a central (4,10) role in feeding. By teaching groups of rats to discriminate the effects of insulin, 2-DG, or food deprivation and then cross- testing with the discriminative stimulus effects of the other procedures, it was possible to determine whether the discriminative stimulus effects of insulin and 2-DG were similar to each other and to those produced by food deprivation.

METHOD

Subjects

Twelve experimentally naive male Sprague-Dawley rats were used as subjects. The rats were about 90 days old and weighed 330-350 g at the start of training. They were housed in individual wire-mesh cages with water continuously available. The room temperature was 22°C, and lights were on from 0600 to 2000.

Apparatus

Sessions were conducted in four standard two-lever Lehigh Valley operant chambers. A houselight was illuminated during the sessions. Liquid reinforcers were presented using a solenoid-operated 0.1-ml dipper located between the two levers and below the houselight. The chambers were enclosed in sound-attenuating cubicles. Masking noise and noise from

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ventilation fans mounted on the back wall of the cubicles masked extraneous noises. Procedures were conducted and data were collected with an MS-DOS-based microcomputer interfaced with MED Associates, Inc. (East Fairfield, NH) interface and programmed under MEDState Notation (9).

Procedure

General procedure. Rats were divided randomly into three groups of four. Rats in one group (insulin group) were trained to discriminate insulin from saline (0.9% NaCl), rats in a second group (2-DG group) were trained to discriminate 2-DG from saline, and the rats in the third group (deprivation group) were trained to discriminate food deprivation from satiation. Initially, rats were trained to approach the dipper and consume a glucose and saccharin mixture (12% glucose and 0.94% saccharin in tap water; Sigma Chemical Co., St. Louis) when they were food-deprived. Rats were trained to press the left lever. The number of lever presses required for each dipper presentation was raised to three (i.e., a fixed ratio 3 [FR 3]), and discrimination training was begun. During the first few sessions the number of lever presses required for dipper presentation was gradually increased to 15 (i.e., FR 15). Insulin (Regular Porcine Insulin, Novo Nordisk Pharmaceuticals, Princeton, NJ) was diluted and 2-DG (Sigma) was dissolved in saline and administered SC in a volume of 1.0 ml/kg. Sessions began 30 min after injections. After insulin injection (insulin group) or 2-DG injection (2-DG group), or when rats were food-deprived (deprivation group), 15 presses on the left lever produced reinforcement and 15 presses on the right lever produced an 8-s period during which the chamber was dark and responses had no effect (i.e., time-out). After saline injection or when rats were satiated the contingencies were reversed; right lever presses were reinforced and responses on the left lever produced time-outs. Sessions ended after 20 consequences, either reinforcers or time-outs. The stimulus conditions varied irregularly from day to day, with no more than two sessions of the same type occurring consecutively. Generalization tests began when rats received a reinforcer in the first ratio (i.e., more than 50% of the responses during the first ratio were on the condition-appropriate lever) for 10 consecutive sessions. Details regarding generalization testing and training conditions are presented for each group separately.

Insulin group. Rats in the insulin (and the 2-DG) group were fed 23 h prior to sessions. During the first weeks of discrimination training, various insulin doses and feeding schedules were used. The final insulin dose (i.e., 1.0 U/kg) was chosen because it did not disrupt lever pressing.

Generalization testing began when rats' performance had reached the criterion for discrimination. In the insulin group, tests of various insulin doses (0.25, 0.5, 1.0, and 1.5 U/kg) and saline were conducted. Each dose test was preceded by at least four consecutive training sessions (two saline and two insulin) in which condition-appropriate responding was obtained. During test sessions, the first 15 responses on either lever produced a reinforcer. For the remainder of the session, 15 responses on that lever produced reinforcers and 15 responses on the other lever produced time-outs.

