The role of the liver and gastrointestinal tract in feeding stimulated after recovery from acute glucoprivation.

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THE ROLE OF THE LIVER AND GASTROINTESTINAL TRACT IN FEEDING STIMULATED AFTER RECOVERY FROM ACUTE GLUCOPRIVATION

A Dissertation Presented
By
JAMES GERARD GRANNEMAN

Submitted to the Graduate School of the University of Massachusetts in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

February 1981

Psychology
THE ROLE OF THE LIVER AND GASTROINTESTINAL TRACT IN FEEDING STIMULATED AFTER RECOVERY FROM ACUTE GLUCOPRIVATION

A Dissertation Presented By
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ABSTRACT

The Role of the Liver and Gastrointestinal Tract In Feeding Stimulated After Recovery From Acute Glucoprivation (February, 1981)

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Injections of insulin and 2-deoxy-D-glucose (2DG) stimulate feeding when food is withheld until after recovery of normoglycemia, six hours post injection (delayed access paradigm). Because the acute energetic emergency produced by these injections presumably has abated by the time rats are allowed access to food, the possibility that feeding is stimulated by enduring alterations in peripheral metabolism, which result from the acute metabolic disturbance, was investigated.

Feeding after recovery from hypoglycemia is associated with depletion of liver glycogen. Intravenous infusions of fructose, a hexose that does not enter the brain, during hypoglycemia inhibited feeding in intact, but not hepatic-vagotomized rats. The effect of fructose was closely associated with changes in liver glycogen, suggesting that sensors in the liver may inhibit insulin-induced feeding in the delayed access paradigm. It was not concluded, however, that the inhibitory action of fructose is mediated by vagal afferents from the liver, because hepatic-vagotomized rats experienced
a greater disruption of hepatic metabolism.

Insulin and 2DG stimulate food intake when compensatory hepatic glucose production is reduced or abolished by adrenal demedullation. Moreover, infusions of adrenaline, which mimic some of the peripheral effects of 2DG, did not stimulate food intake. These results demonstrate that hepatic glucose production or depletion of liver glycogen is neither necessary nor sufficient to elicit feeding in the delayed access paradigm.

It was additionally found that food intake is not dependent on retention or depletion of gastrointestinal contents, or changes in plasma urea, glucose, fatty acids or glycerol. Further, hepatic vagotomy, celiac ganglionectomy, or combined nerve sections did not alter food intake. These results indicate that the liver does not trigger feeding in the delayed access paradigm (but it may inhibit feeding).

In contrast, intraventricular injections of 2DG trigger feeding in the delayed access paradigm. This effect does not depend on hepatic glucose production or depletion of liver glycogen. These results indicate that prior local disruption of cerebral energy metabolism is sufficient to trigger feeding in the delayed access paradigm. However, the cerebral energetic emergency produced by intraventricular 2DG apparently abated by the time animals were given access to food.
It is suggested that prior stimulation of sensors in the brain is necessary to stimulate feeding after insulin or 2DG injection, but a persisting cerebral energetic emergency does not explain why animals eat in the delayed access paradigm. Rather, the results of these experiments indicate that hunger, once stimulated, persists until the animal receives feedback that is appropriate to feeding behavior. Such feedback may be generated when the liver detects an increased supply of fuels.
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Injections of insulin and 2-deoxy-D-glucose (2DG) produce energetic emergencies that stimulate physiological and behavioral compensatory responses. Numerous experiments suggest that insulin- and 2DG-induced feeding behavior is stimulated by receptors in the brain that are activated when cerebral energy metabolism is impaired. This hypothesis is supported by the functional similarity between feeding and the physiological responses that are also stimulated during these emergencies, and are known to be triggered by central receptors. Together, these results have suggested that feeding is one of several responses that helps restore cerebral metabolism during an energetic emergency.

Recent experiments by Ritter, et al. (1978), however, challenge the traditional understanding of insulin- and 2DG-induced feeding. These studies demonstrate that insulin and 2DG provoke feeding when access to food is delayed until after cerebral energy metabolism is apparently restored. One explanation of these results is that compensatory responses to cerebral energetic emergency produce alterations in peripheral metabolism which persist beyond the acute energetic emergency, and provide the stimulus to eat.

The liver and gastrointestinal tract play a central role in the maintenance of fuel homeostasis, and sensory signals about visceral metabolism are believed to be important in the control of food intake.
Because the liver and gut are largely responsible for metabolic counterregulation after insulin and 2DG, enduring changes in these tissues may underlie feeding stimulated after recovery from the acute effects of these treatments. The purpose of the following research, therefore, is to investigate the role of the liver and gastrointestinal tract in the control of food intake seen after recovery from acute energetic emergencies.

Chapter I will develop the hypothesis that feeding during the acute effects of insulin and 2DG (continuous access paradigm) is triggered by an acute cerebral energetic emergency, whereas feeding after recovery from these treatments (delayed access paradigm) may be stimulated by persistent changes in peripheral metabolism. In order to accomplish this, the literature concerning the control of feeding in the continuous access paradigm will be reviewed. Next, the behavioral and physiological properties of the delayed access paradigm will be discussed, and a hypothesis concerning the role of peripheral metabolism in the control of feeding in the delayed access paradigm will be developed.

**Insulin- and 2DG-induced Feeding: Continuous Access Paradigm**

The hunger producing action of insulin has been studied for nearly fifty years. During that time, two basic interrelated questions have been investigated concerning this phenomenon. First, what is the physiological stimulus that triggers feeding? Second, where is the stimulus detected?
Site of Detection

The first experimental work involving insulin-induced hunger was conducted in the context of Cannon's local theory of hunger (1912). According to this theory, hunger was stimulated by contractions of the stomach, and the finding that insulin-induced hypoglycemia provoked both gastric "hunger" contractions and hunger supported this notion (Quigley and Carlson, 1931). However, the finding that vagotomy, which abolishes insulin-induced contractions, failed to prevent hunger in rats (Morgan and Morgan, 1940, dogs (Grossman et al., 1948) and man (Grossman and Stein, 1948) effectively refuted the local gastric theory of insulin-induced hunger.

About this time, advances were made in understanding two neurally-mediated responses to hypoglycemia, adrenal catecholamine secretion and gastric acid secretion. These studies indicated that receptors in the brain were capable of triggering these responses (LaBarre and deCespedes, 1931; Jogi, 1949; Duner, 1953). The idea that the brain stimulates hunger during hypoglycemia first received support from experiments showing that hunger persists despite combined vagotomy and sympathectomy (Grossman, et al., 1948) or high spinal cord section (Grossman and Stein, 1948).

Through the use of 2-deoxy-D-glucose, a non-metabolizable glucose analogue that competitively inhibits glycolysis (Woodward and Hudson, 1954; Wick et al., 1957; Brown, 1962), it became possible to evaluate the location of the receptors controlling feeding more directly. Effective doses of 2DG, like insulin, stimulate feeding
as well as gastric and adrenal secretions (Smith and Gibbs, 1969; Hirschowitz and Sachs, 1965; Hokfelt and Brydgman, 1961). Because of the biochemical action of 2DG and the functional similarity to hypoglycemia, it is believed that the effects of insulin and 2DG are mediated by the same receptor (Smith and Gibbs, 1969; Kadekaro et al., 1975).

Receptors in the brain apparently can trigger feeding after injection of 2DG. Microinjection of 2DG into the lateral cerebral ventricle (in amounts that are ineffective if given systemically) stimulate feeding in rats (Miselis and Epstein, 1975; Berthoud and Mogenson, 1977). The hunger producing action of intraventricular injections of 2DG probably is not secondary to neurally-mediated disruption of peripheral metabolism because adrenal demedullation and vagotomy or both do not reduce feeding to systemic injection of 2DG (Booth, 1972). Further, the finding that infusions of glucose, a hexose that readily enters the brain, inhibit 2DG-induced feeding more effectively than infusions of fructose, a hexose that does not enter the brain, indicates that 2DG has a central site of action (Stricker and Rowland, 1978).

Receptors in the liver are also believed to stimulate hunger by detecting the metabolic effects of 2DG. This conclusion is based on studies that show that hepatoportal injections of 2DG stimulate feeding more effectively than jugular injections in rabbits and rats (Novin et al., 1973; Rowland and Nicolaids, 1974; cf. Russel and Mogenson, 1975). Unfortunately, the interpretation of
the portal-jugular difference is not as clear as it may appear. It has been suggested that jugular injections of 2DG are more debilitating than portal injections (Stricker and Rowland, 1978), and, therefore, rats given jugular injections eat less because of non-specific malaise. Further, the contention that vagotomy eliminates the "special" effectiveness of portal vein injections of 2DG (Novin et al., 1973) cannot be held because the effects of vagotomy have not been shown to be specific to portal injections (see Sawchenko and Friedman, 1979).

