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Effect of Insulin and 2-Deoxy-D-Glucose on Feeding and Plasma Glucose Levels in the Spiny Mouse

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CZECH, D. A. *Effect of insulin and 2-deoxy-D-glucose on feeding and plasma glucose levels in the spiny mouse.* PHYSIOL BEHAV **43**(6) 765–769, 1988.—Adult male spiny mice (*Acomys cahirinus*) were challenged with 2-deoxy-D-glucose (2-DG) or regular insulin, and food intake or plasma glucose concentration was measured. Mice did not increase their food intake over baseline levels following treatment with 2-DG (62.5–1000 mg/kg). In contrast, regular insulin injections (1–50 U/kg) stimulated a modest, but significant increase in feeding, which was apparent within 2 hr at a low dose of 1 U/kg. However, a marked hyper- and hypoglycemia (compared to saline controls), respectively, were induced within 30 min by 2-DG (250 and 500 mg/kg) and regular insulin (1 and 3 U/kg). Reduced glucose levels may not account for the insulin-induced hyperphagia.

Food intake 2-Deoxy-D-glucose Insulin Glucoprivation Spiny mouse *Acomys cahirinus*

ADMINISTRATION of insulin or of the glucose antimetabolites 2-deoxy-D-glucose (2-DG) and 5-thio-D-glucose (5-TG) leads to a robust feeding response in a number of mammalian species (e.g., [11, 12, 14, 17, 18]). It is, however, becoming increasingly clear that this is not a universal phenomenon and that attribution of feeding in these studies to an induced state of glucoprivation is probably premature. Recent reports have revealed species diversity, showing that several common laboratory animals fail to increase food intake in response to 2-DG or 5-TG, but do exhibit some degree of hyperphagia under some conditions following insulin administration; these include golden hamster [16, 19, 22, 25], gerbil [19,22] and deermouse [22,23].

The present study reports a similar dissociation in a desert-dwelling murid rodent, the spiny mouse (*Acomys cahirinus*). This species, believed to be closely related to the gerbil, has been used principally in investigations of diabetes and obesity [10,15] and of renal physiology [3], and may represent a potentially useful model for studying aspects of fluid and energy regulation.

EXPERIMENT 1: FEEDING TESTS

METHOD

Animals

Male adult spiny mice (*Acomys cahirinus*) from the colony maintained at Marquette were individually housed in polypropylene tub-type cages in an air-conditioned room maintained on a 12/12 hr light/dark cycle (lights on 0700–1900 hr). Tap water and ground food (Wayne rodent chow) were available ad lib except as indicated below.

Procedure

2-DG. At approximately 0800 on test days, mice (N=14) were weighed, food and water were removed from cages, and fresh bedding material was provided. Ninety to 120 min later, they were injected with one of three doses of 2-DG (250, 500 and 1000 mg/kg, IP) or normal saline vehicle, and immediately returned to the home cage with access to ground food. Food consumption to the nearest 0.1 g was measured 1, 2, 6 and 24 hr later. Water was not available during the first six hours of testing. All mice were tested under all treatment conditions in a within-subject design with order of treatment counterbalanced. Tests were separated by at least four days.

Insulin. Consistent with preparation and times noted above, mice (N=12) were injected with one of five doses of regular insulin (Lilly, Iletin®) (1, 3, 10, 30 and 50 U/kg, SC) or normal saline vehicle. Food consumption to the nearest 0.1 g was measured at 1, 2, 4, 6 and 24 hr. Water was available during the entire test period. A within-subjects design was again used, with the 0 (vehicle), 3, 10 and 30 U/kg doses run first, followed by the 1 and 50 U/kg doses; order was counterbalanced within each series. Tests were separated by seven days.

Analysis

2-DG. Cumulative food intakes were evaluated separately for each measurement period with repeated measures ANOVA and Dunnett's procedures. Minimally acceptable significance level was set at $p<0.05$.

