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Food Consumption, Plasma Glucose and Stomach-Emptying in Insulin-Injected Hamsters

DAVID DiBATTISTA[1]

University of Waterloo, Department of Psychology, Waterloo, Ontario
Hospital for Sick Children, Research Institute, Toronto, Ontario

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DiBATTISTA, D. *Food consumption, plasma glucose and stomach-emptying in insulin-injected hamsters.* PHYSIOL BEHAV 33(1) 13–20, 1984.—Two experiments were performed to investigate whether insulin-induced hyperphagia (IIH) serves an adaptive function in counteracting hypoglycemia in hamsters. In Experiment 1, insulin-injected hamsters having free access to food during a six-hour feeding test had neither higher plasma glucose (PG) concentrations nor lower frequencies of neurological impairment at the end of the test than did hamsters whose food intake was restricted to control levels. In Experiment 2, it was observed that PG fluctuations did not act as a trigger for meal-onset in insulin-injected hamsters, nor was PG affected by consumption of a meal during IIH. The withholding of food for periods longer than the typical intermeal interval (IMI) of insulin-injected hamsters (=50 min) resulted in a marked increase in the frequency of neurological deficits among hamsters having PG levels lower than about 40 mg/dl. However animals with similarly low PG concentrations, but deprived of food for less than 50 min showed no signs of impairment, suggesting that some alternate metabolic fuel is available during the IMI and prevents the occurrence of behavioural deficits. The results of these experiments suggest that a simple glucostatic interpretation of IIH in hamsters is inadequate, and although hyploglycemia may play a role in hamster IIH, other factors must also be considered. In Experiment 3, the effect of a hyperphagia-inducing dose of insulin upon stomach emptying was investigated. It was found that both insulin-injected and control hamsters have similar amounts of food in their respective pregastric and gastric pouches at both the onset and offset of meals, but that both pouches empty far more rapidly under the influence of insulin. It is suggested that IIH in hamsters may be dependent in large measure upon the acceleration of stomach emptying, which may in turn depend upon the concomitant hypoglycemia. The exact nature of the hypothesized satiety cues arising from the hamster stomach remains to be investigated.

Golden hamster Insulin Hyperphagia Hypoglycemia Stomach-emptying

ADMINISTRATION of exogenous insulin produces both hypoglycemia and hyperphagia in the golden hamster [9, 10, 11, 21, 24], as it does in a variety of species [18, 24, 27]. Although insulin produces a number of metabolic and physiological effects which may influence food intake [13], insulin-induced hyperphagia (IIH) has often been considered to be a response primarily to the concomitant hypoglycemia [2, 17, 21, 31, 32]. A variety of findings support this glucostatic interpretation. For example, chronic insulin administration produces increases in carbohydrate intake, but not fat or protein intake, in rats having access to a choice of nutrient sources [16]. In addition, there is a significant direct relationship between the sensitivity of hamsters to the hypoglycemic effect of a given dose of insulin and their food intake in response to the same dose of insulin [11]. Such findings suggest that the reduction in glucose availability may play an especially important role in IIH.

Given the possible importance of hypoglycemia in the production of IIH, it may be asked whether IIH in turn serves an adaptive function in counteracting hypoglycemia in chow-fed hamsters. While several authors have suggested that IIH may serve as a behavioural mechanism for glucose homeostasis by increasing the supply of nutrients from the gastrointestinal tract [16, 27, 34], relevant data are not available. It is known, for example, that insulin-injected hamsters having free access to food have higher concentrations of plasma glucose (PG) at the end of a six-hour feeding test than do similarly treated hamsters having no access to food [11]. However such data do not properly address the question at hand because the comparison being made is between hyperphagic animals and those which are totally food-deprived. In terms of the adaptive significance of IIH, the appropriate comparison would be between insulin-injected hamsters which are allowed to eat ad lib and those which are restricted to baseline food intake levels.

Experiment 1 was designed to evaluate the adaptive role of hyperphagia in counteracting insulin-induced hypoglycemia in the hamster. Hamsters were injected with a dose of

[1]Requests for reprints should be addressed to D. DiBattista, Concordia University, Department of Psychology, 1455 de Maisonneuve Blvd. West, Montreal, Quebec, Canada, H3G 1M8.

regular insulin known to produce both hypoglycemia and hyperphagia, and measures of PG and neurological integrity were obtained six-hours later. Insulin-injected hamsters having free access to food during the test period did not differ significantly on these measures from animals whose food intake was restricted to the level of saline-injected controls. Both of these insulin-injected groups had higher PG levels and fewer instances of neurological deficits than did insulin-injected hamsters which were denied access to food during the test period.