Recent evidence suggests that central and hepatic receptors may interact to control feeding during hypoglycemia (Stricker et al., 1977; Granneman, 1979). Insulin-induced feeding is inhibited by infusions of fructose, a sugar that is not used by the brain (Stricker et al., 1977; Granneman, 1979), and hepatic vagotomy reverses this inhibition (Granneman, 1979). Further, infusions of glucose, a sugar that the brain uses, readily inhibit insulin-induced feeding in intact and hepatic-vagotomized rats (Granneman, 1979). These results indicate that during hypoglycemia, the brain initiates, and the liver inhibits feeding (see also Granneman and Friedman, 1980). The notion that the liver exerts an inhibitory influence on food intake is consistent with results reported by Russek (1963, 1970) who found that hepatoportal injections of glucose inhibited feeding in hungry dogs more effectively than jugular injections, and the data of Anil and Forbes (1980) who found that hepatic denervation abolished the inhibitory effect of propionate infusions on food intake in hungry sheep.
Stimulus

It is believed that the stimulus triggering feeding after insulin and 2DG injection is decreased glucose utilization in the brain. Because insulin and 2DG have different effects on blood glucose levels, the term "glucoprivation" was adopted to describe the stimulus believed to be common to both treatments. However, because metabolism in the cells that trigger feeding has never been monitored, evidence concerning the stimulus is necessarily indirect. Nevertheless, the case for disruption of cerebral glucose metabolism is straightforward.

Large doses of insulin produce hypoglycemia by facilitating glucose transport into insulin dependent cells (e.g., muscle and adipose tissue), thereby decreasing the supply of glucose to the brain. Only doses of insulin which are large enough to lower blood glucose below the "saturation" point of the facilitated transport mechanism of the brain are sufficient to elicit feeding (and other centrally elicited responses) (Crone, 1965; Steffens, 1969; Collin-Jones and Himsworth, 1969). Likewise, 2DG stimulates feeding only when doses large enough to provoke the centrally-elicited adrenal response are given (Smith et al., 1972). Indeed, the belief that adrenal catecholamine secretion accurately reflects cerebral glucoprivation (the putative stimulus for feeding) has provided the basis for the design and interpretation of some recent experimental research on feeding behavior (e.g., Smith et al., 1972; Stricker et al., 1977; Ritter et al., 1978).
It is not certain whether the receptors that trigger feeding after insulin and 2DG detect glucose metabolism specifically, or whether they sense the metabolism of oxidizable fuels in general. Experiments examining the effects of different nutrient infusions on insulin-induced feeding demonstrate that both glucose and mannose, whose metabolism in brain is virtually identical, inhibit feeding (Stricker et al., 1977). In contrast, infusions of ketones, which are used by certain regions of the brain (Hawkins et al., 1979) and enter metabolism at the Krebs cycle (Newsholme and Start, 1976), are not effective in reducing feeding, yet reliably inhibit adrenal catecholamine secretion (Stricker et al., 1977). The interpretation of these findings is complicated by the fact that the liver does not use ketones (Krebs et al., 1971), and it is possible that the liver triggers feeding under these conditions (Stricker et al., 1977). However, these results are also consistent with the idea that cerebral receptors specifically sense changes in glycolysis. The fact that 2DG, in doses typically used to produce feeding, inhibits glycolysis without suppressing substrate oxidation in the Krebs cycle (Brown, 1962) supports the view that insulin and 2DG stimulate feeding by specifically disrupting glycolysis.
Stimulus-response dynamics

Food intake after insulin injection is dose dependent (Stricker and Rowland, 1978; Booth, 1968) and probably is related to the duration rather than absolute depth of hypoglycemia. In humans, for example, hunger occurs after the nadir of the hypoglycemia (Janowitz and Ivy, 1949) and presumably in the midst of physiological counter-regulation. In fasted subjects, insulin intensifies hunger when blood glucose falls to about 60mg/dl, but then returns to "basal" levels of hunger when this level is exceeded (Janowitz and Ivy, 1949). This observation suggests that hunger is sensitive to threshold levels of blood glucose, and that insulin-induced hunger and deprivation-induced hunger are triggered by different mechanisms.

Steffens (1969) examined the stimulus-response dynamics of insulin-induced feeding by monitoring glucose periodically in conscious, undisturbed rats after injections or infusions of insulin. After insulin injection, there was a greater latency to eat and, like the studies cited above, hunger did not occur until blood glucose began to rise. Hypoglycemia increased meal frequency, and eating was associated with recovery from hypoglycemia, suggesting a feedback relation. This was more clearly shown in animals given infusions of insulin which lowered glucose more slowly. Eating was reliably stimulated when blood glucose dropped below about 50mg/dl, and eating was followed by restoration of glucose back to normal. Upon continuation of the insulin infusion, glucose fell again and the cycle was repeated.
The stimulus-response dynamics of 2DG-induced feeding are less clear for two reasons. First, the critical stimulus (presumably events associated with accumulation of 2DG-6-phosphate in the cell) is not observable in behaving animals. Second, once 2DG enters brain cells and is phosphorylated, it is trapped temporarily (Sokoloff, 1977); therefore, the feedback consequences of counter-regulatory measures (like feeding and adrenal catecholamine secretion) on cerebral glycolysis are difficult to assess. In any case, 2DG, like insulin, stimulates feeding in a dose-related fashion (Strieker and Rowland, 1978).

In summary, feeding during the acute effects of insulin and 2DG appears to be a specific response to a cerebral energetic emergency. Analysis of the stimulus-response dynamics of this phenomenon suggests that feeding, like the physiological responses to insulin and 2DG, comprises a feedback system that maintains cerebral glucostasis.

**Insulin- and 2DG-induced Feeding: Delayed Access Paradigm**

Recent experiments by Ritter et al. (1978) challenge traditional views of insulin- and 2DG-induced feeding behavior. In these experiments, rats were injected with insulin or 2DG and denied access to food until after recovery of normoglycemia, six hours post injection. When given access to food, rats ate significantly more than rats that were similarly food deprived and injected with saline, and amounts comparable to rats injected with insulin or 2DG and
given continuous access to food. These findings are significant because feeding occurs when the stimulus believed to initiate feeding during the acute effects of insulin and 2DG is no longer present.

The weight of evidence indicates that the brain receives an adequate supply of fuels after restoration of normoglycemia. First, functional responses to glucoprivation, the gastric and adrenal responses, terminate upon recovery of normal glucose levels (Kalk and Meyer, 1932; Granneman and Friedman, 1980; Himsworth, 1968). Second, decrements in cerebral energy metabolism induced by moderate hypoglycemia or 2DG treatment are reversed upon restoration of normoglycemia (Tews et al., 1965; Bachelard, 1972). Third, in animals given effective doses of 2DG in the delayed access paradigm, accumulation of 2DG in various tissues at the time animals are given access to food does not exceed levels observed in animals given doses of 2DG that do not stimulate feeding in the continuous access paradigm (Ritter et al., 1978). Finally, glucose injections after recovery from hypoglycemia do not reduce delayed-access food intake (Ritter et al., 1978). Taken together, these results indicate that on-going glucoprivation does not explain why animals eat in the delayed access paradigm.

As mentioned above, insulin and 2DG injection stimulate numerous neural and hormonal counterregulatory responses, any of which could produce enduring changes in peripheral metabolism. Because counterregulation occurs at the expense of hepatic and gastrointestinal reserves, it is possible that enduring alterations in these tissues
trigger feeding in the delayed access paradigm. Indeed, because blood glucose is restored in the absence of food intake, it stands to reason from a functional viewpoint that the sensors controlling food intake when food is restricted should detect depletions and alterations in metabolism, which persist as a result of previous glucoprivation, rather than sense blood glucose or cerebral glucose metabolism.

In order to develop the hypothesis that enduring changes in peripheral metabolism might trigger feeding in the delayed access paradigm, it is necessary to outline the neural and hormonal metabolic responses to insulin and 2DG treatment and discuss how these responses might influence food intake.

**Metabolic Effects of Insulin and 2DG Treatment in the Rat**

Injections of insulin and 2DG provoke a cerebral energetic emergency; thus, metabolic counterregulation functions to increase the supply of utilizable fuels for cerebral oxidation by mobilizing endogenous reserves and facilitating the assimilation of nutrients from exogenous sources. This review will be limited, when possible, to data obtained in rats because important species differences exist in the metabolic response to insulin and 2DG treatment.
Adrenal medulla

In the rat, adrenaline secretion by the adrenal medulla is the principal efferent mechanism by which the brain mobilizes endogenous substrates after insulin and 2DG injection. After insulin treatment, adrenaline is secreted when glucose declines beyond a critical level, and is released exponentially according to the depth of hypoglycemia (Stricker and Rowland, 1978; Himsworth, 1968). Adrenaline secretion after 2DG treatment is also dose dependent once a threshold is exceeded (Smith et al., 1972). Of the endogenous fuels that are mobilized during hypoglycemia, both glucose and ketones inhibit adrenaline output, suggesting the response may be due to a lack of oxidizable fuels for the brain in general, and not glucose specifically (Stricker et al., 1977; Flatt et al., 1974).