Following insulin dose tests, various 2-DG doses (ranging from 25 to 125 mg/kg) and saline were tested in a similar manner. A series of tests was conducted in which rats were fed 12, 23, or 47 h prior to the session. Saline was injected 30 min prior to these sessions. To determine whether doses of insulin (and 2-DG) which were previously indiscriminable would be discriminated under conditions of more severe food deprivation (i.e., whether food deprivation and drug produced additive effects), 0.25 U/kg insulin was tested two more times, once when rats had been fed 47 h prior to the test session and a second time when rats had been fed 23 h prior to the test. Next, to test the specificity of the discrimination, methadone (0.5, 1.0, 2.0, and 3.0 mg/kg) was tested.

Changes in blood-glucose levels following insulin and saline were then measured. Rats were handled as they were prior to training sessions. Thirty minutes after administration of the training dose of insulin or saline rats were placed in the chamber. They were immediately removed and blood was drawn from the tips of their tails. Rats were returned to their home cages and no training sessions were conducted on these days. Glucose levels were determined using the Sigma test system with a Beckman DU65 spectrophotometer.

2-DG group. Four rats were trained to press the left lever after administration of 125 mg/kg 2-DG and to press the right lever after saline administration. Once rats had reached the criterion level of accuracy, generalization testing began. The effects of a range of 2-DG doses (25, 75, 125, and 175 mg/kg) and saline were tested first, followed by insulin tests (0.25 to 2.0 U/kg). The effects of saline were tested after rats had been fed 12, 23, or 47 h prior to the session. Fifty or 75 mg/kg 2-DG was tested two more times, once when rats had been fed 47 h prior to the test session and a second time when rats had been fed 23 h prior to the test. To test the specificity of the discrimination, methadone (0.5, 1.0, 2.0, and 3.0 mg/kg) was then tested. Finally, blood-glucose levels following 2-DG and saline were determined. The procedures followed during generalization tests were the same as those used for the insulin group.

Deprivation group. The procedures used in training for this group were similar to those outlined above, the only difference being that the time that rats were fed their daily ration of food, relative to the start of a session, was modified. The discriminative stimuli for these rats, therefore, were the conditions produced by being fed 2 h or 23 h prior to sessions. Two hours before each session rats were removed from their home cages and weighed. Before half of the sessions, rats were returned to their home cages and given 15 g Purina Rat Chow; right lever presses produced reinforcement during these sessions. Any remaining food was removed 30 min prior to sessions. Thus, rats were allowed 1.5 h to eat the food. Before the other half of the sessions, they were returned to their cages and not fed; left lever presses were reinforced during these sessions. Under both conditions, saline was administered SC 30 min prior to the start of the session.

Generalization testing procedures were similar to those employed in the previous groups, except that tests of all doses of insulin and 2-DG occurred 2 h after rats had been fed. First, the effects of a range of times without food were tested. Rats were fed 2, 6, 14, or 23 h prior to test sessions. The effects of a range of insulin (1.0 to 12.0 U/kg) and 2-DG (25 to 200 mg/kg) doses were then tested. Next, to test the specificity of the discrimination, methadone (0.5, 1.0, 2.0, and 3.0 mg/kg) was tested. Finally, blood glucose levels were determined, as in the other groups, under satiation and deprivation conditions.

RESULTS

All rats learned to respond differentially based on the training stimuli. Rats in the 2-DG group reached the criterion for

