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Glucagon Produces Delayed Increase in Drinking-Associated Food Intake
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ABSTRACT

Glucagon is a pancreatic hormone and a brain-gut peptide thought to function as a short-term satiety signal in control of food intake. This study examined effects of glucagon injection on intake of alcohol, food, and water. Overnight water-deprived female and male Wistar rats (Ns = 10) were given access to 5% w/v ethanol and Purina chow. After adaptation to this schedule, rats received intraperitoneal (ip) injection of 0, 200, or 400 g/kg of glucagon, at 0 or 30 min prior to alcohol access. Food intake was significantly increased by glucagon at 30-60 min after either time of injection (0 or 30 min). Combined total caloric intake (from chow and ethanol) was decreased at 0-30 min after glucagon injection, and increased at 30-60 min after glucagon, reliably in male rats. Immediate declines and delayed increases in feeding after glucagon administration have been reported previously in other feeding paradigms. In addition to acting as a short-term satiety signal for caloric intake, glucagon may function further to sustain blood glucose levels through stimulation of delayed increase in food intake.

Glucagon is a pancreatic hormone and brain-gut peptide implicated in the regulation of food intake. The majority of studies of glucagon's role in the control of food consumption have focused on a short-term inhibitory effect on feeding in lightly- or nondeprived subjects (Geary, 1990; Geary & Smith, 1982; Gibbs, Geary, & Smith, 1993; Martin & Novin, 1977; Weick & Ritter, 1986). However, a few studies have indicated that glucagon administration can also produce increases in food intake in experimental designs involving long-term measurement of feeding behaviors (Bellinger & Williams, 1983; Geary, 1990; Hell & Timo-Iaria, 1985; McLaughlin, Gingerich, & Baile, 1984; Nagai, Thibault, Nishikawa, Hashida, Ootani, & Nakagawa, 1991). One study suggested that glucagon modulates a sequence of ingestive responses, with feeding inhibition seen early after administration, followed later by feeding stimulation (Vanderweele, Haraczkiewicz, & DiConti, 1980). Time-dependent effects of acute administration of glucagon on hepatic glucose metabolism were proposed to underlie this biphasic effect of glucagon on food intake.

In the present experiment, glucagon was assessed as a potential peptide modulator of ethanol ingestion. Glucagon could provide ingestion-contingent neural feedback because alcohol causes rapid glucagon release (Jauhonen, 1978; Poter, Chin, & Rowland, 1980; Shanbour, 1983; Simanowski, Hubalek, Ghatel, Bloom, Polak, & Seitz, 1989). Both glucagon and alcohol produce conspicuous metabolic effects at the liver; glucagon effects hepatic glycogenolysis and gluconeogenesis while ethanol's oxidative metabolism is catalyzed by hepatic enzymes. Further, glucagon has been reported to change the activity of liver alcohol dehydrogenase activity and the alcohol elimination rate in rat hepatocyte culture, with low concentrations increasing and high concentrations decreasing the enzyme activity (Mezey, Potter, & Rhodes, 1986).

Metabolic capacity for ethanol has been described as a factor limiting maximal consumption of ethanol (Kulkosky, 1985). Glucagon could affect alcohol intake indirectly by changing the rate of alcohol metabolism and blood ethanol accumulation. Alternatively, the liver is responsive to both glucagon and alcohol and could serve as a common locus for generation of alcohol ingestion-related neural regulatory feedback to the brain. Thus, it seemed reasonable to expect an effect of glucagon administration on ethanol consumption. Accordingly, the following experiment tested the null hypotheses of glucagon's effect on alcohol intake and associated food and water intakes in water-deprived female and male rats. Glucagon was injected both
immediately preceding ethanol and food access and at 30 min before access to determine if prior ingestion influenced the effects of glucagon.

**Method**

**Subjects**

Ten female and 10 male outbred Wistar rats (Crl:(WI)BR, Charles River Laboratories, Wilmington, MA) were subjects of the experiment. All rats were experimentally naive, approximately 2 months of age and weighed between 189-212 g (females) and 212-237 g (males) at the beginning of the experiment. Rats were individually housed in stainless steel wire-mesh cages in a room with ambient temperature of approximately 23 °C and a 12:12 hr light:dark cycle (0700 on). Each animal had ad libitum access to Purina Rodent Laboratory Chow (5001, Purina Mills, St. Louis, MO) in stainless steel hoppers, and deionized water in polycarbonate bottles with rubber stoppers and valveless stainless steel spouts, except as specified below. All rodents were treated in accordance with the "Ethical Principles of Psychologists and Code of Conduct" (American Psychological Association, 1992).