One of the main effects of adrenaline secretion is stimulation of hepatic glucose production (see "liver," below). Adrenaline also has dramatic effects on pancreatic glucoregulatory hormones. Adrenaline inhibits insulin secretion (Frohman et al., 1973), which in turn facilitates mobilization of glucose by the liver and impairs utilization of glucose by insulin-dependent tissues, thereby sparing glucose for the brain. In addition, release of glucagon, which stimulates hepatic production of glucose, is stimulated by adrenaline secretion (Gerich et al., 1973; Iversen, 1973). In fact, recent studies demonstrate that as much as 50% of adrenaline-induced hyperglycemia is mediated indirectly by glucagon release (Potter et al., 1978; Ellis, 1978). The importance of adrenal medullary
secretions can be appreciated by the fact that adrenergic blockage greatly exacerbates insulin-induced hypoglycemia (Abramson et al., 1966), and adrenal demedullation impairs or abolishes compensatory rises in blood glucose (Frohman et al., 1973; Sacca et al., 1977). Finally, adrenaline mobilizes free fatty acids from adipose tissue (Havel and Goldfein, 1959).

**Endocrine pancreas**

The direct in vivo effects of insulin hypoglycemia and 2DG treatment on pancreatic function differ. With insulin treatment, hypoglycemia per se stimulates glucagon and suppresses insulin secretion (Sacca et al., 1977; Gerich et al., 1978). In contrast, the effects of 2DG are apparently mediated entirely by adrenal catecholamines in rats because prior adrenal demedullation abolishes 2DG-induced suppression of insulin and mobilization of glucose (Frohman et al., 1973).

**Adipose tissue**

Insulin and 2DG treatment stimulate lipolysis, and while a fraction of the rise in blood free fatty acids is due to adrenaline secretion, lipolysis is also mediated by direct noradrenergic innervation of adipose tissue in rats and dogs (Teixeira et al., 1973; Gross et al., 1977, 1979; Frohberg et al., 1964; Richardson and Hokfelt, 1964). The physiological function of lipolysis during hypoglycemia probably is to supply substrate for hepatic ketogenesis
because, unlike fatty acids and glycerol, ketones are a utilizable substrate for cerebral energy metabolism. Consistent with this interpretation is the finding that intravenous infusions of ketones, but not glycerol or fatty acids, suppress insulin-induced lipolysis in dogs (Muller et al., 1976).

Liver

The liver plays a central role in counterregulation after insulin and 2DG injection by mobilizing its carbohydrate reserves, and redirecting substrate traffic. In general, hepatic metabolism shifts from uptake and assimilation of substrate to mobilization and production of substrate. Specifically, adrenaline and high glucagon/insulin ratios stimulate hepatic glycogenolysis and gluconeogenesis (Potter et al., 1978; Pogatsa et al., 1978). Moreover, an increased supply of free fatty acids triggers ketone body production by the liver (Mayes and Felt, 1967), and adrenaline facilitates the process in part by suppressing re-esterification of fatty acids (Himms-Hagen, 1967). Direct neural control of hepatic metabolism during hypoglycemia or 2DG treatment has not been demonstrated in the rat.

Other metabolic/endocrine responses

Injections of insulin and 2DG stimulate release of pituitary and adrenal cortical hormones (Roth et al., 1963; Davis et al., 1965). While secretion of these hormones may be necessary for a normal metabolic response during insulin and 2DG treatment (e.g., Richardson
and Hokfelt, 1964), their direct role in counterregulation is not understood (Young and Landsberg, 1977).

Gastrointestinal effects of insulin and 2DG injection

Hypoglycemia stimulates secretory and motor activity of the gastrointestinal tract; this activity is triggered by the brain and mediated by the vagus nerve (Bachrach, 1953). Of the various aspects of this gastrointestinal activation, gastric activity has been studied extensively, and the activity of the stomach during hypoglycemia is probably representative of the gastrointestinal tract as a whole. After insulin injection, gastric activity is stimulated in an on-off fashion when blood glucose levels drop below about 50 mg/dl (Colin-Jones and Himsworth, 1969; Granneman and Friedman, 1980; Davis et al., 1965). Raising glucose above this value immediately terminates gastric activity (Colin-Jones and Himsworth, 1969; Granneman and Friedman, 1980). Increased gastrointestinal activity during hypoglycemia apparently functions to facilitate assimilation of nutrients from the gastrointestinal tract into the blood, and the sensitivity of the affectors that control the response certainly suggests the system is under feedback control. Although it is clear that insulin injection increases gastric emptying, the actual contribution of insulin-induced gastrointestinal activity to counterregulation has not been examined. Nevertheless, recent observations in this laboratory showing that dietary mannitol, a nonabsorbable sugar that impairs absorption of nutrients from the
intestine, increases the depth of hypoglycemia suggest that absorption of nutrients from the gastrointestinal tract contributes to counterregulation after insulin injection.

**Insulin- and 2DG-induced Changes in Metabolism and Delayed-access Food Intake**

Dramatic shifts in cerebral energy metabolism are necessary to stimulate feeding during the acute effects of insulin and 2DG treatment. However, such alterations in cerebral metabolism rarely occur, and certainly play no role in the normal regulation of food intake. Feeding in the delayed access paradigm appears to differ fundamentally from feeding in the continuous access paradigm, because feeding occurs in the apparent absence of ongoing cerebral glucoprivation. But more significantly, the metabolic alterations that would result from prior insulin or 2DG treatment are essentially the same shifts in metabolism that occur as a result of mild food deprivation (see Friedman and Strickler, 1976). Thus, from a metabolic perspective, feeding in the delayed access paradigm and normal food intake are similar, and may be under similar controls. If food intake in the delayed access paradigm is stimulated by enduring alterations in peripheral metabolism, the most likely sites of these alterations are the liver and gastrointestinal tract because these tissues have been implicated in the control of food intake.
Liver

The liver is centrally important in the maintenance of fuel homeostasis. The liver, unlike the brain, normally experiences dramatic fluxes in the supply of energy substrates. It is crucially important in regulating the supply of nutrients in the circulation in part by altering the balance between uptake and assimilation of nutrients, and the mobilization and production of substrates as fuels for the rest of the body. Because of its unique role in metabolism, it has been proposed that alterations in the utilization of fuels by the liver provides the stimulus to eat under normal circumstances (Friedman and Stricker, 1976). The data that support this notion are largely indirect; however, substantial evidence indicates hepatic receptors monitor metabolic events in the liver and transmit information about them to the brain (Niijima, 1969; Schmitt, 1973a, 1973b). Moreover, a growing literature indicates that hepatic receptors modulate food intake. For example, intraportal injection of nutrients are especially effective in inhibiting food intake in hungry dogs (Russek, 1970) and sheep (Anil and Forbes, 1980). Suppression of food intake by portal injections may occur by altering the animal's sensory reactivity, in this case, to taste (Campbell and Davis, 1974). Further, receptors in the liver may modulate food intake by altering gastrointestinal transit (Granneman and Friedman, 1980; Sakaguchi and Yamaguchi, 1979; Kadekaro et al., 1977), or other visceral events (Niijima, 1975).
Gastrointestinal tract

Perhaps the most obvious change in metabolism that is common to the delayed access paradigm and food deprivation is depletion of nutrients from the gastrointestinal tract. Further, it has been recently shown that alterations in the content of the gut modulates ingestive behaviors in the rat. Deutsch, in a definitive series of experiments (1978; Deutsch et al., 1978), demonstrated that when various amounts of liquid diet were extracted from the stomachs of rats that just ate, the rats quickly consumed the diet in amounts that precisely replaced the amount removed. Because a cuff placed about the pylorus prevented nutrients from entering the intestine, the effects of the nutrients on food intake were mediated by receptors in the stomach. Finally, intestinal control of food intake has been indicated by the work of Davis et al. (1975). In these experiments it was found that mannitol, a sugar that promotes accumulation of fluid in the intestine, inhibited intake of sweetened solutions in proportion to its concentration in the solution.