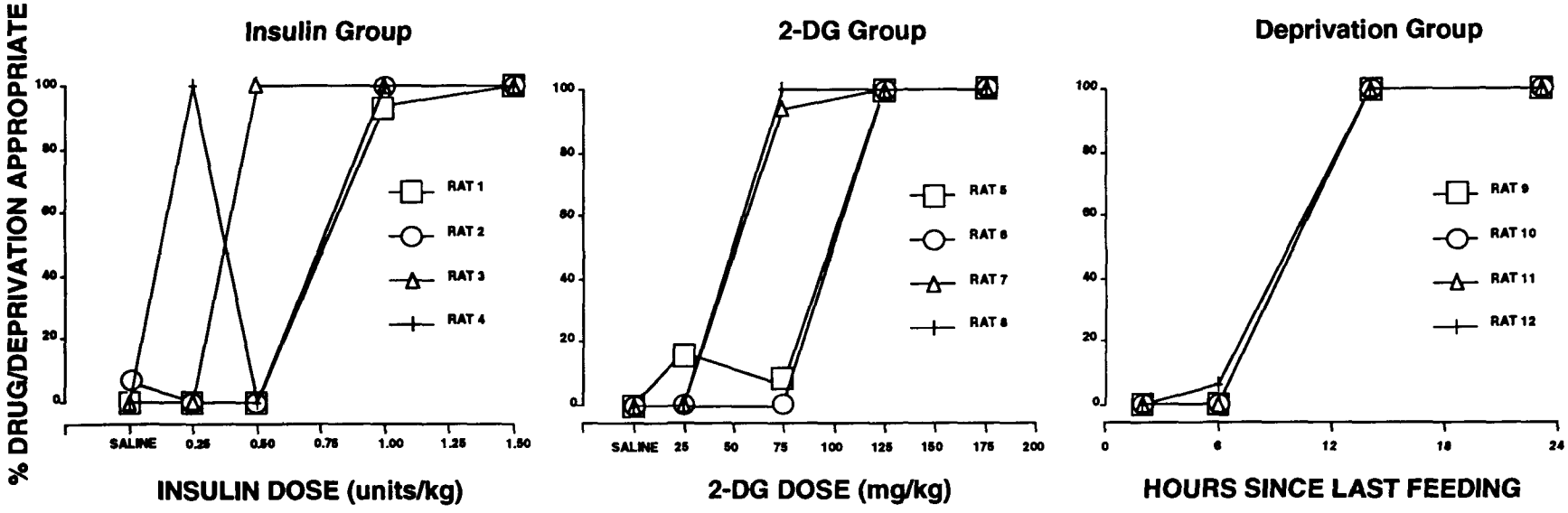

FIG. 1. Percentage of responses occurring on the drug-appropriate (i.e., insulin or 2-DG) or deprivation-appropriate lever prior to the first consequence for each rat in the insulin group (rats 1–4; left panel), the 2-DG group (rats 5–8; middle panel), and the deprivation group (rats 9–12; right panel).

accuracy relatively quickly (mean of 43.8 sessions; range = 35–53). Rats in the insulin group reached criterion in a mean of 129.3 sessions (range = 107–156), and rats in the deprivation group reached criterion in a mean of 120.5 sessions (range = 75–162). Figure 1 shows that drug- or deprivation-appropriate responding was an increasing function of insulin, 2-DG dose, and deprivation. Figure 2 shows the effects of tests of insulin and 2-DG in all three groups. In the upper left panel the effects of 2-DG in insulin-trained rats are depicted. Responses to the insulin-appropriate lever occurred with each rat as the dose approached the training dose used in the 2-DG group (125 mg/kg). Higher 2-DG doses could not be tested because of severe rate-reducing effects. In the lower left panel the effects of insulin on responding by 2-DG-trained rats are shown. 2-DG-appropriate lever pressing was an increasing function of insulin dose. For rats 5, 7, and 8, 2-DG-appropriate responding was observed at the training dose used in the insulin group (1.0 U/kg). Rat 6 responded on the 2-DG-appropriate lever after 1.75 U/kg insulin. The right panels show effects of 2-DG (lower right) and insulin (upper right) in rats trained to respond differentially based on level of food deprivation. Rats 10 and 12 responded on the deprivation-appropriate lever after receiving doses of 2-DG (100 to 200 mg/kg) or insulin (2.0 and 3.0 U/kg in rat 10 and 6.0 and 8.0 U/kg in rat 12). Rats 9 and 11 did not respond on the lever appropriate to food deprivation after any insulin dose, and did so in a manner unrelated to dose following 2-DG administration.