EXPERIMENT 1

METHOD

Subjects

Ninety adult (80–120 g) male golden hamsters (*Mesocricetus auratus*) were obtained from High Oak Ranch, Goodwood, Ontario. The animals were housed individually in hanging wire cages in a single room with lights on from 0700 to 2300 hr. Room temperature was 21–24 degrees Celcius. Water was available ad lib, and Master Laboratory Chow (Master Feeds, Baden, Ontario) was available within the cage at all times, except as noted below.

Solutions

Regular insulin (Insulin Toronto; Connaught Laboratories, Toronto) was diluted in sterile physiological (0.9%) saline to a concentration of 6 units/ml, and was administered at a dosage of 30 units/kg. Sterile physiological saline (0.5 ml/100 g of bodyweight) was used for control injections. All injections were administered subcutaneously.

Groups

Subjects were randomly assigned to one of six treatment groups (n=15/group). Animals in three groups were injected with insulin (Insulin -0, Insulin-4 and Insulin-6), while the remaining animals received saline (Saline-0, Saline-4, and Saline-6).

Hamsters in the Insulin-6 and Saline-6 groups were allowed access to food throughout the six-hour post-injection period, while the Insulin-0 and Saline-0 groups were food-deprived during this time.

The Insulin-4 and Saline-4 groups were allowed access to food during four of the six hours after injection. Food was placed in the cage immediately after injection, removed and weighed at +2 hr, and replaced at +4 hr. This degree of partial deprivation was chosen on the basis of preliminary observations that insulin-injected hamsters allowed access to food in this fashion eat only as much as do saline-injected animals during a full six-hour feeding test.

Procedures

The experiment was conducted over a period of several weeks, with all procedures on a given day beginning at approximately 1000 hr. Hamsters were weighed to the nearest gram, injected with the solution under investigation, and returned to the home cage. Animals in certain groups were given access to a single preweighed pellet of fresh chow during all or part of the next six hours, as described above. The pellet, plus spillage, was weighed to the nearest 0.01 g to determine food consumption.

At the end of the six-hour post-injection period, each animal was carefully observed as it was allowed to explore a table top for 15–20 sec. The animal was considered to be ataxic if its movements were noticeably slow, laboured, uncoordinated, or otherwise not typical of normal hamsters. Animals in the Insulin-4 and Saline-4 groups were also briefly observed for signs of neurological impairment in their home cages at +2 and +4 hr post-injection.

Immediately following observation at +6 hr, a blood sample (0.2 ml) was taken using the cardiac puncture technique under brief, light halothane anesthesia. The sample was centrifuged, and the plasma was frozen for subsequent analysis by the o-toluidine method [12], using a commercially available reagent kit (Pierce Chemical, Rockford, IL).

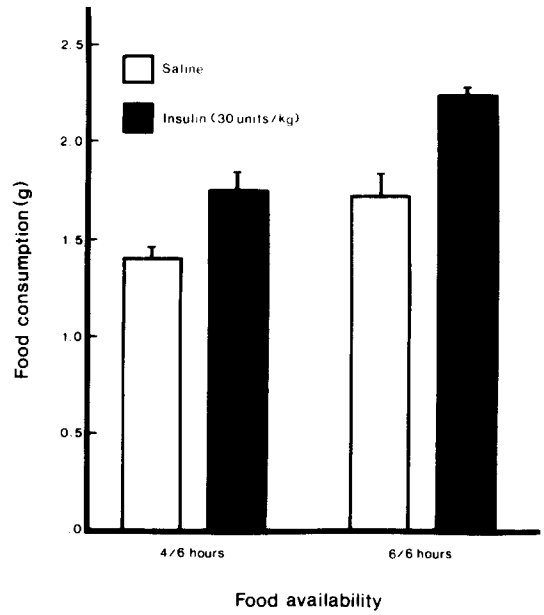

FIG. 1. Effect of insulin upon food consumption under various conditions of food availability during a six-hour feeding test. Values are Mean±SEM, N=15/group.

Statistics

Food intake and PG data were analyzed by the analysis of variance (ANOVA) and by appropriate tests for planned comparisons [19]. Tukey's test was used for post-hoc comparisons [19]. Data concerning neurological impairment were analyzed by non-parametric techniques [29].

RESULTS

Food intake data are summarized in Fig. 1. A two-way ANOVA yielded significant main effects for both the Injection and Food availability variables, $F(1,56)=15.6$ and 13.5, respectively, $p<0.001$, although the interaction was not significant ($p>0.05$). However, as anticipated, intake for the Insulin-6 group was greater than for the Saline-6 group, and for all other groups as well ($p<0.001$).