Insulin. Analysis procedures were the same as used in the

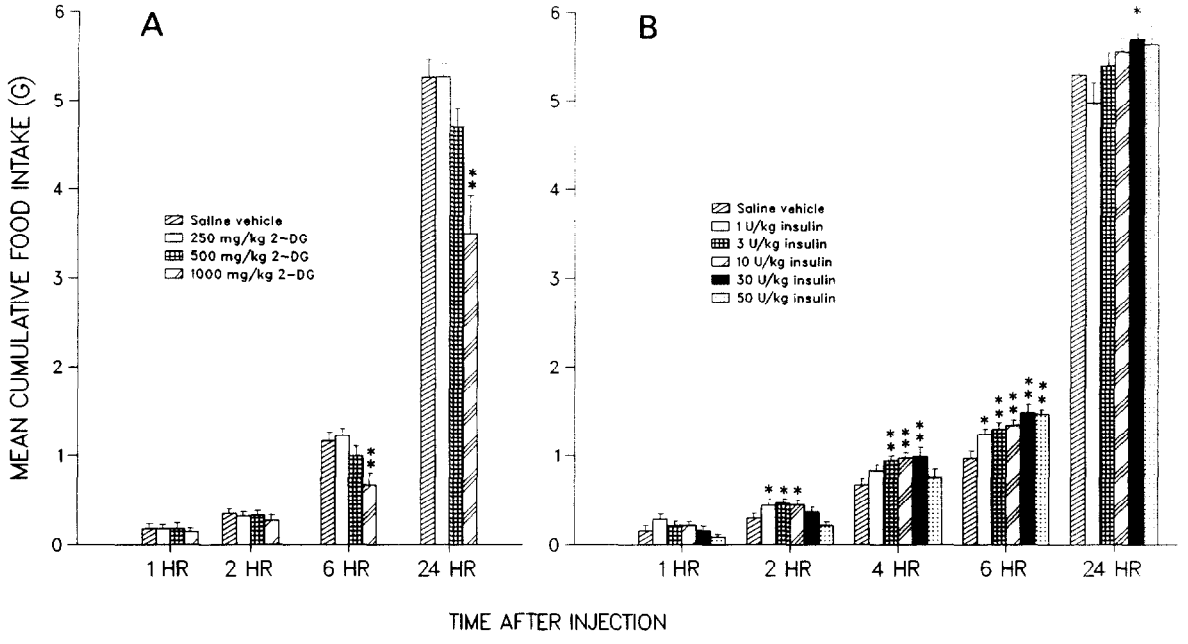

FIG. 1. Mean cumulative food intake following 2-DG (A) or regular insulin (B) and saline vehicle control injections at selected time intervals after access to ground food. Vertical lines reflect ±SEM. Asterisks indicate significantly different from saline control injection: *$p<0.05$, **$p<0.01$, Dunnett's test (two-tailed).

2-DG series, however, only seven mice completed the entire treatment series as reported below.

RESULTS

2-DG

Food intake data for the 2-DG treatment series are summarized in Fig. 1A. When compared to vehicle control injection, no dose of 2-DG increased feeding at any of the time intervals over the 24-hr test period. At the highest dose (1000 mg/kg), food intake was significantly attenuated at 6 and 24 hr (both $p<0.01$). Several animals appeared somewhat lethargic at this dose. These observations, however, were not systematic; no operationalized criteria of general activity were used. Feeding was also depressed, although not significantly, at the 500 mg/kg dose; however, no obvious signs of debilitation were apparent.

While previous work from our laboratory does not support a close association between eating and drinking in *Acomys*, an additional experiment (N=7) was carried out in which water was available ad lib throughout the entire 24-hr data collection period. This experiment (data not shown) also extended the dose range tested (doses were 62.5, 125, 250 and 500 mg/kg SC, and saline vehicle). Again, no dose of 2-DG stimulated feeding, while the 500 mg/kg dose significantly attenuated food intake at all but the first hour (all $p<0.05$).