Summary and overview of experiments

The preceding analysis is based on the model that some enduring change in peripheral metabolism stimulates feeding. From an experimental perspective, the delayed access paradigm makes this proposition uniquely testable. In the continuous access paradigm both physiology and behavior are allowed to vary, and to the extent that the
relation between physiology and behavior is reciprocal, analysis of the relation is exceedingly difficult. In contrast, by delaying access to food one can manipulate and measure the physiological variables that are believed to influence the relation. Further, by restricting access to food until a time one knows the animals will eat yields two more advantages. First, it provides a greater degree of confidence that the physiological variables measured can be associated with eating. Second, it allows one to determine the relation between feeding and physiology by measuring variables that could not be measured easily in the continuous access paradigm (e.g., stomach contents and liver glycogen).
CHAPTER II
EFFECTS OF FRUCTOSE INFUSIONS ON INSULIN-INDUCED FEEDING IN THE DELAYED ACCESS PARADIGM

Experiment 1

Introduction

In experiment 1 the role of the liver in the delayed access paradigm was evaluated by examining the effects of fructose infusions during hypoglycemia. Fructose is a hexose that does not readily enter the brain (Park et al., 1957; Oldendorf et al., 1971), and infusion of this hexose during hypoglycemia creates a pool of utilisable fuel for the periphery, but not the brain (Stricker et al., 1977). In addition, fructose is readily utilized by the liver where it has numerous fates including oxidation and incorporation into glycogen (Newsholme and Start, 1976). Therefore, if alterations in hepatic metabolism are important in stimulating feeding after recovery from hypoglycemia, then infusions of fructose may antagonize or reverse these alterations, and thus inhibit feeding without increasing the supply of fuels for the brain during the hypoglycemic episode. In addition, the effect of hepatic vagotomy on insulin-induced food intake was examined because this nerve has been implicated in the monitoring of hepatic metabolism (Niijima, 1969) and is believed to mediate the inhibitory effects of fructose infusions on insulin-induced gastric activity in rats (Granneman and Friedman, 1980).
Methods

Sprague-Dawley rats (Rat Labs) maintained on a 12:12 light-dark cycle and fed ad libitum were used as subjects. Rats were anesthetized and fitted with jugular catheters as previously described (Granneman, 1979). At the same time, half of the rats were hepatic vagotomized (Granneman and Friedman, 1980) while the remaining rats were either sham-operated (n=12) or received no further surgery (n=9). After recovery of preoperative body weights, rats were adapted to the experimental procedure described below.

Rats were placed in infusion cages at 9 a.m. (time 0) with access to water but not food. Rats were then injected subcutaneously with saline or regular insulin (3U/kg) and immediately infused intravenously with isotonic saline or .6M fructose at a rate of 2.4 ml/h. Infusions lasted for 3 hours after injection because the hepatic compensatory response should be maximal during this time (Sacca, 1973, 1977), and it is during this time that infusions of fructose inhibit feeding in the continuous access paradigm (Granneman, 1979). Further, data obtained in experiments examining the effects of fructose infusions on insulin-induced gastric activity (Granneman and Friedman, 1980), as well as the studies of Ritter et al. (1978), indicate that the timing of the infusions may be critical to any inhibitory effect. Six hours post injection, animals were returned to their home cases, and food intake was recorded over the next two hours. At time 0 and 1, 2, 3, 4 and 6 hours after injection, blood was taken from the tip of the tail for glucose determination.
with a Beckman glucose analyzer. Rats received at least two infusions, and treatments were counterbalanced using each subject as its own control.

One week after completion of the behavioral portion of these experiments, rats were retested as described above except six hours post injection rats were killed in order to determine various physiological parameters. Blood was collected from the neck in chilled heparinized tubes, centrifuged, and plasma was analyzed for glucose and free fatty acids (Ho, 1970). A piece of liver was rapidly excised, frozen in a dry ice-acetone bath, and stored at -40°C until assayed for liver glycogen (Passoneau and Lauderdale, 1974). Finally, stomachs were removed in order to determine the weight of residual stomach contents.

Statistical analysis of the data in this dissertation was made by means of analysis of variance. Contrasts between treatments were made using the Bonferroni t statistic or, when homogeneity of variance was not assumed, the Mann-Whitney U test.

Results

Compared to control (no insulin), insulin injection stimulated feeding in intact and hepatic-vagotomized rats (p < .01, Fig. 1). In intact rats, fructose infusions reliably reduced insulin-induced food intake (p < .01), and the amount consumed did not statistically differ from control levels. Fructose infusions, however, did not significantly suppress insulin-induced food intake in hepatic-vagotomized rats,
Figure 1. Effect of fructose infusions and hepatic vagotomy on plasma glucose and food intake after insulin injection. Values are means ± standard error of mean (S.E.M.).
and these animals ate significantly more than under control conditions (p < .01).

Insulin injection decreased blood glucose in intact and hepatic-vagotomized rats (Fig. 1). In all groups injected with insulin, blood glucose reached the lowest point two hours post injection, rose sharply between 2 and 4 hours, and thereafter increased only slightly. Hepatic-vagotomized rats tended to have lower resting blood glucose levels, and this trend was evident after insulin injection as well. In both intact and nerve-sections animals, fructose infusions did not prevent hypoglycemia, but compared to saline infusions, restored glucose to normal values more rapidly.

Figure 2 shows the physiological parameters measured six hours post injection, when the animals would have been given access to food. In general, changes in the physiological measures across treatments were similar in intact and hepatic-vagotomized rats. There was a tendency for hepatic-vagotomized rats to be more sensitive to insulin treatment; for example, compared to control levels, free fatty acid levels rose higher and liver glycogen was depleted more in nerve-sectioned animals. These results are consistent with the fact that blood glucose fell further in these animals (Fig. 1).

The specific pattern of metabolic responses produced by insulin injection is indicative of previous or on-going counterregulation. Insulin treatment elevated free fatty acids (p < .05) and depleted liver glycogen (p < .05). In addition, there was a clear trend for insulin to reduce residual stomach contents, although the effect was not significant because of extreme variability.
Figure 2. Effect of fructose infusions and hepatic vagotomy on physiological parameters. Values are means ± S.E.M.
Fructose infusions reversed insulin-induced elevation of free fatty acids. The effect of fructose on liver glycogen is less clear. In both intact and hepatic-vagotomized rats, fructose infusions tended to attenuate the depletion of glycogen. In intact rats receiving fructose infusions, glycogen did not statistically differ from either control or saline infused rats. In hepatic-vagotomized rats receiving fructose infusions, glycogen remained well below control levels \( (p < .05) \) and did not reliably differ from saline infused rats. Compared to saline infusions, fructose had no effect on residual stomach contents.

Of the physiological parameters measured, only liver glycogen bore a relation with food intake. This relation is illustrated in figure 3, which represents mean food intake as a function of mean liver glycogen across the six treatments of the experiment. The relation is linear and statistically significant \( (p < .01) \). It should be recognized that the strength of the relation is enhanced by the fact that the relation is among mean values. An alternative method inferring a relation between food intake and glycogen might be to search for significant differences in food intake among the various groups which are paralleled by significant contrasts in liver glycogen. However, because the statistical power of the tests used to evaluate these differences is not the same for food intake \( (n=14-17) \) and liver glycogen \( (n=5-6) \), the failure to find statistically significant contrasts in liver glycogen that parallel those in food intake does not mean that liver glycogen could not account
Figure 3. Relation between mean food intake and mean liver glycogen across the 6 conditions of experiment 1.
for the changes in food intake. For this reason mean values for liver glycogen and food intake are used to illustrate the possible relation.

Discussion

Infusions of fructose inhibited insulin-induced feeding in the delayed access paradigm. This finding suggests that feeding in the delayed access paradigm may be influenced by receptors located in the periphery. Moreover, because feeding in the delayed access paradigm is correlated with changes in liver glycogen (Fig. 3), fructose may inhibit feeding by antagonizing insulin-induced alterations in hepatic metabolism. Whether the liver monitors liver glycogen per se cannot be determined from these experiments; however, it is likely that glycogen levels are indicative of the state of hepatic metabolism, especially with respect to hepatic glucose production.

Fructose infusions failed to inhibit insulin-induced feeding in hepatic-vagotomized rats (Fig. 1). It is possible that these animals eat despite fructose infusions, because afferent information about the effects of fructose in the liver is disrupted. However, because hepatic-vagotomized rats experienced a greater depletion of glycogen, it is likely that the elevated food intake of nerve-sectioned animals was due to a greater disruption of hepatic metabolism, although disruption of sensory information cannot be ruled out. Consistent with this interpretation is the fact that
feeding in nerve sectioned and intact animals infused with fructose is described by the same function with liver glycogen (Fig. 3).

The strategy behind using fructose infusions was to antagonize or reverse insulin-induced alterations in hepatic metabolism without affecting the cerebral energetic emergency brought about by hypoglycemia. However, because fructose infusions raised blood glucose levels, it is possible that the effects of fructose could be mediated in part by changes in blood glucose. The data presented in Figure 1 illustrate an apparent relation between average level of hypoglycemia and food intake. Intact rats receiving fructose infusions were least hypoglycemic and ate the least, whereas hepatic-vagotomized rats given fructose infusions and intact rats given saline infusions had comparable glucose responses and ate similar amounts. Whether fructose infusions and hepatic-vagotomy have effects on food intake that are independent of their effects on blood glucose is difficult to resolve. However, there is evidence to indicate that these effects are not totally dependent on changes in blood glucose levels. First, if rats from the various groups are matched on the basis of glucose levels (Fig. 4), the effects of fructose infusions and hepatic-vagotomy, although somewhat diminished, are still present. Second, although changes in glucose levels may be correlated with food intake, other data suggest that such differences may not be sufficient to produce changes in feeding behavior. In these studies it was found that doses of insulin that produced systematic changes in the depth and duration of hypoglycemia stimulated similar amounts of food intake in the delayed access
paradigm (Friedman and Granneman, unpublished observations).

