Effect of hepatic glucose infusion on glucose intake and licking microstructure in deprived and nondeprived rats

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Baird, John-Paul, Harvey J. Grill, and Joel M. Kaplan. Effect of hepatic glucose infusion on glucose intake and licking microstructure in deprived and nondeprived rats. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1136–R1143, 1999.—The effects of hepatic-portal glucose or saline infusions on intake and the temporal distribution of licking (lick microstructure) were evaluated in nondeprived and in 20.5-h food-deprived rats. Rats received portal infusions of isotonic glucose or saline (0.1 ml/min) for 2 h before and then throughout a 90-min period of access to a spout that delivered 12.5% glucose. Overall, a significant treatment-related intake suppression was obtained in nondeprived but not in deprived groups. For both groups, however, there was a significant positive linear relationship between the amount of individual rats consumed under the saline (baseline) infusion condition and the extent to which portal glucose infusion suppressed intake. The linear fit for the deprived group was similar in slope, but right shifted, relative to the best fit for the nondeprived group. The individual-subject and group differences in response to portal glucose infusion are discussed in relation to the inconsistent literature on this treatment’s short-term intake effects. We focused analysis of the licking pattern on those rats for which a prominent portal glucose infusion effect was obtained (i.e., nondeprived rats with higher than average baseline intakes). Features of the licking pattern associated with taste evaluation (1st min lick rate; lick burst duration) were not significantly affected by portal glucose infusion. Rather, the minute-by-minute rate of ingestion under glucose infusion declined more rapidly than under baseline tests, indicating that portal glucose infusion enhanced the inhibitory influence of the accumulating postgestive load.

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between baseline intake and intake suppression after portal glucose infusion may offer insight into the general problem of treatment effectiveness. For this study, it provided a rationale for focusing the lick-microstructure analysis on the subset of rats for which a prominent portal-glucose infusion effect on meal size was obtained.

METHODS

Subjects

Male Sprague-Dawley rats (Charles River) weighing between 382 and 625 g at the time of surgery were tested. Rats were maintained in individual hanging wire cages on a 12:12-h light/dark schedule. Food (Purina Rat Chow #5001) and water were available ad libitum in the home cage. Rats were tested at the same time each day, between 5 and 8 h after lights on.

Surgery

Rats were anesthetized (ketamine hydrochloride (80 mg/kg) and xylazine (8 mg/kg)) for surgery, which was conducted under aseptic conditions. Hepatic-portal catheter implantation was modified from the method of Tordoff and colleagues (31–33; see also Ref. 3). Briefly, the cecum and distal intestine were exposed via midline laparotomy and placed onto saline-moistened gauze to expose the main branch of the ileocolic vein. The hepatic catheter (Silastic tubing; 0.037 in. OD, 0.02 in. ID) was inserted ∼5 cm through the ileocolic vein toward the liver with the tip situated just distal to the last intestinal tributary and secured via silk ligatures. The distal end of the catheter was led subcutaneously to the top of the head where it was fitted with 19-gauge tubing and attached to the skull with jeweler’s screws and dental acrylic. Catheter patency and placement was confirmed via postmortem analysis after catheter infusion of an overdose of ketamine mixed with food coloring. If the coloring was found in the peritoneal cavity or if the catheter tip was occluded, then data for that rat were discarded.

Apparatus

Rats were tested (up to 6 at a time) in individual hanging wire test cages. A drinking spout (Girton) was introduced to the test chamber at the beginning of each test. The tip of the spout lay 4 cm from the floor and 1–2 mm behind a slit (28 × 8 mm) in a metal plate attached to the front of the cage. A lickometer circuit consisting of a custom interface and an 80286 processor running a program of our own design was used to record the time of occurrence of each lick. The circuit passed <50 μA through the rat when the tongue made contact with the spout (27). Fluid was delivered through a PE-100 tube with a flared tip fit flush against the tip of the spout. Each lick registration resulted in delivery of a precalibrated volume of 12.5% glucose, as follows. Fluid for each spout lick registration resulted in delivery of a precalibrated volume of 12.5% glucose, as follows. Fluid for each session, the number of licks in the analysis period (whole meal; single minutes) by the session-average lick volume and dividing by the duration of the analysis period.