Mean intakes for the Insulin-4 and Saline-6 groups were virtually identical, indicating that the food intake of animals in the Insulin-4 group had been successfully restricted to baseline levels for a six-hour feeding test. A paired *t*-test

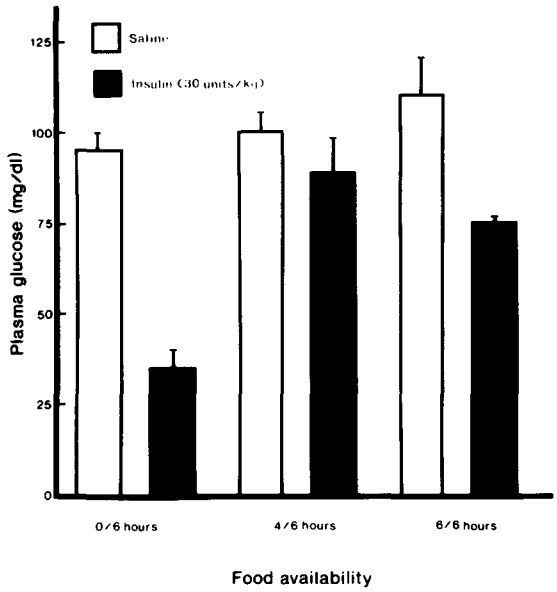

FIG. 2. Effect of insulin upon plasma glucose concentration under various conditions of food availability during a six-hour feeding test. Values are Mean±SEM, N=15/group.

further indicated that the Insulin-4 subjects ate rather similar amounts in the two two-hour periods during which food was available to them (0.77 vs. 0.99 g; $t(14)=1.15$, $p>0.05$). In addition, all of these hamsters consumed appreciable amounts of food during these periods (range=0.27–2.40 g), and none of them was observed to be ataxic at either +2, +4 or +6 hr post-injection. The data thus indicate that hamsters in the Insulin-4 group were not neurologically impaired during the portions of the test interval during which food was available to them.

ANOVA of the PG data, shown in Fig. 2, indicated a significant Injection × Food availability interaction, $F(2,84)=6.30$, $p<0.005$. The mean of the Insulin-0 group was lower than that of all other groups ($p<0.01$), but the Insulin-4 and Insulin-6 groups did not differ from each other ($p>0.05$). Although the difference in PG between the Insulin-4 and Saline-4 groups was not significant ($p>0.05$), PG was significantly lower for the Insulin-6 group than for the Saline-6 group ($p<0.01$).

In addition to having the lowest PG levels, hamsters in the Insulin-0 group were more frequently neurologically impaired at +6 hr than were the other insulin-injected animals (Fisher's exact test, $p=0.0006$). Six of fifteen hamsters in the Insulin-0 group demonstrated signs of impairment ranging from moderate ataxia to coma, the impaired hamsters having PG concentrations in the range 14–46 mg/dl (median=17). All hamsters in both of the other insulin-injected groups and in the various saline-injected groups had PG levels above this range and were never observed to be impaired.

DISCUSSION

This experiment replicates the finding that insulin-injected hamsters which are allowed to become hyperphagic have higher PG concentrations and show fewer signs of neurological impairment six hours after injection than do insulin-injected food-deprived hamsters [11]. Thus the availability of nutrients to be absorbed from the gastrointestinal tract serves to offset both insulin-induced hypoglycemia and its neurobehavioural sequelae in hyperphagic hamsters. However it is clear from the data presented here that IIH is no more effective in allowing hamsters to offset hypoglycemia than is consumption of a merely normal amount of food. In fact, insulin-injected hamsters restricted to baseline levels of food consumption show no signs of neurological impairment and do not have different PG concentrations at +6 hr post-injection than do hyperphagic hamsters. Therefore, under the conditions of Experiment 1, hyperphagia does not serve an adaptive function in counteracting insulin-induced hypoglycemia in hamsters.

It should be noted that, because of species differences in feeding patterns, a procedure similar to that of Experiment 1 might produce very different results in the more commonly studied rat than in the hamster. The degree of IIH in the hamster is quite small compared to that typically observed in rats. The 30% increase in food consumption by the free-feeding insulin-injected hamsters of Experiment 1 is similar to increases previously observed in this species [10, 11, 21, 24]. In contrast, rats frequently increase food consumption by up to 400% when insulin is administered during the daytime [13,22], when they normally eat very little [37]. Because their normal daytime level of food intake is very low, rats might suffer considerably more than hamsters in terms of both hypoglycemia and neurological impairment if restricted to baseline levels of food consumption following insulin administration. Therefore it would still be reasonable to hypothesize that IIH would serve an adaptive function in counteracting hypoglycemia in rats, although such is not the case in hamsters.