**Procedures**

Rats were initially deprived of water for 23 hr with continued ad lib access to food. On each of five adaptation days, at 1200, rats were weighed, returned to their home cages and given access to 5% w/v ethanol (from U.S.P. 95% deionized water) and food for 30 min, followed by access to water and food for 30 min. On the final baseline day, rats received a 1.0 ml/kg intraperitoneal (ip) injection of 0.9% NaCl (saline) prior to presentation of ethanol. Following this adaptation procedure, rats were randomly assigned to receive ip injections of 0, 200, or 400 g/kg of glucagon (human, Bachem, Torrance, CA) at either 0 or 30 min prior to ethanol access. The sequence of doses was randomized across test days for each animal and counterbalanced with the restriction that each rat receive all doses.

Fluid and food intake data were analyzed with 2 (between subjects: sex; female, male) x 3 (within subjects: dose; 0, 200, 400 g/kg) x 2 (within subjects: time of injection; 0, 30 min prior to ethanol access) x 2 (within subjects: measurement interval [food and total caloric intakes only]; 0-30, 30-60 min after ethanol access), mixed factorial analyses of variance. Post hoc comparisons of means were performed with the least significant differences (LSD) test at an alpha significance level of $p < .05$. Ethanol and food intake data were transformed into caloric intakes (7 kcal/g for ethanol, 3.3 kcal/g for Purina chow) and combined to provide measures of total caloric intake in the 0-30 and 30-60 min intervals after presentation of alcohol.

**Results**

Table 1 displays mean (±SE) 5% ethanol intake of female and male rats injected at 0 or 30 min prior to ethanol access, as a function of ip dose of glucagon. Analysis revealed only a significant interaction of sex and time of injection, $F(1, 18) = 13.43, p < .05$. The main effects of sex, time of injection, and dose were not reliable. Other interactions of these factors were also not reliable. Females consumed more ethanol when injected 30 min prior to access than when injected immediately before ethanol presentation, $p < .05$.

Table 1 also displays mean (±SE) water intake of female and male rats injected at 0 or 30 min prior to ethanol access, as a function of dose of glucagon. Analysis revealed a significant main effect of time of injection, $F(1, 18) = 10.24$, and a reliable interaction of sex and time of injection, $F(1, 18) = 9.39, ps < .05$. All other main effects and interactions were not statistically significant, $Fs < 2.4, ps > 0.05$. Rats consumed more water at 30-60 min after ethanol presentation when injected immediately prior to presentation of ethanol; this effect was only reliable in males, $p < .05$.

Mean (±SE) intake of Purina chow at 0-30 and 30-60 min after ethanol presentation is shown as a function of dose of glucagon in Table 1 for female and male rats injected at 0 or 30 min prior to ethanol access.
Table 1

Summary of Ethanol, Water, and Food Intakes.

<table>
<thead>
<tr>
<th>Intake</th>
<th>Sex</th>
<th>Time of Injection</th>
<th>Dose of Glucagon 200µg/kg</th>
<th>400µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Male</td>
<td>-30 min</td>
<td>57.76 (59.30)</td>
<td>59.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0 min</td>
<td>63.62 (55.92)</td>
<td>61.68</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>-30 min</td>
<td>55.06 (44.73)</td>
<td>52.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0 min</td>
<td>59.31 (14.00)</td>
<td>49.82</td>
</tr>
<tr>
<td>Water</td>
<td>Male</td>
<td>-30 min</td>
<td>58.26 (30.44)</td>
<td>49.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0 min</td>
<td>31.58 (47.15)</td>
<td>37.42</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>-30 min</td>
<td>58.26 (30.44)</td>
<td>49.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0 min</td>
<td>24.46 (34.47)</td>
<td>34.51</td>
</tr>
<tr>
<td>Food</td>
<td>Male</td>
<td>-30 min</td>
<td>5.87 (8.09)</td>
<td>5.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0 min</td>
<td>8.41 (6.10)</td>
<td>7.02</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>-30 min</td>
<td>6.18 (6.99)</td>
<td>6.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0 min</td>
<td>5.97 (11.07)</td>
<td>5.90</td>
</tr>
</tbody>
</table>

Note. Mean (±SE) intake of 5% w/v ethanol and water by water-deprived male and female rats (Ns = 10) after ip doses of glucagon. Glucagon was injected at 0 or 30 min prior to presentation of ethanol for a duration of 30 min. Water was presented 30-60 min after the ethanol. Mean (±SE) food intake (Purina Chow) of male and female rats as a function of ip doses of glucagon, injected at 0 or 30 min prior to access to ethanol. Data represents intakes during the initial 30 min access to ethanol (0-30 min) and the second 30 min period when water was available (30-60 min).