Rats trained to respond differentially to the stimuli produced by insulin or 2-DG selected the saline-appropriate lever when food was witheld for 12, 23, or 47 h prior to the test session (data are not shown). Because food deprivation may interact with decreased glucose availability under insulin and 2-DG, a low dose of these drugs was tested again after 23-h (training conditions) and 47-h food deprivation. Figure 3 shows that doses of insulin (left panel) and 2-DG (right panel) that produced saline-appropriate responding when rats had been fed 23 h before testing produced drug-appropriate responding when they had been fed 47 h before testing. The 2-DG dose tested in rat 5 differed because 50.0 mg/kg pro-

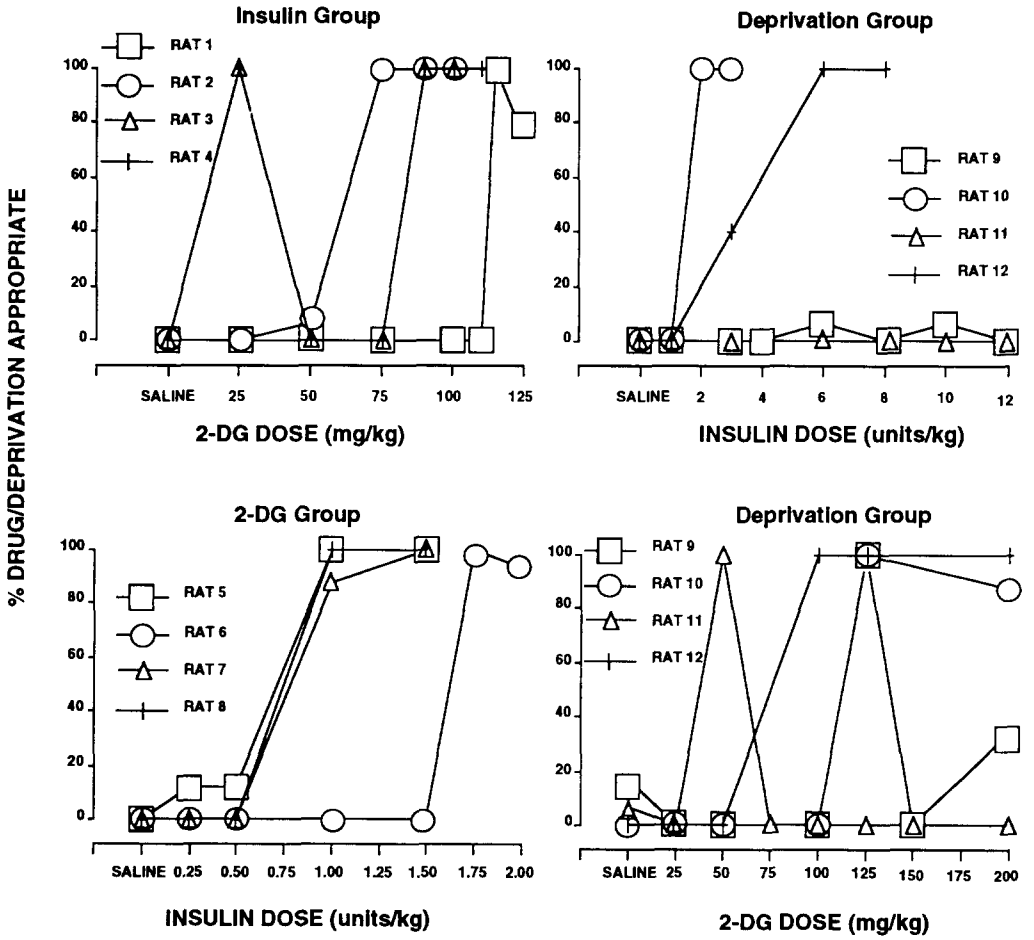

FIG. 2. Effects of insulin or 2-DG on the percentage of responses occurring on the drug-appropriate (i.e., insulin or 2-DG) or deprivation-appropriate lever prior to the first consequence. 2-DG was tested in rats trained to discriminate insulin (upper left panel), insulin was tested in rats trained to discriminate 2-DG (lower left panel), and both insulin (upper right) and 2-DG (lower right) were tested in rats trained to discriminate food deprivation from relative satiation.