It must be noted that the technique used in Experiment 1 to restrict the food intake of insulin-injected hamsters to control levels was both arbitrary and artificial. Hamsters normally consume meals at intervals of 60–80 min, and in response to an effective dose of insulin, reduce this interval to 35–50 min during a six-hour feeding test [11]. Thus a more appropriate method of restricting the food consumption of insulin-injected hasters would involve a pair-feeding design, with insulin-injected hamsters being allowed access to food during the feeding test only when their paired, saline-injected controls initiate and consume meals. Such a design would ensure that insulin-injected animals would space their meals like control animals and eat approximately as much as controls throughout the entire feeding test. Unfortunately, when it was attempted in this laboratory, this pair-feeding design proved impractical and had to be abandoned. All too often 8/14 cases), insulin-injected hamsters showed signs of neurological impairment at the time their saline-injected matched controls initiated meals, and they did not, and presumably were not able to, eat food when it was made available to them. This observation appears anomalous when considered in conjunction with the results of Experiment 1. Recall that hamsters in the Insulin-4 group were food-deprived for two full hours in the middle of the feeding test, and yet they never showed signs of neurological impairment at the end of this deprivation period (i.e., at +4 hr post-injection). It is somewhat surprising then that insulin-injected hamsters in the abortive pair-feeding experiment were so often impaired after being without food for only the normal intermeal interval of 60–80 min. A clue to the understanding of this situation

may lie in the fact that the neurological deficits observed in the pair-feeding tests occurred at between 3–5 hours post-injection. It is during these hours that the PG concentrations even of free-feeding insulin-injected hamsters are at their lowest [11]. The hamster may therefore be particularly vulnerable to insulin-induced neurological deficits during this time, and failure to have access to food at more frequent than normal intervals may trigger symptoms such as ataxia. Such behavioural deficts might be rather short-lived however, due perhaps to the well-known sympathoadrenal activation which occurs in response to hypoglycemia [1,4]. It is relevant in this regard that hamsters injected with only a small dose of insulin (1.25 units/kg) and denied access to food are significantly hyperglycemic six hours later [11]. This finding suggests that the hamster may indeed possess potent physiological mechanisms to counteract insulin-induced hypoglycemia, and this ability may account for the hamster's lack of neurological deficits after the two-hour period of food-deprivation used in Experiment 1.

EXPERIMENT 2

Experiment 1 indicates that hyperphagia is no more effective than normophagia in allowing insulin-injected hamsters to achieve normal PG concentrations and neurobehavioural status at the end of a six-hour period. However the increased meal frequency associated with IIH [11] may allow the hamster to maintain more nearly normal levels of PG and neurological function within the shorter term, i.e., throughout the period of hyperphagia rather than merely at the end of this period. Steffens [32], for example, has observed a cyclical pattern in the blood glucose (BG) concentration of insulin-infused rats. When BG falls to a level of about 50 mg/dl, the rat consumes a meal and BG increases rapidly to a nearly normal level. BG soon begins to fall, and the cycle is repeated. This cyclic pattern suggests that IIH in the rat results from the taking of more frequent meals in response to recurring short-term reductions in glucose availability, and that the taking of these meals acts to offset the existing hypoglycemia in the short term. Behavioural evidence that IIH in hamsters is the result of increased meal frequency is compatible with this interpretation [11], but there is no direct evidence regarding the possible cyclicity of PG in insulin-injected hyperphagic hamsters. Indeed previous data suggests that fluctuations in PG concentrations, if they occur at all in insulin-injected hamsters, are not as large as those observed in insulin-infused rats [11].

Experiment 2 was undertaken to assess the importance of periodic reductions in PG as a stimulus for meal initiation in insulin-injected hamsters and to determine if PG concentrations are affected by meal consumption. The effects of withholding food from insulin-injected hamsters for periods of up to 100 minutes were also examined. It was observed that fluctuations in PG do not act as a trigger for the onset of meals in hyperphagic hamsters, nor is PG greatly affected by the consumption of a meal. However the withholding of food for periods of 60 min or longer results in an increase in the frequency of neurological deficits, although mean PG levels are unaffected by this treatment.

METHOD

Subjects

Nine groups of hamsters (n=8/group) were randomly selected from among the subjects of Experiment 1. Several weeks intervened between the completion of the first experiment and the start of the second.

Procedure

Animals were tested in batches of 9–18. All hamsters were injected on one occasion only with either insulin (30 units/kg) or saline at about 1000 hr and returned to their home cages with food and water available. Visual observation began at 3 hr post-injection, at which time the insulin-injected animals would be expected to be hyperphagic [11].