It is doubtful, however, that the effects of fructose infusions are totally independent of blood glucose levels. For example, it has recently been shown that fructose infusions inhibit food intake during mild but not moderate hypoglycemia (Stricker and Rowland, 1978; Rowland and Stricker, 1979). This interaction is probably related to the ability of the liver to metabolize fructose during hypoglycemia. Alterations in hepatic metabolism, increased substrate production and decreased substrate utilization, are mediated by sympathoadrenal catecholamine secretion (Sacca et al., 1977). This response is related to the depth of hypoglycemia and is not suppressed by fructose infusions (Hetenyi et al., 1972; Stricker et al., 1977). Thus, it can be expected that the effect of fructose to raise blood glucose (Fig. 1) may, at the same time, increase utilization of fructose by the liver by reducing the sympathoadrenal response.

Although the results of Experiment 1 suggest that the liver may modulate insulin-induced feeding, it is not clear whether receptors in the liver act to elicit or inhibit feeding in the delayed access paradigm. The fact that food intake is correlated with depletion of glycogen suggests that enduring alterations in hepatic metabolism trigger feeding in this paradigm. Specifically, these results indicate that hepatic events associated with glycogenolysis and gluconeogenesis stimulate hunger.
Figure 4. Effect of fructose and hepatic vagotomy on insulin-induced feeding in rats matched, post hoc, for level of hypoglycemia. Intact + saline vs. intact + fructose p < .046; intact + fructose vs. hepatic vagotomized + fructose p < .062. (Mann-Whitney U test, one tail).
Alternatively, receptors in the liver may act to inhibit rather than elicit feeding in the delayed access paradigm. In this regard it is important to note that the pattern of results across treatments in the present experiment is similar to that obtained in the continuous access paradigm (Granneman, 1979). Specifically, fructose infusions inhibited feeding in intact but not hepatic-vagotomized rats. In the continuous access experiments it was found additionally that glucose infusions inhibited food intake in hepatic vagotomized rats. Because the differential effectiveness of the hexoses to inhibit feeding in hepatic-vagotomized rats reflects the ability of these sugars to enter the brain, these findings indicate that feeding during hypoglycemia is controlled by receptors in the brain and liver. Further, these results suggest that these receptors interact such that cerebral receptors initiate and terminate feeding while receptors in the liver terminate feeding. While direct comparisons between paradigms is difficult, the parallels between paradigms raise the possibility that cerebral and hepatic receptors interact to control food intake in the delayed access paradigm.
ROLE OF HEPATIC GLUCOSE PRODUCTION AND CEREBRAL ENERGY METABOLISM IN INSULIN- AND 2DG-INDUCED FEEDING IN THE DELAYED ACCESS PARADIGM

Experiment 2: Role of Hepatic Glucose Production in the Delayed Access Paradigm

Introduction

Experiment 1 examined whether alterations in hepatic metabolism could be associated with feeding behavior in the delayed access paradigm. The results of this experiment indicate that feeding stimulated after recovery from insulin-induced hypoglycemia is associated with depletion of liver glycogen. Further, infusions of fructose, which antagonize this depletion, reduce feeding. These findings suggest that hepatic production of glucose or depletion of glycogen or both could stimulate feeding in the delayed access paradigm. Indeed, the finding that food intake across the six treatments of the experiment is correlated with liver glycogen content supports this hypothesis.

The role of changes in hepatic glucose metabolism in the delayed access paradigm was investigated in Experiments 2a and 2b by examining the effects of adrenal demedullation on insulin- and 2DG-induced feeding. In rats, adrenaline secretion by the adrenal medulla is the principal mechanism by which the brain mobilizes endogenous substrates after insulin and 2DG treatment. Adrenal demedullation completely abolishes the compensatory rise in blood glucose after 2DG
injection (Forhman et al., 1973) by preventing stimulation of hepatic glycogenolysis and gluconeogenesis (Friedman and Wertheimer, 1966). Glucose counterregulation after insulin injection, on the other hand, is mediated by both glucagon and adrenaline. Nevertheless, adrenalectomy attenuates compensatory glucose production after insulin injection in rats (Sacca, 1977). If hepatic glucose production, or its consequences, stimulate feeding in the delayed access paradigm, adrenal demedullation should prevent feeding to the extent it inhibits this response. In the case of 2DG, feeding should be abolished, whereas insulin-induced feeding should be reduced but perhaps not abolished.

Experiments 2a and 2b determined whether shifts in hepatic metabolism are necessary to stimulate feeding in the delayed access paradigm. Experiment 2c determined whether shifts in hepatic metabolism are sufficient to elicit feeding by examining the effect of adrenaline infusinos, which mimic some of the peripheral effects of 2DG observed in intact rats yet do not disrupt cerebral energy metabolism.

Methods

Experiment 2a. Rats maintained with lab chow and water ad libitum, on a 12:12 light cycle (lights on 8 a.m.) were used as subjects. Under ether anesthesia, half of the rats were adrenal demedullated and half were unoperated controls. Before testing, three weeks were allowed for revascularization of the adrenal cortex, during which time animals had access to a 3% NaCl solution. Completeness of demedulation was determined by the lack of a hyper-
glycemia after 2DG injection (Frohman et al., 1973). Before testing, all rats were adapted to handling and blood sampling.

On the day of testing rats were deprived of food at 9 a.m. and injected with saline or 2DG (300mg/kg ip). Six hours later, food was returned and intakes over the next two hours were recorded. Injections were counterbalanced for order, and each subject served as its own control. Blood samples were taken from the tail at various times for glucose determination. One week after behavioral testing, the experimental procedure was repeated and rats killed six hours post injection for determination of various physiological parameters. Blood, collected from the neck, was analyzed for glucose and plasma urea (Sigma). Both glucose and urea rise as a result of 2DG-induced glycogenolysis and gluconeogenesis (Friedman and Wertheimer, 1966) and changes in these measures may be indicative of enduring shifts in hepatic glucose metabolism. A piece of liver was excised for glycogen determination as described in Experiment 1. Finally, the stomach was removed for determination of residual contents.

Experiment 2b. At 9 a.m. demedullated rats were deprived of food and injected subcutaneously with saline or insulin (3U/kg). Six hours later, food was returned and intakes over the next two hours recorded. Injections were counterbalanced for order, using each subject as its own control. One week after behavioral testing, the experimental procedure was repeated and rats killed for determination of urea, glucose and glycogen as described above.
Experiment 2c. Under ether anesthesia, intact rats were implanted with chronic cardiac catheters via the jugular vein. Before testing, rats were allowed a recovery period of at least 4 days, during which time body weight was recovered to preoperative levels. Rats were deprived of food at 8:30 a.m., and 3.5 hours later saline or adrenaline (800ng/min) was infused for two hours. Pilot data indicated that adrenaline infusions which started at 9 a.m. strongly inhibited gastric emptying. In order to more closely reproduce the effects observed after 2DG injection, the 3.5 hour delay was adopted to permit gastric emptying. One hour after infusions terminated, rats were given access to food and intakes were recorded over the next two hours. At various times during and after the infusions, blood samples were taken from the tail for glucose determination. One week after behavioral testing, rats were killed for determination of liver glycogen and stomach contents as described above.

Results

Experiment 2a. The effects of adrenal demedullation on plasma glucose and feeding behavior after 2DG injection are shown in Figure 5. 2DG provoked marked hyperglycemia in intact rats which abated by six hours post-injection. The hyperglycemia observed in intact rats was indicative of adrenaline mediated hepatic glucose output and was completely abolished by adrenal demedullation. 2DG stimulated feeding in intact rats, and, despite the absence of hepatic glucose production, 2DG-induced feeding was significantly potentiated (p < .05) in demedullated rats.
Figure 5. Effect of adrenal demedullation on plasma glucose and food intake after 2DG injection. Values are means ± S.E.M.
The values of various physiological parameters measured six hours post injection are given in Figure 6. In general, none of the measures varied consistently with changes in feeding behavior. Liver glycogen, which is depleted after recovery from hypoglycemia (Experiment 1), was not significantly affected by 2DG or adrenal demedullation. The fact that no differences in glycogen were observed does not indicate that glycogenolysis did not occur; rather, it indicates that 2DG produces no net depletion of glycogen once glucose values return to normal (Pogasta et al., 1978; see also Experiment 2c). There was a clear trend for plasma urea levels to be elevated in intact rats given 2DG (.05 < p < .10), suggesting gluconeogenesis was occurring in these animals. This trend, however, was absent in adrenal demedullated rats. Compared to saline treatment, gastric contents were significantly elevated in intact rats given 2DG (p < .05). This effect may have been due to inhibition of gastric motility by adrenaline because the effect was absent in demedullated rats (see also Experiment 2c).