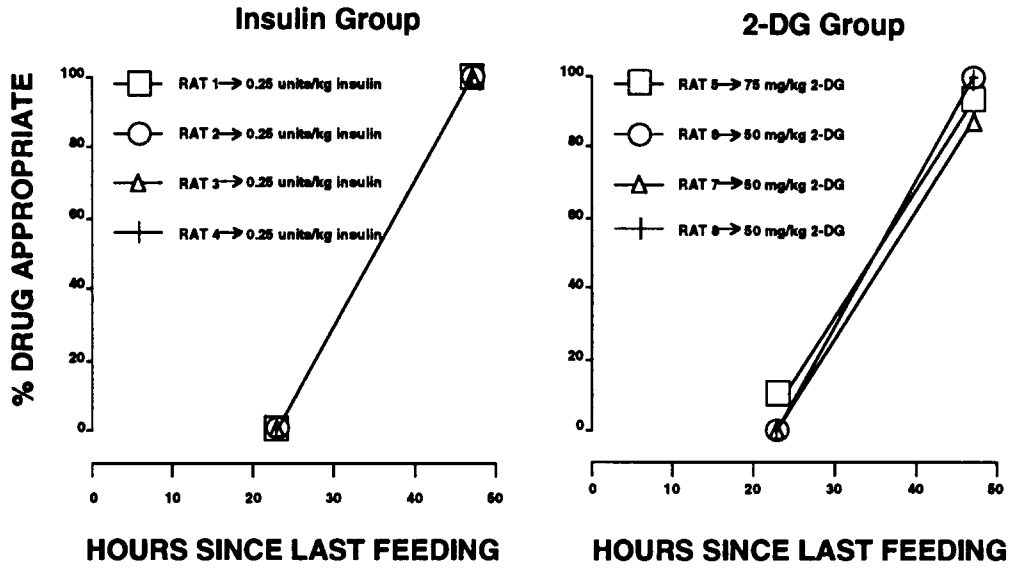

FIG. 3. Effects of low doses of insulin (left panel) or 2-DG (right panel) on the percentage of drug-appropriate responses prior to the first consequence when rats had been fed 23 h or 47 h prior to testing.

duced saline-appropriate responding in this rat after both levels of deprivation initially.

Rats in all three groups selected the saline-appropriate lever after 0.5, 1.0, and 2.0 mg/kg methadone (data are not shown). Methadone 3 mg/kg produced ataxia precluding lever pressing in the first few animals tested and was omitted from further testing.

Figure 4 shows that blood glucose levels were modified by insulin, 2-DG, and food deprivation. Insulin (left panel) significantly lowered blood glucose from a mean of 120 mg% to 53 mg% (matched-sample t, $p < .01$). 2-DG significantly increased blood glucose levels in all four rats (middle panel). The mean level was 148 mg% after 2-DG and 120 mg% after saline (matched-sample t, $p < .01$). When rats were food-deprived (right panel), the mean levels were not significantly different from satiation conditions.

Response rates (lever presses/min) during each test are depicted in Table 1. Although high 2-DG doses typically reduced response rates, changes in rates were not reliably correlated with insulin dose or different levels of food deprivation.

DISCUSSION

The present study showed that three common feeding-inducing operations (insulin and 2-DG administration and food deprivation) all produce discriminable interoceptive stimulus effects. 2-DG was consistently discriminated after a mean of 43.8 sessions, but rats required a mean of 129.3 sessions to consistently discriminate insulin, and 120.5 to discriminate food deprivation. This suggests that conditions produced by 2-DG are more readily discriminated than those of insulin or food deprivation. Rates of lever pressing were not reliably altered by insulin or food deprivation (see Table 1). 2-DG generally reduced response rates in a dose-dependent manner.