Experimental Design

Experiment 1. Experimentally naive rats received a series of six consecutive daily 90-min test sessions. On days 2 and 5 an hepatic-portal infusion (0.3 osM saline or glucose at 0.1 ml/min; see Refs. 3, 31, 33) was initiated 2 h before the intake test and continued until the end of the test session. Presentation order for infusion condition (saline or glucose) was counterbalanced across rats. On days 1, 3, 4, and 6, “mock infusion” tests were run in which the infusion line was connected but nothing was infused. Only data from rats (n = 16) that completed all experimental conditions and whose catheter placements were confirmed postmortem were included in the analysis.

Experiment 2. Experimentally naive rats received a series of eight consecutive daily 90-min test sessions. Portal infusions of glucose or saline (see Experiment 1) were delivered on days 2 and 6, with condition order counterbalanced across rats. Rats were deprived of food for 20.5 h before the portal glucose or saline infusion. Mock infusion tests were conducted on the remaining test days, with ad libitum food access between these tests. Only data from rats that completed all experimental conditions with patent catheter placements (n = 14) were included in the analysis.

Statistical Analysis

A one-way repeated-measures ANOVA was performed for each experimental parameter, followed by planned contrasts to evaluate the effects of venous infusion condition (glucose versus saline), differences between venous and mock infusion tests, and differences among mock infusion tests. Individual-subject differences were explored via Pearson r, relating amount consumed under saline infusion conditions with the
intake difference between glucose and saline infusion conditions. Kendall’s coefficient of concordance (W; 19) was used to assess stability of individual intake differences across saline and mock infusion conditions. A two-way repeated-measures ANOVA (minute × infusion condition) was used for analysis of ingestion rate over successive minutes.

RESULTS

Experiment 1: Nondeprived Rats

One-way ANOVA revealed a significant effect of test condition on meal size [F(5, 75) = 3.96, P < 0.005]. As shown in Fig. 1, portal glucose infusion suppressed intake by 24% relative to meal size under the saline infusion condition, an effect the planned contrast showed was significant [F(1, 15) = 7.49, P < 0.025]. Intake under glucose infusion was also significantly lower than that for each mock infusion test (P < 0.05) and significantly lower when contrasted against all four mock tests combined [F(1, 15) = 20.21, P < 0.0005]. Intake under the saline infusion condition did not differ significantly from that under any or all mock infusion conditions. Moreover, there were no significant differences between mock condition intakes, whether mock tests were grouped by infusion condition or in relation to actual testing order (i.e., test days 1, 3, 4, 6).

The extent to which glucose infusion reduced intake relative to saline baseline was positively and significantly correlated with the amount consumed under the saline infusion condition [saline condition intake (ml) × saline condition minus glucose condition intake (ml): Pearson r = 0.85; P < 0.0001; see Fig. 2]. Baseline intake was also significantly correlated with the treatment effect when the effect was expressed as percent difference between infusion conditions [(saline condition minus glucose condition intake) /100 /saline condition intake; Pearson r = 0.66, P < 0.005]. Individual differences in pretest body weight were not a significant correlate of either baseline intake (r = 0.40; NS) or of the difference (ml) in intake between glucose and saline infusion conditions (r = 0.32; NS).