Experiment 2b. The effects of insulin hypoglycemia on feeding, liver glycogen and plasma urea are shown in Figures 7 and 8. Insulin produced hypoglycemia in demedullated rats, which reversed by six hours post injection. Insulin stimulated feeding in demedullated rats despite the absence of significant changes in liver glycogen or plasma urea.
Figure 6. Effect of 2DG on physiological parameters in intact and demedullated rats.
Figure 7. Effect of insulin on plasma glucose and food intake in demedullated rats. Values are means ± S.E.M.
Figure 8. Effect of insulin on physiological parameters in demedullated rats. Values are means ± S.E.M.
Experiment 2c. The effects of adrenaline infusions on blood glucose, gastric contents and liver glycogen are shown in Figure 9. Adrenaline infusions altered the physiological parameters in a pattern similar to that observed in intact rats given 2DG. A two hour infusion produced sustained hyperglycemia, which returned to normal within one hour after the infusion was discontinued. As with intact rats given 2DG, adrenaline infusions did not result in a net depletion of glycogen once glucose values returned to normal. However, adrenaline significantly decreased gastric emptying (p < .002). Despite these similarities in the pattern of physiological responses between adrenaline infusions and 2DG treatment, adrenaline did not alter feeding behavior.

Discussion

The results of these experiments demonstrate that prior stimulation of hepatic glucose output or depletion of liver glycogen are not necessary to stimulate insulin- and 2DG-induced feeding in the delayed access paradigm. The fact that feeding is significantly potentiated in animals in which the hepatic response has been prevented by adrenal demedullation indicates that adrenaline-mediated hepatic glucose production and feeding behavior may be complementary responses to a common stimulus. Further, the fact that intact rats eat less than demedullated rats after 2DG injection is strong evidence that 2DG-induced shifts in hepatic metabolism do not stimulate feeding, but rather reduce it through compensatory glucose production.
Figure 9. Effect of adrenaline infusions on food intake and physiological parameters. Values are means + S.E.M.
The ability of hepatic glucose production to decrease 2DG-induced feeding is most likely due to less 2DG entering critical tissues because of increased competition with plasma glucose. This suggests that food intake in the delayed access paradigm may be related to the degree of the prior disruption in energy metabolism. However, at six hours post-injection, feeding behavior of demedullated rats given 2DG is surely not due to the contemporaneous, direct accumulation of 2DG in these tissues, because insulin, which does not depend on accumulation of an antimetabolite, also triggers feeding in the delayed access paradigm (Experiment 2b; see also Ritter et al., 1978).

The contention that feeding in the delayed access paradigm is related to the degree of prior disruption of cerebral energy metabolism, but not to the hepatic consequences of that disruption is reinforced by the results of Experiment 2c. These results demonstrate that, in the absence of a prior cerebral energetic emergency, infusions of adrenaline, which mimic some of the peripheral effects of 2DG, fail to stimulate feeding behavior.

Another peripheral mechanism by which feeding may have been stimulated in the delayed access paradigm is through depletion of gastric contents (McHugh and Moran, 1979; Hunt, 1980). Depletion of nutrients from the stomach stimulates feeding in sated rats (Deutsch, 1978), and both insulin and 2DG elicit gastric acid secretion and stimulate gastric motility (Davis et al., 1965; Hirschowitz and Sachs, 1965). However, it is clear from Figure 3
that simple differences in residual gastric contents cannot explain feeding behavior across the conditions of the experiment. 2DG-induced feeding in intact rats was associated with a significant elevation of residual contents; however, 2DG did not affect residual contents in demedullated rats, yet food intake was significantly potentiated.

**Experiment 3: Effect of Intraventricular 2DG Injections on Drinking and Feeding in the Delayed Access Paradigm**

**Introduction**

The results of Experiment 2 indicate that eating in the delayed access paradigm is related to the degree of the previous metabolic emergency. Sensors which stimulate feeding by detecting the direct actions of 2DG and insulin are believed to be located in the brain (Miselis and Epstein, 1975) and liver (Stricker et al., 1977; Novin et al., 1973). However, the finding that feeding behavior and the centrally elicited sympathoadrenal response appear to be complementary responses to a common stimulus implies that the receptors which stimulate feeding in the delayed access paradigm may be located in the brain. In order to investigate whether stimulation of receptors in the brain is sufficient to elicit feeding in the delayed access paradigm, the effects of intraventricular injections of 2DG were examined. Further, to separate the local effects of these injections from the peripheral sympathoadrenal response on the liver, these experiments were conducted in intact and adrenal demedullated rats.
Methods

Rats were adrenal demedullated or unoperated and maintained as described in Experiment 2a. One week before testing, rats were implanted with cannulae (25 Ga.) in the right lateral cerebral ventricle according to the method of Berthoud and Mogenson (1977). Guide cannulae were placed 1.5mm lateral and 5.2mm anterior to interaural zero, then lowered 1.7-2.2mm below the dura mater. Injector cannulae extended beyond the guide cannulae by 1.0-1.5mm, and teh tips were cut at a 60° angle. Thus, when injections were made, the injector pierced the upper wall of the ventricle. Placement of the cannulae was verified histologically at the conclusion of the experiments by injecting dye through the cannulae. Before testing, rats were adapted to having their heads lightly restrained and blood samples taken from the tail.

On the day of testing, rats were deprived of food at 9 a.m. (time 0). The first intraventricular injection was made at this time, followed by a second injection 30 minutes later. Two injections were made in order to more closely simulate the effects observed after systemic injections of 2DG. Ventricular injections were composed of isotonic saline or 2DG (.5mg/ul saline) and delivered in a 7ul volume by microsyringe over 45 seconds. At 3 p.m., animals were given access to food and intakes over the next hour were recorded. At time 0, 1, 2, 4, and 6 hours, water intakes were recorded and blood samples taken from the tail for determination of glucose. One week after completion of behavioral testing, the experimental
procedure was repeated for determination of liver glycogen, plasma glycerol\(^1\) (Wieland, 1957), and gastric contents.

Results

Intraventricular injections of 2DG produce hyperglycemia in intact rats, but had no effect on blood glucose in adrenal demedullated rats (Fig. 10). 2DG stimulated water intake in demedullated, but not intact, rats (Fig. 11). The time course of 2DG-induced drinking behavior, which by six hours averaged over 4ml (range 0-14ml) in demedullated rats (p < .05), is shown in Figure 11. 2DG stimulated feeding in intact and demedullated rats (p < .05) (Figure 10). There was no significant effect of demedulation on food intake, nor did demedulation significantly potentiate 2DG-induced eating.

Figure 12 illustrates the physiological parameters measured six hours post injection. 2DG treatment tended to result in higher residual stomach contents, but this effect was not statistically reliable (p = .156). Liver glycogen and glycerol were not significantly affected by either adrenal demedullation or intraventricular 2DG injection.

Discussion

The finding that intraventricular injections of 2DG, like systemic injections, stimulate feeding in the delayed access paradigm in intact and demedullated rats indicates that stimulation of receptors located in the brain is sufficient to elicit feeding in the delayed access paradigm. Average food intake after intra-
Figure 10. Effect of intraventricular 2DG injection on plasma glucose and food intake in intact and demedullated rats. Values are means ± S.E.M.
Figure 11. Effect of intraventricular injections of 2DG on water intake in intact and demedullated rats. Values are means ± S.E.M.
Figure 12. Effect of intraventricular 2DG injection on physiological parameters in intact and demedullated rats. Values are means ± S.E.M.
ventricular injection of 2DG was somewhat less than that observed after systemic injections (Figures 5 and 10). This difference could arise from numerous factors including differences in the distribution and kinetics of 2DG in the brain, stimulation of extracerebral receptors, or non-specific effects associated with intraventricular injections. The latter possibility may be significant because eating after saline injection was also somewhat lower, a phenomenon observed in two other replications of this experiment (data not presented).

In any case, the fact that demedullation did not alter feeding indicates that adrenaline-mediated shifts in peripheral metabolism, like hepatic glucose output or suppression of insulin (Frohman et al., 1973) are not necessary for the response.