Six groups of insulin-injected hamsters were allowed to consume their first meal of the observation period normally, and food was removed at the end of the meal, defined as a cessation in eating of two minutes duration. Either immediately or up to 100 min after the meal, the animals were observed carefully for signs of impairment, and a blood sample was taken for PG analysis. The post-meal food deprivation periods for these six groups were +0, +20, +40, +60, +80 and +100 min.

Hamsters in the Pre-first meal and Pre-second meal groups were also injected with insulin. Animals in the Pre-first meal group were taken from their home cages just as they were about to begin their first meal of the observation period. The animals were observed for symptoms of impairment, and a blood sample was taken. Hamsters in the Pre-second meal group were allowed to consume their first meal of the observation period normally, but were removed from their cages just before beginning their next meal. The duration of the intermeal interval (IMI) was noted, and following observation, a blood sample was taken. For these groups, the manipulation of a food pellet in apparent preparation to eat was considered to indicate the incipient onset of a meal.

A single group of hamsters was injected with saline and allowed to eat ad lib, with the duration of the interval between the first and second meals of the observation period being noted. Animals in this Saline group were not observed for signs of neurological deficits, nor were blood samples taken.

RESULTS

As expected, the mean IMI during the observation period was shorter for the Pre-second meal group than for the saline group, $t(12)=4.07$, $p<0.01$ (see Fig. 3). Therefore, although food consumption was not measured directly, we may conclude that insulin-injected hamsters were under the influence of a hyperphagia-inducing stimulus during the observation period.

Mean PG levels were consistently well below the normal range for all groups (Fig. 4). A one-way ANOVA confirms the impression that there was no significant effect of treatment upon PG, $F(7,56)=0.87$, $p>0.05$. Planned comparisons further indicate that PG did not increase following a meal, nor did it decline significantly even when animals were food-deprived past the mean IMI of the Pre-second meal group (=50 min). PG did appear to be making a slight recovery toward normoglycemia levels at plus 100 min, but this PG value was not significantly different even from the lowest of the other PG means.

Interestingly, food deprivation beyond the normal 50 min IMI of insulin-injected hamsters adversely affected behavioural integrity, although mean PG levels were unaffected. There was a significantly greater frequency of neurological impairment among hamsters deprived for 40 min or

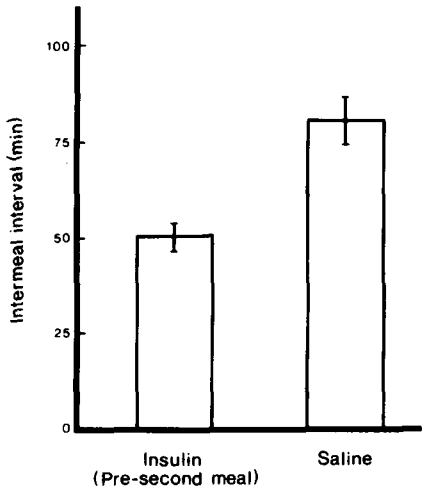

FIG. 3. Mean (±SEM) intermeal interval for hamsters injected with either insulin (30 units/kg) or saline. Intermeal interval was determined by observations beginning at +3 hr following injection, with food available at all times. N=8/group.

TABLE 1

FREQUENCY OF IMPAIRMENT AMONG INSULIN-INJECTED HAMSTERS DEPRIVED FOR EITHER LESS(+20 and +40 MIN GROUPS) OR MORE (+60, +80 AND +100 MIN) THAN THE TYPICAL INTERMEAL INTERVAL FOR SUCH HAMSTERS (=50.1 MIN)

	Duration of Food Deprivation	
	+20 and +40 min	+60, +80 and +100 min
Impairment	0	10
No impairment	16	14

Results are significant (Fisher's exact test, $p=0.002$).