The relationship between individual differences in baseline intake and degree of intake suppression after glucose infusion prompted us to perform a median split of the overall group based on saline baseline intake (median = 9.46 ml) and contrast results for the “higher-baseline” and “lower-baseline” subgroups. For the higher-baseline subgroup, there was a significant overall effect of condition on amount consumed [F(5, 35) = 5.55, P < 0.001; Fig. 2]. The 48% reduction in intake from saline to glucose infusion conditions was significant [F(1, 7) = 18.07, P < 0.01]. (Intake under glucose infusion was also significantly different from intakes under any and all mock infusion conditions. There were no significant differences between saline and mock infusion conditions.) For the lower-baseline subgroup, intakes under saline and glucose infusion conditions did not significantly differ [F(1, 7) = 1.14, NS]. There was an overall condition effect on intake in this subgroup [F(5, 35) = 6.63, P < 0.0001], however, which was largely attributable to somewhat lower intakes under saline and glucose infusion conditions (mean = 7.4 ml) than under mock infusion conditions (mean = 9.2 ml; F(1, 7) = 10.12; P < 0.02).

As described, the higher- and lower-baseline groups were composed on the basis of individual rat intakes under the saline infusion condition. It is important to add that the individual-subject differences were stable across saline and mock infusion conditions, as shown by a significant coefficient of concordance [Kendall’s W = 0.413; χ²(15) = 30.98, P < 0.01]. It follows, then, that results similar to those described above should be obtained if the split was made on intakes under any baseline (saline or mock) condition. We explicitly evaluated this suggestion with the grouping based on intake under the first mock infusion test day (i.e., before any portal infusion was delivered) and found a nearly identical pattern of results.

For the higher-baseline subgroup, there was no overall effect of condition on meal duration [F(5, 35) = 1.58, NS; Table 1] or on latency [F(5, 35) = 1.60, NS; Table 1]. Although meal duration was reduced by 26% under the glucose compared with the saline infusion, the difference was not statistically significant according to the planned comparison [F(1, 7) = 0.49, NS]. For the lower-baseline subgroup, the overall ANOVAs and planned comparisons yielded no significant effect on meal duration [F(5, 35) = 1.18, NS] or on latency [F(5, 35) = 1.66, NS], respectively.

The average rate of ingestion (meal size divided by meal duration) was reduced 25% in the higher-baseline subgroup and was increased 22% in the lower-baseline subgroup under glucose relative to saline infusion conditions (Table 1). These differences, however, were not statistically significant [higher baseline: F(5, 35) = 2.53, NS; lower baseline: F(5, 35) = 1.62, NS].

Lick microstructure. Analyses of behavioral results for the lower-baseline subgroup yielded no significant effect of treatment condition for any parameter (mean values for the two venous infusion conditions are...
The ANOVA yielded no significant effect on within-burst lick frequency \(F(5,35) = 0.66, \text{NS}\) or on number of bursts in the meal \(F(5,35) = 0.46, \text{NS}\). The mean differences between saline and glucose infusion conditions for these two parameters were quite small (Table 1) and not significant according to the respective planned comparisons.

There was a large difference in mean burst duration between the two portal infusion conditions (Table 1). However, significant results were not obtained with the overall ANOVA \(F(5,35) = 0.78, \text{NS}\) or for the planned comparison between infusion conditions \(F(1,7) = 0.32, \text{NS}\). The ingestion rate (minute-by-minute) curves for glucose and saline conditions, however, did diverge in the subsequent minutes (Fig. 3). Inspection of the group ingestion rate curves (Fig. 3) suggests that the separation, in general, was sustained, but only until about the 12th min of the meal. Such group curves, however, should not be taken at face value insofar as the later descent of the curves reflects, to a large extent, the growing number of rats whose meals have already ended. This subject attrition issue prompted us to limit the ANOVA to the 5-min range, throughout which all rats in both conditions were actively ingesting. The two-way ANOVA (infusion condition × minute) on ingestion rate for the first 5 min of the respective meals showed no overall effect of minute \(F(4,28) = 1.95, P = 0.14\) but a significant main effect of infusion condition \(F(1,7) = 18.02, P < 0.004\) and a significant two-factor interaction \(F(4,28) = 7.56, P < 0.0003\). Post hoc tests showed that ingestion rate became significantly slower for glucose compared with saline infusion condition by