Insulin

Food intake data for the insulin series are shown in Fig. 1B. In sharp contrast to the 2-DG data, regular insulin stimulated feeding. A modest, but significant increase in food intake occurred within 2 hr of injection of the lowest dose (1 U/kg) used. With the exception of 1 U/kg at 4 hr and 30 U/kg at 2 hr, doses ranging from 1–30 U/kg were consistently significantly higher than vehicle injection over the first 6 hr. Some fluctuation over time might be expected since *Acomys* ordinarily eats frequent small meals [2]; an interaction of insulin treatment and the timing of animals' ongoing meal-taking patterns could probably produce shifts in intake sufficient to produce observed fluctuations, particularly since obtained p values were very close (both above and below) to the rejection criterion ($p<0.05$) values for the 1 U/kg condition at 1, 2 and 4 hr. The somewhat lower food intakes over the first 2 hr at 30 U/kg suggested some debilitation during this earlier period. Indeed, 50 U/kg was generally debilitating and was fatal in 5 of 12 (40%) mice. Consequently, the insulin data are based on a sample size of seven animals. For these seven subjects, debilitation at 50 U/kg is suggested by relatively low intakes during at least the first 4 hr, when locomotor activity appeared to be markedly lower than usual. From hr 4 to 6, insulin stimulated feeding. With the exception of the 30 U/kg dose, food intake of insulin treated mice was no longer significantly different from control treatment at 24 hr. This likely reflects the vehicle condition intakes "catching up" by 24 hr to cumulative intakes induced by the lower insulin doses.

EXPERIMENT 2: BLOOD SAMPLING

In the first experiment, it was shown that acute insulin treatment can lead to increased food intake in the spiny mouse, while 2-DG fails to stimulate eating over a wide dose range. In Experiment 2, we asked if treatment with these agents triggers the expected robust hypoglycemic (insulin) or hyperglycemic (2-DG) response widely documented in other species and, if so, its time course. These data were not currently available for *Acomys*.

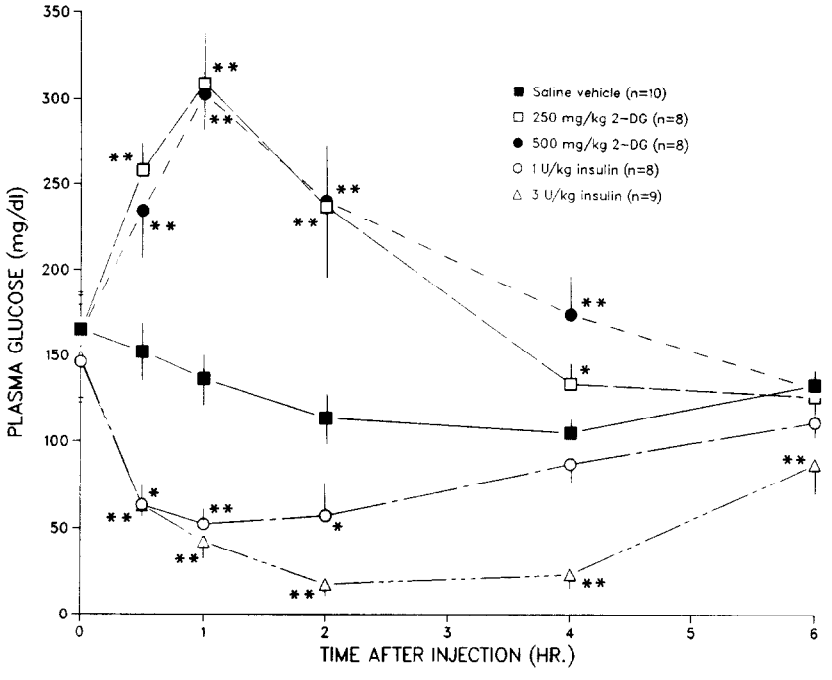

FIG. 2. Mean plasma glucose concentration (mg/dl) at selected time intervals following a single injection of 2-DG or regular insulin, or saline vehicle control injection, administered at time 0. Vertical lines reflect ±SEM. Asterisks indicate significantly different from control group: $*p<0.05$, $**p<0.01$, Dunnett's test (two-tailed).

