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RAPID COMMUNICATION

Vagally Mediated Feeding Responses to Phloridzin Infusion in the Rabbit

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SANDERSON, J. D., D. A. VANDERWEELE AND P. J. GEISELMAN. *Vagally mediated feeding responses to phloridzin infusion in the rabbit.* PHARMACOL BIOCHEM BEHAV **43**(3) 919-923, 1992.—Rabbits were infused with the plant glycoside, phloridzin, which blocks absorption of glucose across a number of bodily tissues. Feeding was dramatically increased in the first 0.5 h following phloridzin infusion into either the duodenum or the hepatic-portal vein of intact rabbits. Food intake covaried inversely with glycemic levels after phloridzin infusion into the hepatic-portal vein, but rabbits did not show a systematic relationship between blood glucose levels and food intake following duodenal infusion of phloridzin. When administered into the general circulation via the jugular vein, phloridzin did not elicit feeding. Finally, vagotomized rabbits did not show the hyperphagic response to phloridzin that was observed in intact rabbits. It was concluded that the feeding response to phloridzin is vagally mediated and appears to be induced by glucose transport inhibition at some peripheral site.

Phloridzin Feeding Rabbit Liver Duodenum Hepatic-portal vein Jugular vein
Vagus nerve Subdiaphragmatic vagotomy Glucose transport Glycemia Glycosuria

DATA indicating that the liver may play a crucial role in mediating the feeding response to decreased glucose utilization were first presented in 1973 (31). That study used 2-deoxy-D-glucose [(2-DG), a glucose analog that blocks intracellular utilization of glucose]; 2-DG has been studied extensively for its physiological effects and its ability to stimulate food intake (2,6,18,39). Despite the data reported in that study and a number of related findings (9,11,29,35,41), the role of the liver in the short-term control of food intake remains controversial.

Perhaps the two most serious challenges to the role of the liver in the control of food intake are the findings that a) complete denervation of this organ does not alter food intake (5,26) and that b) portal-caval shunts (attachment of hepatic-portal vein to the ascending inferior vena cava, thereby allowing absorbed nutrients to bypass the liver) do not alter short-term feeding (22). Neither finding can be dismissed, and one might argue that those results indicate that animals that have recovered or are recovering from these manipulations can control food intake without accurate signals from the liver. However, one cannot infer from those data that the intact, normally functioning liver does not play a role in short-term feeding.

The feeding effects of glucose administered directly into the liver of intact animals are also controversial. Studies showing that hepatic-portal infusion of glucose does not affect food intake have provided a basis for arguments against the role of the liver in the control of feeding (3,4,10,24,28). However, other investigations have found that hepatic-portal infusion of glucose, depending upon the infusion parameters, can significantly increase or decrease the animal's subsequent food intake (11,34,36,37,42). It is noteworthy that similar results, again dependent upon the infusion parameters, have been reported following glucose infusions into the duodenum, an organ well documented in the short-term control of food intake (13,17,30).

An additional criticism of the role of the liver, or any peripheral loci, in the control of food intake stems from a series of experiments suggesting that the area surrounding the

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fourth ventricle is crucial for the increased feeding observed following 2-DG administration, especially when access to food is delayed for a period of hours (32,33). These findings, however, are also consistent with a redundant signal arising from the liver, and/or other peripheral organs, participating in the control of food intake following glucoprivation because the major afferent, first synaptic area of the vagus is in the nucleus tractus solitarius, located at the floor of the fourth ventricle.

In the present studies, we offer further evidence indicating that peripheral loci, including the liver, play a role in mediating the feeding responses to changes in glucose availability. We used the plant glycoside phloridzin to challenge glucostatic regulation in the rabbit. The action of phloridzin as an inhibitor of glucose transport in several tissues is well established in vitro and in vivo. Transport inhibition of glucose has been demonstrated for the intestinal epithelium (21), renal tubules (27), and the liver (20). In isolated brush border membrane fragments of the intestine, phloridzin has been shown to possess a greater affinity for the carrier than does the normal substrate, glucose (1). As previously shown with 2-DG, the rabbit responds to glucoprivation by vigorous eating (31). Studies in the rat indicate that phloridzin increases food intake, perhaps by blocking glucose transport into glucoreceptors in the CNS (8,19). We demonstrate here that the rabbit also increases eating following phloridzin treatment and that this increase in food intake is vagally dependent and appears to be induced by peripheral inhibition of glucose transport.