access. Analysis indicated a significant main effect of sex, F(1, 18) = 10.86, and interactions of time of injection (0 or 30 min prior to ethanol access) and measurement interval (0-30 min or 30-60 min), F(1, 18) = 7.77, and time of injection, measurement interval, and dose of glucagon, F(2, 36) = 4.77, ps < .05. All other main effects and interactions were not reliable, Fs < 2.7, ps > .05. Post hoc tests indicated that both doses of glucagon, when injected immediately prior to ethanol, increased food intake in male rats, during the 30-60 min measurement interval. Glucagon also increased food intake in males at 200 g/kg when injected 30 min prior to ethanol during the 0-30 min measurement interval, ps < .05. Males consumed more food than females (in g/kg), and when data from both sexes were combined, glucagon (400 g/kg) increased food intake at 30-60 min after ethanol access, when injected immediately prior to access. Food intake reliably increased 30-60 min after either dose or time of glucagon injection, F(2, 78) = 4.91, p < .05, (see Figure 1) which combines food intake data for both sexes, measurement intervals, and times of injection (0-30 min if injected 30 min prior to access, 30-60 min if injected 0 min prior to access).

When data from ethanol and food intakes were transformed into total caloric intake, analysis revealed main effects of sex, F(1, 18) = 11.98, and measurement interval, F(1, 18) = 112.15, and interactions of time of injection and measurement interval, F(1, 18) = 7.17; analysis also revealed main effects of time of injection, measurement interval, and dose of glucagon, F(2, 26) = 5.7, ps < .05. No other main effect or interaction was statistically significant, Fs < 2.0, ps > .05. In males, whose data are depicted in Figure 2, injection of 200 g/kg glucagon immediately prior to alcohol access decreased total caloric intake at 0-30 min.
Figure 2. Mean (±SE) total caloric intake of male rats as a function of ip dose of glucagon. Rats were injected at 0 or 30 min prior to ethanol access of 30 min duration, which was followed by water access for a second 30 min period.

After access. This decrease was followed by increased caloric intake at both doses glucagon during 30-60 min after access to ethanol access (ps > .05). No reliable effects of glucagon on total caloric intake were seen in female rats, whose data is ethanol (ps < .05). Whereas, injection of 200 g/kg of glucagon 30 min before ethanol access increased caloric intake at 0-30 min after access (ps < .05), there was no effect on intakes at 30-60 min after depicted in Figure 3 (ps > .05). Although, when data from both sexes were combined, glucagon (200 g/kg) injected immediately prior to ethanol access decreased caloric intake at 0-30 min after access, and at 400 g/kg increased caloric intake during 30-60 min after access, ps < .05.

Discussion

The data show that glucagon administration has no reliable effect on ethanol consumption alone in water-deprived female and male rats. Similarly, there was little effect of glucagon on water consumption in the period following the access to 5% ethanol. Thus it is concluded that injection of large doses of glucagon does not affect fluid intake in overnight water-deprived, ad lib fed rats given successive 30-min accesses to ethanol first and then water. Glucagon does not affect deprivation-induced drinking behaviors under these conditions of administration. Despite the aforementioned functional connections between glucagon and ethanol, no evidence was found that glucagon serves as a signal of satiation with alcohol.

However, there were interesting biphasic effects of glucagon injection on drinking-associated food intake and total caloric intake, as indicated by statistically significant higher-order interactions. Glucagon reliably increased food intake in male rats at 30-60 min after injection, whether the rats were consuming water (when injected just before alcohol presentation) or were consuming ethanol (when injected 30 min before alcohol presentation). This delayed stimulatory effect of glucagon on feeding was substantial (+34.8% at 400 g/kg in males and females combined) and did not depend on prior food or alcohol ingestion.

When food and ethanol intake were
GLUCAGON PRODUCES

transformed and combined into total caloric intakes, a biphasic action of glucagon injection was revealed. Acutely, ip glucagon reduced caloric intake at 0-30 min after injection, but increased caloric intake at 30-60 min after injection, reliably in male rats. At 60-90 min after injection, there was no longer a reliable effect of glucagon on food-derived caloric intake. The above-described effects were seen whether or not food and ethanol had been consumed earlier; the effect seemed time-dependent, and not dependent on antecedent ingestive behaviors. Thus, the delayed increase in feeding by glucagon could not be simply a rebound compensatory increase after an acute anorexigenic effect of glucagon.