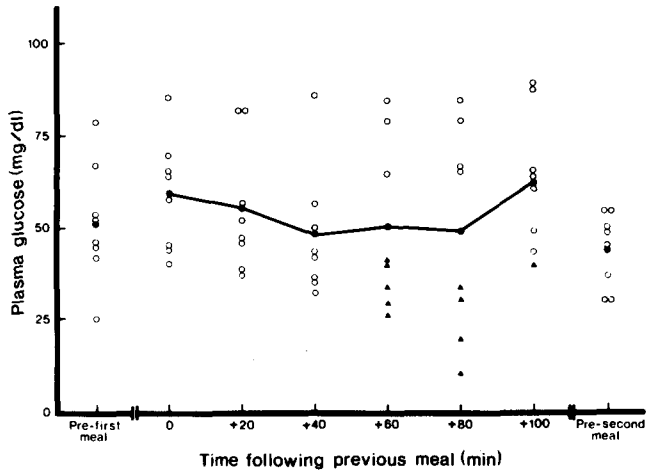

FIG. 4. Effect of food availability upon plasma glucose concentration in insulin-injected hamsters. Observations of food intake began at +3 hr following injection of 30 units/kg of insulin. Samples were taken from animals in the Pre-first meal and Pre-second meal groups immediately before consumption of a spontaneous meal. Animals in other groups had samples taken after food was removed for various intervals following consumption of a spontaneous meal. Symbols: ●=group mean PG concentration; ○=PG concentrations of individual animals showing no symptoms of neurological impairment; ▲=PG concentrations of individual animals showing; clear symptoms of neurological impairment.

less than among those deprived for 60 min or longer (Table 1). In addition, only one of the hamsters in the plus 100 group was impaired, suggesting that behavioural recovery may have been occurring by this time despite the absence of food and despite the fact that the mean PG was not significantly above that of hamsters deprived for shorter periods. A careful inspection of Fig. 4 also indicates that hamsters which had PG levels less than about 40 mg/dl, and which had been food deprived for 60 min or longer, were always impaired. In contrast, individual animals in the various other groups which had similarly low PG values did not show symptoms of impairment.

DISCUSSION

Insulin-injected hamsters do not show the same pattern of PG cyclicity in conjunction with hyperphagia as is shown by insulin-infused rats [32]. Mean PG levels do not increase following a meal, nor do they fall appreciably either within or beyond the typical IMI of insulin-injected hamsters. It may be concluded that fluctuations in PG do not play an important role in the initiation of more frequent meals by insulin-injected hamsters, nor does the increased meal frequency allow the hamster to maintain its PG at a more nearly normal level.

Although food deprivation of insulin-injected hamsters past their normal IMI of 50 min does not greatly influence mean PG levels, it does result in a marked increase in the frequency of neurological impairment, but only in those animals having PG concentrations of about 40 mg/dl or less. While this hypoglycemia may be involved in the occurrence of the behavioural symptoms, it cannot account for them entirely because hamsters having equally low PG levels, but deprived for periods shorter than 50 min, are not neurologically impaired. This pattern of results suggests that some alternate metabolic fuel is available during the IMI to prevent the occurrence of behavioural deficits; beyond the normal IMI, the supply of this alternate fuel may be exhausted, leading to impairment in those animals with low PG concentrations. The alternate metabolic fuel in question may be volatile fatty acids, which are derived from fermentation in the hamster pregastric pouch and are absorbed directly into the circulation from the stomach [14].

The failure to observe a cyclic pattern in PG levels in insulin-injected hamsters does not necessarily imply that hypoglycemia plays no role at all in IIH. Certainly the degree of hypoglycemia produced in hamsters by a hyperphagia-inducing dose of insulin is profound. Although hamsters have a digestive system which contains a pregastric pouch similar to that of ruminants, basal PG concentrations of hamsters are well above those of ruminants and are similar to those of monogastric animals [14]. A hyperphagia-inducing dose of insulin produces decreases in hamster PG which are

at least as large as those observed in the monogastric rat [27,32], and although no cyclicity of PG was observed in Experiment 2, mean PG levels were well below the normal range for all groups of animals. Other evidence for a role of hypoglycemia in the production of IIH in hamsters comes from the finding of a significant direct relationship between the sensitivity of hamsters to the hypoglycemic effect of a given dose of insulin and their food consumption in response to that dose of insulin [11]. The occurrence of IIH in hamsters may thus be interpreted according to the glucostatic theory, which suggests that the insulin-induced reduction in the availability of glucose stimulates specialized receptor cells in the brain, which in turn directly trigger the feeding response [17].

There are however certain difficulties with this interpretation. First, hamsters do not become hyperphagic in response to either 2-deoxyglucose [21, 24, 28, 30] or 5-thioglucose [9], although both of these glucose analogs produce glucoprivation [3, 5, 36] and cause hyperphagia in other species [20, 23, 33, 35]. It seems therefore that glucoprivation may not be a sufficient stimulus for the production of hyperphagia in hamsters. Furthermore a dose of insulin which produces hyperphagia in rats has a number of effects, causing not only hypoglycemia but also marked decreases in plasma ketone concentrations and an acceleration in gastric emptying, both of which may be factors in IIH in rats [13]. It has not been established whether similar effects occur in hamsters, although it is known that insulin produces decreases in plasma levels of free fatty acids and probably also of ketones [10,26]. It appears then that the interpretation of IIH in hamsters based upon the glucostatic theory represents an oversimplification of the situation, and while hypoglycemia may certainly play a role in the production of IIH in hamsters, other factors must also be sought.