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Higher-Baseline Subgroup</th>
<th>Lower-Baseline Subgroup</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Glucose</td>
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<tr>
<td>Latency, s</td>
<td>15.52 ± 4.55</td>
<td>89.94 ± 38.27</td>
</tr>
<tr>
<td>Meal duration, s</td>
<td>823 ± 107</td>
<td>609 ± 146</td>
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<tr>
<td>Ingestion rate, ml/min</td>
<td>1.04 ± 0.12</td>
<td>0.78 ± 0.10</td>
</tr>
<tr>
<td>Mean burst duration, s</td>
<td>15.04 ± 3.37</td>
<td>8.65 ± 2.31</td>
</tr>
<tr>
<td>Burst count</td>
<td>41.25 ± 12.74</td>
<td>38.00 ± 12.16</td>
</tr>
<tr>
<td>Lick frequency, licks/s</td>
<td>7.00 ± 0.94</td>
<td>6.24 ± 0.13</td>
</tr>
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Results are means ± SE.
the 3rd min of the meal and remained so for the 4th and 5th min.

Experiment 2: Deprived Rats

Meal size varied significantly as a function of testing condition \(F(7,91) = 3.08, P \leq 0.006\). This overall treatment effect, however, was attributable to differences between mock conditions (rats not deprived) and infusion conditions (rats deprived of food). The difference in intake between saline and glucose infusion conditions was quite small (5%) and not significant \(F(1,15) = 0.39; \text{NS}\).

Despite no effect on group-average meal size, glucose infusion did systematically affect meal size in a manner that depended on the amount that individual rats ingested under the saline infusion condition \(r = 0.57, P < 0.035; \text{see Fig. 4}\). The correlation between saline baseline intake and degree of intake suppression under glucose infusion expressed as a percent reduction from baseline was also significant \(r = 0.64, P < 0.01\).

Pretest body weight was not a significant correlate of baseline intake \(r = -0.23, \text{NS}\) or of the difference in intake between glucose and saline infusion conditions \(r = 0.12; \text{NS}\).

The individual differences in intake were stable across saline and the six mock infusion conditions, as shown by a significant coefficient of concordance [Kendall’s \(W = 0.506; x^2(13) = 46.05, P < 0.0001\)].

As in experiment 1, the overall group was divided in half on the basis of intake under the saline infusion condition (median = 15.3 ml). Glucose infusion tended to reduce intake relative to saline values in the higher-baseline subgroup (from 18.57 to 14.54 ml), but tended to increase intake in the lower-baseline subgroup (from 11.84 to 14.33 ml; see Fig. 4). These trends, however, did not achieve statistical significance according to the

Fig. 3. Average minute-by-minute ingestion rate for higher-baseline subgroup of experiment 1 after portal infusion of saline (●) and glucose (○) infusion. Error bars (+SE) are shown for first 5 min, during which all rats were ingesting. For each subsequent minute, symbol represents mean intake of all 8 rats, and adjacent value indicates number of rats that were ingesting during that minute (i.e., whose meals had not yet ended).

Fig. 4. Mean (+SE) 12.5% glucose intake after portal infusion of saline (solid bar) and glucose (open bar) for deprived rats whose baseline intakes fell in upper half of intake range (A; \(n = 7\)) and for those falling in lower half of range (B; \(n = 7\)). C: scatter plot of intake after portal saline infusion (x-axis) versus magnitude of treatment effect on intake (i.e., difference (ml) between intakes under saline and glucose infusion conditions (glucose – saline); y-axis). Line of best fit is shown (slope = −0.79 per ml baseline intake; \(r = 0.57, P < 0.035\)).
respective planned comparisons [higher baseline: \( F(1,6) = 5.60, P < 0.10 \); lower baseline: \( F(1,6) = 2.22, P < 0.25 \)].

None of the analyses (whole group ANOVA, ANOVAs for higher- and lower-baseline subgroups, planned comparisons) yielded significant effects on meal duration, latency, average ingestion rate, or lick microstructural parameters.