The results of the generalization tests indicate these feeding-inducing operations produce overlapping stimulus effects. Insulin and 2-DG were mutually substitutable in rats trained to discriminate one or the other from saline. This observation is consistent with knowledge indicating that both of these procedures produce intraneuronal glycopenia. Insulin in larger doses, such as those used in this study, produces hypoglycemia and lowers brain intracellular glucose by driving glucose into peripheral tissues. Glucose utilization within cells is chemically blocked by 2-DG, thus producing the same effect within cells as seen with lack of glucose availability. There is some evidence that the response to glucoprivation may involve different neural substrates for insulin and 2-DG. Walsh and Grossman (13) found the 2-DG glucoprivic response required an intact zona incerta and midbrain reticular formation, but the response to insulin glucoprivation did not. In the animals in the present experiment no attempts were made to separate these neural pathways. The results of the present study suggest that the reduction of intracellular glucose utilization is the starting point for a series of events, as yet undetermined, that results in the similar stimulus events. Deprivation does not appear to produce substantial alterations in blood glucose levels, at least in the sense that hypoglycemia is not produced. The effect of deprivation on intraneuronal glucose utilization is less clear.

Insulin and 2-DG substituted for 23-h food deprivation in half the rats in the deprivation group (rats 10 and 12). Performance of these two subjects under these conditions may be due to factors unrelated to the stimulus effects of food deprivation. Two findings suggest this variability is not simply due to across-subject individual differences, however. First, deprivation-appropriate responding was produced by insulin and 2-DG in a dose-dependent manner. Second, the two rats that responded as if they were food-deprived when given insulin responded the same way after 2-DG. A possible reason for the differences across subjects may be that food deprivation

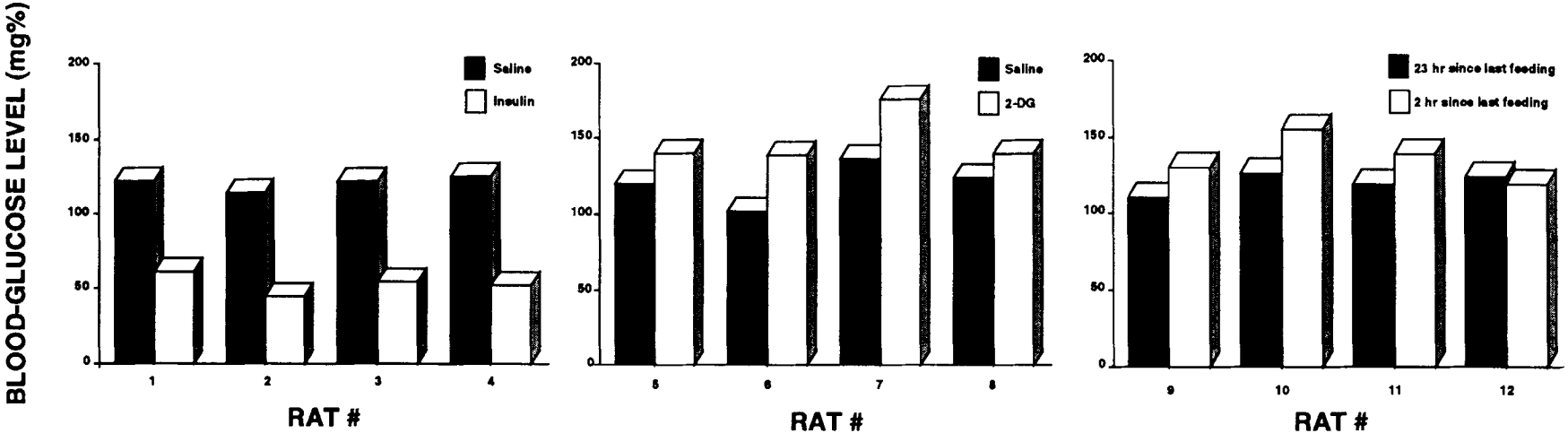

FIG. 4. Blood-glucose levels (mg%) following insulin (left panel), 2-DG (middle panel), or food deprivation (right panel) in each rat. Effects of saline and food deprivation are depicted by filled bars; effects of insulin, 2-DG, and relative satiation are depicted by clear bars.