None of the physiological parameters measured correlated with the observed changes in food intake. It is interesting to note that 2DG did not significantly alter plasma glycerol levels in demedullated rats by stimulating lipolysis via noradrenergic innervation of adipose tissue (Coimbra et al., 1979; Gross and Migliorini, 1977). The lipolytic action of 2DG is similar to the centrally elicited adrenaline response in that both are indicative of an on-going cerebral energetic emergency. Thus, it appears that such an emergency was not present when demedullated rats were given access to food, and therefore, an existing metabolic emergency in the brain does not explain why rats eat after intraventricular 2DG in the delayed access paradigm.
Intraventricular injections of 2DG also stimulate water intake. This phenomenon was first reported by Berthoud and Mogenson (1977) who produced the effect in intact rats given continuous access to food and water. In contrast to these findings, intact rats did not significantly increase water intake in the present experiment. Because animals in the present experiment did not have access to food, it is possible that the drinking observed previously in intact rats after intraventricular injection of 2DG may be related to the feeding response that occurred shortly after the bout of water intake. It is more likely, however, that 2DG stimulates thirst, and this effect is potentiated by adrenal demedullation. Consistent with this interpretation are the recent findings of Thompson et al., (in press) who found that intravenous injection of 2DG stimulates thirst in humans, and this effect was dramatically potentiated in a subject in which peripheral sympathoadrenal responses were absent due to cervical spinal cord section.

Berthoud and Mogenson suggested that intraventricular injection of 2DG may stimulate thirst in part by local increases in cerebral osmolarity. This does not appear to be the case in the present experiments because both intact and adrenal demedullated rats received equiosmotic injections yet only demedullated rats drank. Moreover, the increase in plasma osmolarity attendant with hyperglycemia (in addition to the cerebral osmotic effects of 2DG) was not sufficient to elicit drinking in intact rats. Rather, 2DG-induced thirst appears to be stimulated by the metabolic effects of 2DG in the brain because compensatory glucose production reduces thirst.
This conclusion is supported further by the fact that thirst induced by intravenous injection of 2DG in humans is not due to alterations in plasma osmolarity, catecholamines or angiotension (Thomson et al., in press).
CHAPTER IV
EFFECT OF PERIPHERAL NERVE SECTIONS ON INSULIN- AND 2DG-INDUCED FEEDING IN THE DELAYED ACCESS PARADIGM

Experiment 4

Introduction

If alterations in hepatic metabolism are responsible for feeding in the delayed access paradigm, then it is possible that information about these alterations is transmitted to the central nervous system over a neural route. In the rat, the liver receives parasympathetic and sympathetic innervation from the vagus and splanchnic nerves, respectively (see Sawchenko and Friedman, 1979 for review). These nerves are known to carry afferent information about hepatic metabolism (e.g. Niijima, 1969; Schmitt, 1973a, 1973b; Orbach and Andrews, 1973), and a growing literature indicates that information transmitted over these nerves may influence feeding behavior. For example, systemic hypoglycemia alters firing rates of neurons in the thalamic taste nucleus of cats, and this effect is significantly reduced by spinal cord section (Emmers, 1979). More direct evidence implicating these nerves in the control of food intake is provided by studies showing that hepatic vagotomy abolishes the inhibitory effects of fructose infusions on insulin-induced feeding in rats (Granneman, 1979), and that removal of the hepatic plexus abolishes the inhibitory effect of propionate infusions on feeding in hungry sheep (Anil and Forbes, 1980).
This experiment was conducted to determine whether information transmitted over the major parasympathetic and sympathetic innervation of the liver is necessary for feeding in the delayed access paradigm by examining the effects of hepatic vagotomy, celiac ganglionectomy and combined hepatic vagotomy and celiac ganglionectomy. In addition, these experiments determined whether alterations produced by the efferent actions of these nerves (e.g., Shimazu, 1971; Shimazu and Amakawa, 1975) are essential for feeding in this paradigm.

Methods

Rats maintained with chow and water ad libitum were used as subjects. Hepatic vagotomy was performed as previously described (Granneman, 1979). Celiac ganglionectomy was executed under pentobarbital anesthesia. The abdominal cavity was exposed through a midline incision, and intestines were moved gently to one side. The celiac ganglion was located using an operating microscope by finding the bifurcation of the renal artery and the abdominal aorta, upon which the ganglion is situated. The ganglion was removed by first isolating it from all visible connections, and then removing the ganglion proper. Rats which underwent laparotomy served as controls. All animals were allowed three weeks to recover from surgery before testing.

On the day of testing, rats were deprived of food at 9 a.m. (time 0), and injected subcutaneously with saline, insulin (3U/kg), or
2DG (200mg/kg). Each rat received each injection, and treatments were counterbalanced for order. At 3 p.m., food was returned and intakes over the next two hours recorded. Blood samples were taken from the tail at time 0, 2, 4, and 6 hours post injection for determination of glucose.

Results and Discussion

Figure 13 shows the effects of hepatic vagotomy, celiac ganglionectomy and combined nerve section on blood glucose and food intake in the delayed access paradigm. The time course of insulin-induced hypoglycemia and 2DG-induced hyperglycemia were similar in all nerve section groups. Analysis of variance (Table 1) revealed a strong effect of injection on food intake across nerve section groups; however, nerve section did not affect food intake, nor did nerve section interact with the injections.

Because hepatic vagotomy, celiac ganglionectomy and combined nerve section did not alter feeding in the delayed access paradigm, afferent information mediated by these nerves is not necessary for feeding to occur in this paradigm. Further, alterations in hepatic metabolism which may be mediated by the efferent action of these nerves is not essential for feeding. These findings do not support this hypothesis that enduring alterations in hepatic metabolism elicit feeding in the delayed access paradigm.
Figure 13. Effect of various nerve sections on plasma glucose and food intake after insulin and 2DG injection. Values are means ± S.E.M.
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CHAPTER V

THE ROLE OF THE GASTROINTESTINAL TRACT IN INSULIN-INDUCED FEEDING IN THE DELAYED ACCESS PARADIGM

Experiment 5

Introduction

Experiment 5 was conducted to determine whether accumulation of nutrients in the gastrointestinal tract could influence feeding in the delayed access paradigm. In 1978, Mei identified receptors in the small intestine of cats that are sensitive to carbohydrates and transmit afferent information via the vagus nerve. That receptors in the intestine may be involved in the control of food intake has been indicated by several studies. Davis et al. (1975), for example, found that mannitol, a nonabsorbable sugar that promotes accumulation of fluid in the intestine, inhibited intake of sweetened solutions in proportion to its concentration in the solution. Moreover, a connection between intestinal events and feeding in the delayed access paradigm can be drawn by considering recent observations by Bellin and Ritter (1979) and Meyers and McCaleb (1980). Bellin and Ritter observed that insulin-induced hypoglycemia increases noradrenaline turnover in areas of the hypothalamus, and this effect persists until animals are allowed to eat, six hours post injection. Myers and McCaleb observed that injection of nutrients into the intestine of hungry rats decreases noradrenaline turnover in these same areas of the hypothalamus. Although the latter experiments did not
control for the absorption of nutrients from the intestine into the blood, they suggest that receptors in the intestine may reverse events in the brain that are associated with hunger in the delayed access paradigm.

In the following experiment, the role of the intestine in the delayed access paradigm was investigated by examining the effects of dietary mannitol on food intake and gastrointestinal contents after insulin injection.

**Methods**

In order to produce changes in intestinal contents, a stock solution of sweetened condensed milk (Borden's Eagle Brand with water 1:1) that contained no or .2M mannitol was substituted for regular laboratory chow 18 hours prior to testing in two groups of rats. Because hypoglycemia increases gastrointestinal activity (Bachrach, 1953), it was expected that mannitol would accumulate in the intestine after insulin injection, impair absorption of other nutrients and, compared to control animals, result in a greater retention of nutrients in the gastrointestinal tract.

At the start of the experiment, rats were deprived of the liquid food and injected subcutaneously with saline or insulin (3U/kg). Six hours later, rats were given access to laboratory chow and intakes over the next hour were recorded. Saline and insulin treatment were counterbalanced, using each animal as its own control. At various times before and after injection, blood samples were taken from the tail for glucose determination.
Upon completion of the behavioral portion of these experiments, rats were retested and killed six hours post injection for determination of residual contents of the stomach and small intestine, as well as liver glycogen.

Results

Compared to controls, insulin reduced blood glucose more in rats treated with mannitol, suggesting that during hypoglycemia mannitol interferes with absorption of nutrients from the intestine (Fig. 14). Analysis of variance indicated that insulin produces a modest stimulation of food intake ($p < .05$). Mannitol, however, had no significant effect on food intake and did not interact statistically with the injection.

Figure 15 shows the effects of insulin and mannitol on residual gastrointestinal contents and liver glycogen. Contrary to expectation, it was discovered that by six hours post injection, mannitol treated animals had significantly less stomach and intestinal contents than controls ($p = .001, .012$, respectively). Similarly, it was found that liver glycogen was reliably reduced by mannitol treatment ($p = .016$). No significant effect of insulin on gastrointestinal contents was observed, nor did the injections interact with the dietary treatment. In contrast, insulin produced a modest depletion of glycogen ($p = .052$).