**DISCUSSION**

Hepatic-portal glucose infusion reduced glucose intake in nondeprived rats (experiment 1). The effect was significant for the overall group (24% suppression relative to saline infusion), but the brunt of the effect was carried by a subset of the rats tested. A strong correlate of the degree of intake suppression was the intake magnitude under baseline conditions (Fig. 2). This correlation did not result from a “floor effect” on intake reduction (i.e., rats with small baseline intake could only show a small absolute suppression), because it held when intake suppression was taken as a percentage of baseline intake. We therefore divided the overall group in half on the basis of intake under the saline infusion condition and found that portal glucose infusion reduced intake of rats in the higher-baseline subgroup by 48% after portal glucose infusion. Rats in the lower-baseline subgroup, by contrast, showed no effect of portal glucose infusion on meal size. The individual rat differences in sensitivity to portal nutrient infusion may be relevant to the inconsistent literature on this treatment’s intake effects (see discussion below). The outcome of the median split also prompted us to emphasize, for purposes of discussion of the lick-microstructure results, those rats for which a prominent portal glucose infusion effect on intake was obtained.

**Behavioral Correlates of Taste Evaluation and Satiation**

The initial (1st min) ingestion rate, the feature of the licking pattern most widely held as an indicator of taste evaluation, did not vary with portal infusion condition for any group or subgroup in either experiment and was not a significant correlate of the influence of portal glucose infusion on amount consumed. This lack of an effect contrasts with findings reported by Campbell and Davis (7), where lick counts during 2-min periods of spout access to 3.2% glucose test were significantly reduced after a brief (1 min) portal glucose infusion. Novin et al. (21) also showed an effect of portal glucose infusion on the initial rate of 10% glucose ingestion, although there was no treatment effect on amount consumed by the end of their 30-min test. Any of a number of methodological differences (e.g., test duration, parameters of the portal glucose infusion protocol) may account for the contrasting results. We have provided, in any event, a clear counterexample to the notion that an effect of portal nutrient infusion on intake (where obtained) entails an effect on ingestion rate at the beginning of the meal.

Another parameter sometimes taken as a sensitive measure of taste evaluation is the average burst duration for the meal (9). Spector et al. (28), for example, showed that increases in initial lick (ingestion) rate as sucrose concentration was raised, reached asymptote at ~0.1 M, whereas average burst duration increased linearly over the full concentration range (0.03–1.0 M) tested. Here we found that burst duration was not significantly reduced by portal glucose infusion. There was, however, a trend in this direction of appreciable magnitude (50% reduction) that, moreover, was observed only in the higher-baseline subgroup of experiment 1. We view this trend as suggestive and therefore hesitate to rule out the possibility that portal nutrient infusion affects taste processing under the present testing conditions.

More clear was an action of portal nutrient infusion on the satiation process. Such judgments are typically derived from differences in the slopes of ingestion rate curves for treatment and control conditions. This was apparent here in the form of a significant interaction between venous infusion condition and ingestion rate over the first 5 min of the meal, due to the greater decline in ingestion rate over minutes when glucose was intraportally delivered. Post hoc comparisons showed that it was not until the 3rd min of the meal that ingestion rates under the two infusion conditions significantly diverged (see Fig. 3), suggesting a synergy between portal nutrient treatment and the intake-inhibitory influence of the accumulating postingestive load.