TABLE 1

MEAN RATES OF RESPONDING (LEVER PRESSES/MIN ± SD) DURING GENERALIZATION TESTING IN THREE GROUPS OF RATS TRAINED TO DISCRIMINATE INSULIN, 2-DG, OR FOOD DEPRIVATION

Insulin (U/kg)	Insulin Group	2-DG Group	Deprivation Group
0.0	57.3 (19.2)	53.4 (7.9)	64.1 (28.7)
0.25	59.8 (9.5)	56.3 (8.3)	–
0.5	56.4 (15.7)	52.7 (13.4)	–
1.0	55.8 (9.4)	31.8 (18.3)	56.9 (26.0)
1.5	45.8 (21.6)	47.7 (5.3)	–
1.75	–	46.6*	–
2.0	–	48.0*	54.9*
3.0	–	–	55.4 (24.2)
4.0	–	–	51.4*
6.0	–	–	70.8 (33.2)†
8.0	–	–	69.8 (29.8)†
10.0	–	–	78.1 (29.7)‡
12.0	–	–	82.1 (32.9)‡
2-DG (mg/kg)			
0.0	58.9 (12.3)	53.2 (13.7)	58.5 (23.1)
25.0	57.4 (8.8)	51.9 (8.5)	54.7 (18.3)
50.0	62.5 (14.7)	–	58.2 (22.9)
75.0	50.6 (21.9)	47.1 (9.4)	96.0*
90.0	30.9 (23.2)†	–	–
100.0	25.0 (19.1)	–	57.8 (24.2)
110.0	42.6 (36.6)‡	–	–
115.0	1.07*	–	–
125.0	0.8*	36.1 (14.9)	60.0 (19.8)
150.0	–	–	73.8 (16.8)‡
175.0	–	18.4 (7.4)	–
200.0	–	–	48.0 (34.9)
Hours + insulin/2-DG			
23	53.1 (20.3)	50.0 (9.4)	–
47	56.5 (11.2)	41.5 (8.6)	–

*Only one subject tested. †Mean based on two subjects. ‡Mean based on three subjects.

produces more than one discriminable interoceptive stimulus change, only one of which is produced by insulin and 2-DG. The variation across subjects in the deprivation group's response to insulin and 2-DG may have occurred because subjects' performance was under control of different aspects of the food deprivation state.

Increasing food deprivation levels in the rats in the insulin and 2-DG groups did not produce insulin- or 2-DG-appropriate responding. This may reflect the fact that these substances produce more rapid changes in glucose availability during the 30 min prior to the session, contrasted with the gradual change in glucose availability produced by being without food for two days. This explanation is supported by the fact that doses of both substances that did not produce drug-appropriate responding after 23-h deprivation reliably did so after 47-h deprivation (Fig. 3). Forty-seven-hour food deprivation may have combined with the small but rapid change in glucose availability produced by low doses of insulin and 2-DG to produce conditions that were similar to those produced by larger doses of the drugs. More research will be required to determine whether the rapidity of the onset of changes in glucose availability is an important determinant of the discriminative stimulus effects of insulin and 2-DG.

Discrimination of internal cues produced by food deprivation has been studied previously in numerous ways (1,2,5–8). Recently, Corwin, Woolverton, and Schuster (3) used a procedure similar to the present one to teach rats to respond differentially based on the conditions produced by 3-h or 22-h food deprivation. Cholecystokinin, lithium chloride, *d*-amphetamine, and fenfluramine were each tested after animals were 22-h food-deprived. Cholecystokinin produced satiation-appropriate responding, suggesting stimuli produced by this putative satiety factor mimic those produced by food satiation. The other drugs, noted for their anorectic effects, did not reliably produce satiation-appropriate responding. The effects observed in this study and in the present experiments suggest similarities in the stimulus effects of procedures which are thought to produce hunger or satiation.

ACKNOWLEDGEMENTS

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