METHOD

Animals

Male adult spiny mice were maintained as before, on a 12/12 hr light/dark cycle in tub cages. Tap water was available ad lib; food in pelleted form was available ad lib except during tests.

Procedure

On the test day, food was removed approximately 80–100 minutes prior to drawing an initial (time 0) blood sample, which served as the control sample. Immediately after the time 0 drawing, mice were injected with one of two doses of regular insulin (Iletin®) (1 and 3 U/kg, SC) or 2-DG (250 and 500 mg/kg, IP) or normal saline vehicle (IP or SC), and returned to the home cage with only water available. Additional blood samples were drawn at 0.5, 1, 2, 4 and 6 hr (within-subjects). Blood sampling (40 μl) was done by retro-orbital puncture technique under light ether anesthesia. Samples were quickly centrifuged and the plasma was drawn off and refrigerated (or frozen) for subsequent analysis. Plasma glucose concentration was determined by the glucose oxidase method (Sigma Chemical Kit 510-A) on a Coleman (model 6/20) Junior II spectrophotometer. Testing was carried out between 0830 and 1500 hr.

Analysis

Plasma glucose levels at 0.5, 1, 2, 4 and 6 hr were converted to percent of glucose level at time 0. Both absolute (mg/dl) and "percent of control" concentrations were analyzed in 5 (type of injection) × 6 (sampling time) mixed factorial ANOVAs, with replications on the sampling factor. Post hoc pairwise comparisons were carried out using Dunnett's procedures. Rejection level was again set at $p<0.05$.

RESULTS

Figure 2 reflects shifts in plasma glucose levels following the various treatments. A significant hyperglycemia (comparison to saline injected controls) occurred within 30 min of injection with 2-DG; plasma glucose concentrations were highest at the 60 min sampling point, reaching 196% (309 mg/dl) and 188% (303 mg/dl) of preinjection baseline levels, respectively, for 250 and 500 mg/kg 2-DG. Both insulin doses produced a significant hypoglycemia, also within 30 min. Plasma glucose in the 1 U/kg group dropped to 37% (52 mg/dl) of preinjection level within the first hour, while the 3 U/kg group dropped to 12% (18 mg/dl) by hr 2. Mean preinjection glucose concentrations were 165, 163, 163, 146 and 149 mg/dl, respectively, for vehicle, 2-DG (250 and 500 mg/kg) and insulin (1 and 3 U/kg). Only the 3 U/kg insulin group remained significantly different from controls at the end of 6 hours. Analyses of the "percent of control" data (not shown) yielded essentially the same outcome, differing only in that 1 U/kg insulin at 2 hr, 250 mg/kg 2-DG at 4 hr and 3 U/kg insulin at 6 hr did not differ significantly from saline controls.

GENERAL DISCUSSION

Results of the present study clearly show a dissociation between the ability of 2-DG and insulin to stimulate feeding in the spiny mouse. As previously observed in a number of species [19,23], and most extensively investigated in the

golden hamster [16, 19, 25], even a very large dose of 2-DG failed to stimulate feeding. Seemingly nondebilitating doses of 2-DG, however, produced the expected hyperglycemic response. While 2-DG-induced feeding in the rat appears to be somewhat dependent on diet palatability [27], in hamster even a highly preferred sunflower seed diet is ineffective [24].

It is conceivable, however, that vigorous sympathetic activation, indicated by the pronounced hyperglycemia observed, might have interfered with a feeding response. In light of this possibility, we tested two lower doses of 2-DG (62.5 and 125 mg/kg), which also failed to stimulate feeding. These doses were subsequently tested for their effect on blood glucose levels, as well. Following injection of a 125 mg/kg dose, mean blood glucose level was highest at the 2 hr sampling point, reaching 127% of preinjection baseline before dropping back to 114% by hr 5; with 62.5 mg/kg, mean blood glucose was slightly below baseline at all postinjection sampling points (1, 2 and 5 hr). Considering these data, it would seem unlikely that response interference or competition is responsible for the absence of enhanced feeding observed in *Acomys*.