In this light, the possibility that insulin may influence the rate of stomach emptying in the hamster is intriguing. It has been noted that the pregastric pouch of the free-feeding hamster fills and empties with a periodicity similar to that of the normal meal cycle, suggesting that cues from the pregastric pouch, whether of a mechanical or a chemical nature, may play an important role in satiety in the hamster [25]. If insulin accelerates gastric emptying in hamsters as it does in rats [13], this might trigger the more frequent meals which occur during IIH. Experiment 3 was conducted to determine whether gastric emptying is affected by insulin in hamsters. It was found that both insulin-injected and control hamsters have similar amounts of food in their stomach compartments at both the onset and offset of a meal, and that both the pregastric and gastric pouches empty more rapidly in insulin-injected hamsters.

EXPERIMENT 3

METHOD

Subjects

Ten groups of hamsters (n=6/group) were randomly selected from among those previously used. Experiment 3 began several weeks after the end of Experiment 2.

Procedure

Hamsters were injected in batches of 10–15 with either insulin (30 units/kg) or saline at about 1000 hr and returned to their home cages with food and water available. Visual observation began three hours later.

Animals in the Insulin-Premeal and Saline-Premeal groups were removed from the cages just as they were about to begin their first meal of the observation period, and sacrificed immediately. Three groups of insulin-injected hamsters were allowed to consume their first meal of the observation period. After the meal, food was removed from the cage and the animals were sacrificed either immediately or up to 50 min after the end of the meal (Insulin +0, Insulin +25, Insulin +50). Five groups of saline-injected hamsters were similarly allowed to consume their first meal of the observation period, and were then food-deprived until being sacrificed up to 80 min later (Saline +0, etc.). Meal durations were recorded.

At the times indicated above, animals were deeply anesthetized with halothane and sacrificed by cervical dislocation. The abdomen was immediately opened and the stomach dissected free. To prevent mixing of their contents, a ligature was placed around the constriction between the pregastric and gastric pouches. Each pouch was opened carefully, and its contents were rinsed into a preweighed glass tube. The tube was centrifuged, the supernatant was discarded, and the stomach contents were dried at 80 degrees Celsius until a constant weight was achieved.

All data were analyzed by the one-way ANOVA and by Tukey's test for multiple comparisons [19].

RESULTS

Stomach contents data are illustrated in Fig. 5. Significant treatment effects were found for all measures of stomach contents (Pregastric: $F=11.5$; Gastric: $F=3.0$; Total=27.8, $df=9,50$; $p<0.001$ for all analyses). The Insulin-Premeal and the Saline-Premeal groups did not differ on any measure, nor were there any differences between the Insulin +0 and the Saline +0 groups. However insulin-injected hamsters had significantly less food than their saline-injected controls in the pregastric pouch at +25 min ($p<0.025$) and at +50 min ($p<0.01$), in the gastric pouch at +50 min ($p<0.01$), and in the total stomach at +50 min ($p<0.01$). In addition, the stomachs of hamsters in the Insulin +50 group were virtually empty. In contrast, animals in the Saline +80 group had more food in their gastric pouch ($p<0.01$) and in their total stomach ($p<0.025$) than did animals of the Insulin +50 group, although the pregastric pouches of the Saline +80 hamsters contained very little material.

There were no significant differences in meal duration among the various groups which were allowed to consume meals, $F(7,40)=1.23$, $p>0.05$; range=9.0–14.0 min.

DISCUSSION

The data confirm that the emptying time of the pregastric pouch of untreated hamsters is similar to the typical 80 min IMI of free-feeding hamsters observed in Experiment 2. Furthermore saline-injected hamsters do not initiate meals until the pregastric pouch is virtually empty, supporting the notion that cues from the pregastric pouch play a role in normal satiety in the hamster [25]. In contrast, the fact that there is no change in the contents of the gastric pouch during the typical IMI of untreated hamsters suggests that this structure is not involved in the control of normal food intake.