Discussion

The pattern of residual gastrointestinal contents observed in
Figure 14. Effect of dietary mannitol on food intake and plasma glucose after insulin injection. Values are means ± S.E.M.
Figure 15. Effect of dietary manitol on physiological parameters after insulin injection. Values are means ± S.E.M.
this experiment is difficult to explain. Rats tended to consume less of the mannitol diet than the stock solution. However, this fact does not appear to account for the differences observed six hours post injection because control and mannitol treated rats killed at the beginning of the test (data not shown) had similar amounts of nutrients in their gastrointestinal tract. Despite these anomalies, the results of this experiment suggest that simple differences in gastrointestinal contents do not influence feeding in the delayed access paradigm for the following reasons. First, insulin had no reliable effect on gastrointestinal contents, yet significantly stimulated food intake. Second, residual gastrointestinal contents of control rats was significantly greater than that of mannitol treated rats, yet this difference was not reflected in the feeding behavior of these groups. Thus, changes in gastrointestinal contents appear neither necessary nor sufficient to alter feeding behavior in the delayed access paradigm.
CHAPTER VI
GENERAL DISCUSSION

Insulin- and 2DG-induced feeding, like the physiological responses to these treatments, have been understood in terms of a simple feedback model. This model assumes that reversal of the metabolic events which elicit a response must be sufficient to terminate the response. For example, insulin-induced gastric and adrenal activity is triggered when glucose falls beneath a critical value and terminates when glucose rises above this value. The delayed access feeding phenomenon is problematic for the traditional interpretation of insulin- and 2DG-induced eating because feeding can be temporally dissociated from the physiological responses which are believed to be triggered by the same stimulus. One would therefore have to assume that feeding in the continuous access and delayed access paradigms are different because reversal of the metabolic event that elicits feeding in the continuous access paradigm (cerebral energetic emergency) is not sufficient to terminate hunger in the delayed access paradigm. From this model, the hypothesis that enduring shifts in peripheral metabolism trigger feeding in the delayed access paradigm was developed.

Whether specific changes in peripheral metabolism, which endure beyond the acute energetic emergency produced by insulin and 2DG, account for feeding in the delayed access paradigm was investigated in the experiments described above. Specifically, liver glycogen, hepatic glucose production, gastric contents and various plasma
metabolites were measured to determine whether changes in these parameters were associated with food intake. It was found that food intake in the delayed access paradigm was not dependent on hepatic glucose production, depletion of liver glycogen, retention or depletion of gastrointestinal contents, or changes in plasma urea, glucose, fatty acids or glycerol. Further, various peripheral nerve sections failed to alter insulin- and 2DG-induced feeding in the delayed access paradigm. These findings do not support the hypothesis that enduring alterations in peripheral metabolism trigger feeding in the delayed access paradigm. Moreover, the findings that food intake was significantly potentiated when the prime peripheral-hepatic response to and 2DG was prevented by adrenal demedullation, and that infusions of adrenaline, which mimic some of the peripheral metabolic effects of 2DG, failed to stimulate feeding, strongly indicate that peripheral metabolic responses mediated by adrenal catecholamines to insulin and 2DG are neither necessary nor sufficient to elicit feeding in the delayed access paradigm.

In contrast, the results of Experiment 3 demonstrates that local disruption of cerebral energy metabolism is sufficient to elicit feeding in the delayed access paradigm. Further, this response occurs independently of hepatic glucose production, depletion of glycogen or changes in residual stomach contents. These results indicate that stimulation of receptors in the brain is sufficient to trigger feeding in the delayed access paradigm. However, enduring cerebral glucoprivation probably does not explain why animals eat when given
access to food six hours post injection because glycerol levels, which rise during acute glucoprivation, were not altered by 2DG at this time. This conclusion is supported by the findings of Ritter et al. (1978) who found that, six hours post injection, the accumulation of 2DG in the brains of rats given effective systemic doses of 2DG does not exceed levels observed in rats given doses of 2DG that do not stimulate feeding in rats given continuous access to food.

Given that 1) specific alterations in peripheral metabolism are neither necessary nor sufficient to stimulate feeding in the delayed access paradigm, 2) local stimulation of the brain is sufficient to trigger feeding in the absence of specific alterations in peripheral metabolism, but 3) on-going cerebral energetic emergency does not explain why animals eat in the delayed access paradigm, it is possible that feeding induced by insulin and 2DG is triggered by the brain yet is not a simple feedback response to disruption of cerebral energy metabolism. Specifically, I suggest that simple reversal of the metabolic events in the brain which are sufficient to elicit feeding after insulin and 2DG may not be sufficient to terminate hunger.

An explanation of why the physiological responses to insulin and 2DG are organized as simple feedback responses, while hunger persists beyond the acute energetic emergency may be based on fundamental differences in the nature of feeding and the physiological responses. Feeding behavior is constrained by both external and internal events. For example, while a cerebral energetic emergency
may initially elicit hunger, the feeding response depends on the availability of food. As a consequence, the cerebral energetic emergency that initiates hunger may not always be contemporaneous with the consequences of feeding. Thus, because cerebral energy metabolism is restored regardless of whether animals eat, restoration of cerebral energy metabolism may not be a useful feedback signal for the termination of hunger in the delayed access paradigm. In contrast to feeding, the physiological responses (e.g., gastric activity and adrenal catecholamine secretion) are not constrained by the external environment; therefore, the consequences of the physiological responses can be directly coupled to the stimulus that elicits the response.

The difference in the nature of the feedback required to terminate hunger and the physiological responses may be explained by the function that each response serves. The physiological responses are the first line of defense and counter the effects of insulin and 2DG by drawing on endogenous reserves. Whereas feeding may contribute to acute counterregulation (Steffens, 1969), feeding also replenishes deficits in energy substrates brought about by the primary, physiological responses. Moreover, feeding serves this function independently of whether animals have continuous or delayed access to food. Thus, from this perspective, it is not surprising that hunger persists beyond acute glucoprivation, until the animal receives feedback that is relevant to the response of feeding.

Feeding in the delayed and continuous access paradigms may
be under similar controls, because in both paradigms it appears that a cerebral energetic emergency is both necessary and sufficient to elicit feeding (see Chapter I for review). However, a question that is crucial to the present discussion is: what stimulus or feedback is necessary to terminate hunger, and is it the same in both paradigms? Such comparisons would be difficult if not impossible to make if the procedures of the paradigms were rigidly followed. Recent experiments by Ritter et al. (1980), however, demonstrate interactions between food intake in the continuous and delayed access paradigms. In these experiments it was found that a one hour feeding bout in the midst of hypoglycemia prevented food intake six hours post injection. Eating was associated with rapid recovery of normal glucose values. Further, drinking glucose solutions, but not saccharin solutions, during hypoglycemia also prevented hunger six hours after injection. Thus, some nutritive consequence of feeding is sufficient to terminate hunger after insulin injection.

In this context, the results of Experiment 1, in which it was found that fructose infusions inhibited insulin-induced feeding, may be particularly relevant. During hypoglycemia, feeding and fructose infusions are similar in that both provide the animal with exogenous energy and thereby reduce depletion of internal reserves. Because the liver is the first internal organ to detect the consequences of ingested nutrients and has been implicated in monitoring the effects of fructose infusions (Stricker et al., 1977; Granneman and Friedman, 1980), it is possible that sensors in the liver provide feedback
information to the brain which prevents insulin-induced feeding six hours post injection. The specific metabolic events in the liver which constitute the stimulus under these conditions may be correlated with liver glycogen (Experiment 1), but probably is not liver glycogen per se. One possibility is that suppression of hunger is due to increased hepatic oxidation of fuels (Friedman and Stricker, 1976).

It should be emphasized that this model of delayed-access food intake assumes that food intake is determined by the interaction of cerebral inputs, which initiate hunger, and peripheral feedback, which terminates hunger. Because control rats (no insulin or 2DG) did not experience a cerebral energetic emergency, comparing the peripheral metabolic parameters of these animals with those of rats treated with insulin and 2DG may not reveal information about the nature of the peripheral feedback. Rather, differences in these measures among rats treated with insulin and 2DG may reveal important feedback stimuli. For example, the potentiation of 2DG-induced food intake in demedullated rats may be due in part to greater depletion of gastric contents (Figs. 5 and 6).

Because prior disruption of cerebral metabolism is necessary to stimulate feeding in the delayed access paradigm, feeding under these conditions and feeding under normal circumstances probably are triggered by different events. However, while central energetic emergencies may not elicit feeding under normal conditions, the feedback needed to terminate hunger in the delayed access paradigm and under normal conditions may be similar. In this regard, the
results of the present experiments may be instructive in the analysis of feeding under normal conditions. The present experiments demonstrate that while feeding ultimately serves to maintain fuel homeostasis, its control, unlike the physiological responses, is not necessarily related to moment-to-moment fuel homeostasis. Rather, feeding behavior is best understood by considering the relation of the response to the external environment as well as the relation of the consequences of the response to the internal environment. This approach suggests that behavioral and physiological responses that are often termed "homeostatic," are indeed governed by factors which reflect the unique position of each response in the external and internal ecologies of the animal.
1. Plasma glycerol levels were generously assayed by Dr. Israel Ramirez.
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