In sharp contrast, regular insulin induced a highly reliable and significant, although modest, feeding response in *Acomys*. Compared to the rat, which has shown increases in intakes upwards of 400% over baseline in a 2-hr feeding test following administration of 5 U/kg of regular insulin [9], *Acomys'* maximal mean increase was 53% (range: 28–53%, excluding 50 U/kg dose) as measured at 6 hr. Comparable increases have been reported for the hamster [5,16]. Relatively low percent increases might be partially accounted for in higher baseline intakes. While concentrating its feeding in the dark period, the spiny mouse eats frequent small meals at somewhat irregular intervals throughout the 24 hr period. Consequently, daytime intakes might be expected to be somewhat high even under control conditions. Relative increases in food intake are also less in rats when insulin is given during the dark period, when baseline intakes are higher [13]. *Acomys*, however, is quite sensitive (1 U/kg) to insulin stimulation. Considerably larger doses have often been reported as necessary to significantly increase feeding in hamster (e.g., ranging from 2 U/kg [16] to >40 U/kg [19]), gerbil (>100 U/kg [19]), and deermouse (100 U/kg [23]). Direct comparison of threshold dose is generally precluded, however, owing to limited range of doses used in individual studies with animals other than rat and, to a lesser degree, hamster.

While insulin led to both feeding and hypoglycemia, it does not necessarily follow that the spiny mouse possesses a functional glucoprivic feeding mechanism, a point that has been made for the hamster as well. In connection with this issue, e.g., it will be noted that while there appears to be a distinct trend toward dose-relatedness in the insulin data by hr 6, the differences are nonetheless quite small. At 6 hr, mean intakes are 1.24, 1.30, 1.34, 1.48 and 1.47 g, respectively, for the 1, 3, 10, 30 and 50 U/kg doses; indeed, the slightly higher intakes for the two highest doses might simply reflect "overcompensation" following earlier depressed feeding rather than dose-related increases. Limited, or absence of, dose dependency associated with regular insulin-induced feeding is also seen with the hamster [5, 6 16]. In both rat [9,26] and hamster [7], insulin accelerates gastric emptying, which could also promote feeding. Further, Rowland has shown that filling and emptying of hamster forestomach occurs with a periodicity similar to that of spontaneous meal-taking [20], and insulin-induced hyperphagia in hamsters appears to be a function of increased meal frequency rather than meal size [6]. In contrast, 2-DG had no effect on, and 5-TG retarded forestomach emptying [8]. We have seen (unpublished observations) both accelerated stomach emptying and increased meal frequency, without change in meal duration, following acute insulin treatment in *Acomys* as well. The role of gastric emptying in insulin-induced hyperphagia is unknown. Our preliminary anatomic observations show that *Acomys* does not possess a two-chambered stomach.

In addition to producing hypoglycemia, insulin injections have been shown to lower plasma free fatty acid (FFA) levels [4, 5, 21], thus reducing the availability of these metabolic fuels as well (in contrast, 5-TG increases plasma FFA [4]). DiBattista [5] points out that reduced FFA would also limit availability of ketone bodies as an alternate energy source. Consequently, reduced glucose availability is just one of several physiological responses to insulin administration which could influence feeding behavior in *Acomys*. Experimental protocols used in the present study, however, do not permit pursuing these issues at this time. It might be instructive to assess the potential role of FFA and ketone bodies in *Acomys'* feeding response to insulin or glucose antimetabolites.

In summary, very little is known about feeding behavior in the spiny mouse, and it will be important to expand our data bases to determine similarities/differences in the behavior, physiology and morphology of *Acomys* relative to other species. We recently reported that *Acomys* possesses functional opioid-sensitive feeding and drinking systems [1] and, like the gerbil and the rat, is an effective postdeprivation compensatory feeder [2].

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