One may entertain the simple notion that the liver itself is a source of signals that contribute to the termination of an ongoing meal and that the portal nutrient infusion affects intake directly by exaggerating the liver’s normal contribution to the satiation process. Our previous work (3), however, suggests that an appropriate interpretation of the hepatic contribution to meal size control is not likely to be so straightforward. We showed, using the intraoral intake model, that portal glucose infusions that begin with the onset of ingestion do not affect the amount consumed. To be effective at limiting meal size, portal infusions had to begin ~1 h before the onset of meal taking. The degree of intake suppression, moreover, did not vary with the amount of glucose infused into the portal vein. Thus the same degree of intake suppression was obtained with a 2-h premeal glucose infusion that was sustained during the intake test as was obtained with a 15-min infusion initiated 1 h before the test. These characteristics contrast with the results from studies where nutrient delivered to or withdrawn from the stomach and/or duodenum affects intake in a graded manner, as a function of the amount infused during the test (e.g., Refs. 8, 16, 17, 36). We argued on the basis of these results that the action(s) of effective portal infusions probably do not reflect the engagement of a primary hepatic satiation mechanism but rather affect the amount that will be ingested in a subsequent meal. In this light, the present data suggest that, via as yet unidentified central and/or peripheral mechanisms,
greater baseline intake would favor a greater intake. One might reasonably expect that conditions that favor a greater baseline intake would favor a greater intake suppression after portal glucose infusion. In experiment 2, portal infusions of glucose or saline were delivered to rats that had been deprived of food for 20.5 h. By contrast with results obtained in nondeprived rats, no significant effect of portal nutrient infusion on the elevated group-average meal size was obtained, nor were there any significant effects on parameters of licking behavior (initial rate, burst duration, etc.).

Others have investigated the ingestive effects of portal nutrient infusion in deprived animals, with conflicting results. Portal nutrient infusion effects have been obtained in the deprived rabbit (22). Nondeprived rabbits, in fact, failed to show the effect (22). A survey of the literature, however, shows that the majority of studies employing deprivation failed to obtain an effect of portal nutrient infusion on intake (e.g., Refs. 2, 4, 5, 29, 37; cf. Refs. 24, 25). These failures may reflect methodological choices (e.g., infusion parameters, test paradigm, etc.; see Ref. 33) or, alternatively, may be related specifically to the metabolic/physiological sequence of food deprivation. Here comparable designs were used in the respective experiments with nondeprived and deprived rats. Our finding that an effect of portal nutrient infusion on average meal size was obtained only in nondeprived rats is consistent with the suggestion that deprivation per se reduced the potential of hepatic-portal nutrient infusion to suppress intake under the current testing conditions.

Although deprivation muted or eliminated the portal nutrient effect for the group taken as a whole, a significant correlation was obtained linking the amount consumed under the saline baseline condition with intake change after treatment. This regression result can be contrasted with that obtained in nondeprived subjects. One can see from Figs. 2 and 4 that the slopes of the respective best-fit lines for deprived and nondeprived rats were quite comparable (respectively, 0.79 and 0.85 ml intake suppression per additional ml baseline intake). The best-fit lines, however, occupied different positions. Their separation can be characterized in terms of the respective points of intersection with the dashed horizontal (“zero suppression”) line. For the deprived group, we observe a 7.7 ml rightward shift in the baseline intake point, above which intake is suppressed and below which increased intake is the expected outcome of portal nutrient infusion. An overall treatment effect arose for the nondeprived group, where the distribution of the baseline intakes was more balanced across the intersection point, resulting in no net effect of portal nutrient infusion.

The individual difference correlations suggest that a common factor or factors underlie both the sensitivity to portal nutrient infusion and the tendency of individuals to ingest either more or less glucose during baseline tests. The identity of the relevant factor, however, is a matter of speculation. We know that body weight is not a relevant variable, as it was not a significant correlate of either baseline intake or sensitivity to the treatment. Variables that may merit attention include baseline insulin level, and insulin response to portal glucose infusion and to ingested glucose (see Refs. 6, 23). Portal nutrient infusion triggers a cascade of events [including changes in portal glucose and insulin levels, net hepatic glucose uptake, glycogen synthesis, hepatic oxidative metabolism, and hepatic-vagal afferent activity (e.g., Refs. 20, 23, 26, 32)], some unknown subset of which must be relevant to the intake response. When the relevant variables are identified we will be in a better position to address the individual differences in response to portal nutrient infusion, the influence of deprivation, and the interaction of these factors and the central processes by which taste and postingestive signals are integrated to affect meal size.

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REFERENCES