The immediate decrease in caloric intake produced by glucagon is consistent with the extensive literature which describes glucagon as a short-term satiety factor in the regulation of feeding (Geary, 1990; Gibbs et al., 1993). This effect is consistent with the rapid stimulation of glucagon secretion by food and ethanol (DeJong, Strubbe, & Steffens, 1977; Jarrousse, Lardeaux, Bourdel, Girard-Globa, & Rosselin, 1980; Jauhonen, 1978; Langhans, Pantel, Muller-Seheli, Eggenbergen, & Scharrer, 1984; Potter et al., 1980, Shanbour, 1983; Simanowski et al., 1989). Presumably, these intakes acutely stimulate release of glucagon, which signals satiety, in proportion to calories ingested, at neural elements responsible for control of feeding. Many studies have described a rapid food satiation effect of glucagon administration, usually in lightly- or nondeprived animals (Geary et al., 1982, Gibbs et al., 1993). In the present experiment such an acute satiation effect of glucagon injection is demonstrated in overnight water-deprived, ad lib fed female and male rats, but only when total calories are combined from intake of both 5% ethanol solution and Purina chow. A more robust effect of glucagon in this experiment was the delayed increase in caloric intake, due largely to increased feeding on chow at 30-60 min after injection.

Delayed increases in feeding by glucagon have been reported previously, sometimes surprisingly in the course of a study of glucagon as an inhibitory factor in feeding. For example, food intake of obese Zucker rats was increased 50% after 400 g/kg of glucagon across a 0-60 min measurement period, but not across a 0-30 min period, and the injection also increased daily food intake, although intake of lean Zucker rats was decreased (McLaughlin, Gingerich, & Baile, 1986). Also, immunization of female Zucker obese and lean rats against pancreatic glucagon decreased food intake and body weight, which is consistent with an appetite-stimulating effect of glucagon (McLaughlin et al., 1984). Glucagon (.5-1 mg/kg) produced in ground squirrels a 37% increase in feeding cumulated over 0-4 hr, which was preceded by a decrease in intake cumulated over 0-30 min (Nizielski, Morley, Bartness, Seal, & Levine, 1986). Injection of glucagon at 540 g/kg increased 2-hr cumulative food intake in either liver-denervated or sham-operated, food deprived male rats (Bellinger & Williams, 1983), which suggests the orexigenic effect of glucagon does not depend on hepatic innervation.

Glucagon enhancement of feeding was described as a new phenomenon in 1980 in a study of operant feeding in the rat (Vanderweele et al., 1980). Then it was determined that ip glucagon injection (.35 mg/kg) suppressed 3-hr food intake if injected following a 3-hr fast, but such injection also increased daily food intake. Intracerebroventricular administration of glucagon to rats or baboons was also reported to produce a relative enhancement of caloric intake, which was selective for protein intake in the rat (Nagai et al., 1991; Woods, Lotter, McKay, & Porte, 1979). It has been speculated that the stimulation of feeding by glucagon in these experiments may reflect a behavioral action of the hormone that corresponds to its ability to sustain blood glucose levels over periods of fasting.

A biphasic effect of glucagon on feeding can be viewed as the function of an integrative brain-gut peptide in the coordination of sequences of adaptive responses. According to this model, food-stimulated, acute glucagon secretion
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inhibits feeding as a proportional signal of satiation, perhaps working synergistically with other food-stimulated brain-gut neuropeptides (Hinton, Rosofsky, Granger, & Geary, 1986). A subsequent action of glucagon secretion is to increase the likelihood of feeding later in the postprandial interval. The net result of such a biphasic behavioral action would be to ensure relatively constant blood glucose levels over long intervals. This behavioral effect would mirror and complement the metabolic actions of glucagon to promote gluconeogenesis and glycolysis during a fast. This biphasic model of glucagon's behavioral effects is consistent with the acute stimulation of glucagon by food intake and the later, prolonged rise in glucagon with fasting. However, further experimentation is required to clarify the circumstances of glucagon's biphasic action and to describe the essential neural and endocrine concomitants of this hypothesized complex effect.

In summary, a time-dependent effect of glucagon administration on caloric intake associated with drinking in the water-deprived rat was observed. Glucagon acutely decreased caloric intake and later stimulated intake. This complex action of glucagon may be viewed as an integrative function of a hormone which sustains energy homeostasis in both the fed and fasted state.

References


Author Note

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