Emptying of both gastric pouches is markedly accelerated by a dose of insulin known to produce hyperphagia in hamsters. Like control hamsters, insulin-injected animals eat

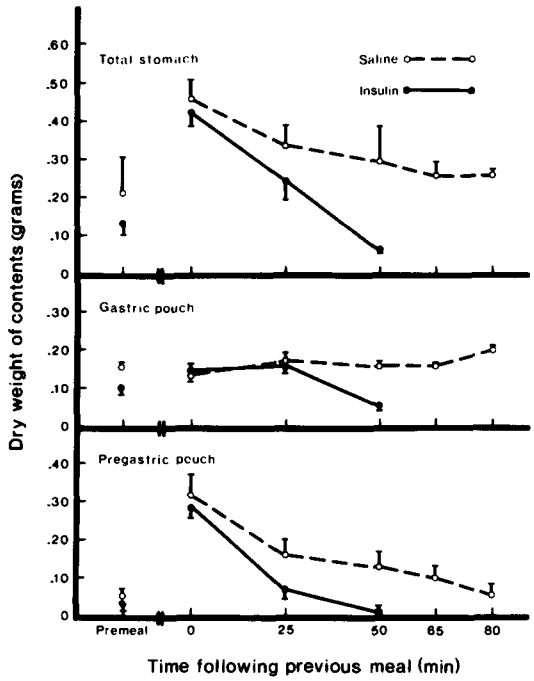

FIG. 5. Effect of insulin (30 units/kg) upon stomach emptying in hamsters. Observations of meal consumption began at +3 hr following injection. Values are Mean±SEM for six animals.

only when the pregastric pouch is empty, but this pouch empties far more rapidly than usual under the influence of insulin. Indeed, the pregastric pouch is empty 50 min after the end of a meal, which corresponds to the typical IMI of insulin-injected hamsters under these circumstances. The gastric pouch also contains less food than normal at this time, and it may therefore play some role in the initiation of the more frequent meals of insulin-injected hamsters, although it is not implicated in the control of normal food intake. The nature of the satiety cues from the hamster stomach is not known. Such cues may be mechanical, based simply upon the degree of stomach distention or they may be based on the absorption of nutrients such as volatile fatty acids from the stomach [7, 8, 14]. Cycles in either or both types of cue may play a role in the production of IIH in hamsters. It is also of interest that stomach emptying in hamsters is not accelerated by 2DG and is significantly retarded by 5TG (DiBattista, submitted for publication). While it is not possible entirely to rule out the existence of a glucoprivic system for feeding in the hamster, the failure of these glucoprivic agents to produce hyperphagia in hamsters [9, 21, 24, 28, 30] reinforces the notion that IIH may be mediated largely by insulin's accelerative effect upon stomach-emptying rather than by direct stimulation of a glucoprivic feeding system.

The similarity in the IMI and the gastric emptying times of insulin-injected hamsters may account for the pattern of occurrence of neurological impairment in the previous experiments. In Experiment 2, the frequency of impairment increased dramatically in insulin-injected hamsters deprived for 60 minutes or longer, when both the pregastric and gastric pouches would be expected to be virtually empty. Thus the absorption of nutrients from the stomach probably plays an important role in maintaining neurological integrity in insulin-injected hamsters during their normal IMI. The most relevant nutrient in this regard is probably not glucose, as the mean levels of PG do not fluctuate during IIH and insulin-injected hamsters having very low PG concentrations within their normal IMI do not show signs of impairment. Because hypoglycemia thus seems to be a necessary but not a sufficient condition for the occurrence of impairment, it is likely that the absorption from the stomach of some energy metabolite other than glucose, such as volatile fatty acids [14], serves to prevent impairment during the IMI.

The effect of insulin upon gastric emptying in hamsters may be dependent upon the occurrence of hypoglycemia, although further research is needed to clarify this point. However it is known that insulin greatly increases gastric acid secretion in rats, and that this effect is mediated by the vagus nerve [15] and can be abolished by the administration of glucose [6]. If the same is true in hamsters, it could account for the insulin-induced acceleration of emptying of the gastric pouch, which is analogous to the stomach of rats [14]. Although the origin of the acceleration of emptying of the pregastric pouch is more obscure, it too may be dependent upon the hypoglycemic effect of insulin. Thus the chronically low PG concentrations occurring in insulin-injected hamsters may serve as the metabolic situation which causes increases in meal frequency and food intake via the acceleration of stomach emptying. If this is the case, an interpretation of IIH in the hamster which is based upon the glucostatic theory is clearly inadequate. For example, the inverse relationship between food intake and PG in insulin-injected hamsters [11] would most economically be interpreted in terms of the effects of hypoglycemia upon the speed of stomach emptying. There may indeed be specialized cells in the hamster central nervous system which are responsive to the availability of glucose and are involved in the production of IIH, as the glucostatic theory postulates [17]. However the primary function of these neurons may not be to stimulate feeding behaviour directly. Rather they may act to increase gastric acid secretion and to accelerate stomach emptying, with the result that the IMI is reduced and hyperphagia occurs. Further investigations to more clearly delineate the role of the stomach in the control of normal food intake and IIH in the hamster are obviously in order.

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