EXPERIMENT 1: PHLORIDZIN INFUSION INTO INTACT RABBITS

METHOD

Silastic cannulae were chronically implanted into either the duodenum ($n = 9$), the hepatic-portal system via a mesenteric vein ($n = 9$), or the jugular vein ($n = 7$) in intact, female New Zealand rabbits in the manner outlined in previous publications (11,43). Following recovery of normal feeding (defined as ingestion of 100 g or more of chow per day for 1 week), each rabbit received a 10-ml infusion of 2.5×10^{-3} M phloridzin and a 10-ml control infusion of isosmotic saline in the diurnal and nocturnal portions of the nychthemeral cycle. Phloridzin and saline infusions were each delivered at the rate of 1 ml/min and were presented in counterbalanced order during each portion of the light-dark cycle. The present dose of phloridzin was chosen because it is 2.5 times greater than that needed to sufficiently block intestinal glucose absorption in vivo in the rat (21). Food intake (g) was measured at 0.5, 1, 2, and 24 h postinfusion.

Following the behavioral study, an additional group of 10 intact rabbits was assessed in an acute study for urine and blood glucose effects produced by the infusion of phloridzin. Nonfasted rabbits were anesthetized with pentobarbital (Nembutal) and prepared with cannulae entering the jugular and hepatic-portal veins. The bladder was ligated to prevent urination. Phloridzin was then infused into either the liver via the hepatic-portal cannula or the duodenum by enterocentesis at the same rate and concentration as used in the behavioral studies. Blood samples from the jugular vein and urine samples were collected from all rabbits prior to administration of phloridzin and at 10, 20, 30, 60, 90, and 120 min following the drug. In addition, eight rabbits were treated in the same manner but received either saline infusion or no drug administration and were sampled for fluids at the same time intervals.

RESULTS

In the behavioral study, food intake was dramatically increased in the first 0.5 h following administration of phloridzin into either the duodenum or the hepatic-portal system (see Fig. 1, top and middle panels). Ingestion was similarly affected during the dark and light cycles. Half-hour food intake was 83/94% (dark-light) greater for rabbits given duodenal phloridzin and 144/117% (dark-light) greater for those receiving the drug via hepatic-portal cannulae, compared to saline infusions via the same routes (p's < 0.05). Further statistical analyses revealed that the increase in the first 0.5 h of food intake following duodenal phloridzin infusion was not significantly compensated during the subsequent 1.5 h, during which time food intake did not differ from control levels. Although the initial 0.5 h food intake following hepatic-portal phloridzin was significantly elevated, food intake across the subsequent 1.5 h was depressed (p's < 0.05). Infusion of phloridzin via the jugular vein had no significant effects on short-term feeding (p's > 0.10). Food intake measured 24 h after either duodenal, hepatic-portal, or jugular infusion of phloridzin did not statistically differ from that in the saline control conditions.

In the acute study, as expected (1,20,21,27), administration of phloridzin via the hepatic route produced glycosuria at all postinfusion measurement periods (p's < 0.05). However, duodenal infusions produced no glycosuria, indicating little, if any, transfer of phloridzin from the lumen of the intestine into the blood stream reaching the renal tubules (23,40). The two routes of administration also demonstrated substantial differences in their effects upon glycemia. Duodenal administration of the glycoside produced a significant, prolonged increase in blood glucose concentration throughout the 2-h sampling period (p's < 0.05). This glycemic rise is most likely due to glycogenolysis occurring in response to phloridzin-induced inhibition of glucose transport across the intestinal epithelium. Hepatic-portal phloridzin produced a fall in blood glucose reaching a nadir at 20 min postinfusion ($p < 0.05$), rising to baseline at 1 h postinfusion, and then increasing to 114 and 129% of baseline at 1.5 and 2 h, respectively, following infusion.

EXPERIMENT 2: PHLORIDZIN INFUSION INTO SUBDIAPHRAGMATICALLY VAGOTOMIZED RABBITS

METHOD

A follow-up study was conducted to determine whether or not the vagus was involved in producing the hyperphagic response to inhibition of glucose transport in peripheral tissue. Seven rabbits were subdiaphragmatically vagotomized as previously described (15,31). After recovery, vagotomized rabbits received phloridzin into the liver through the hepatic-portal vein, using the same procedure as described above for intact rabbits.

RESULTS

Vagotomized rabbits did not show the hyperphagic response to hepatic-portal infusion of phloridzin as reported in intact rabbits (see Fig. 1, bottom panel). Results did not statistically differ between the dark and light cycles, and data

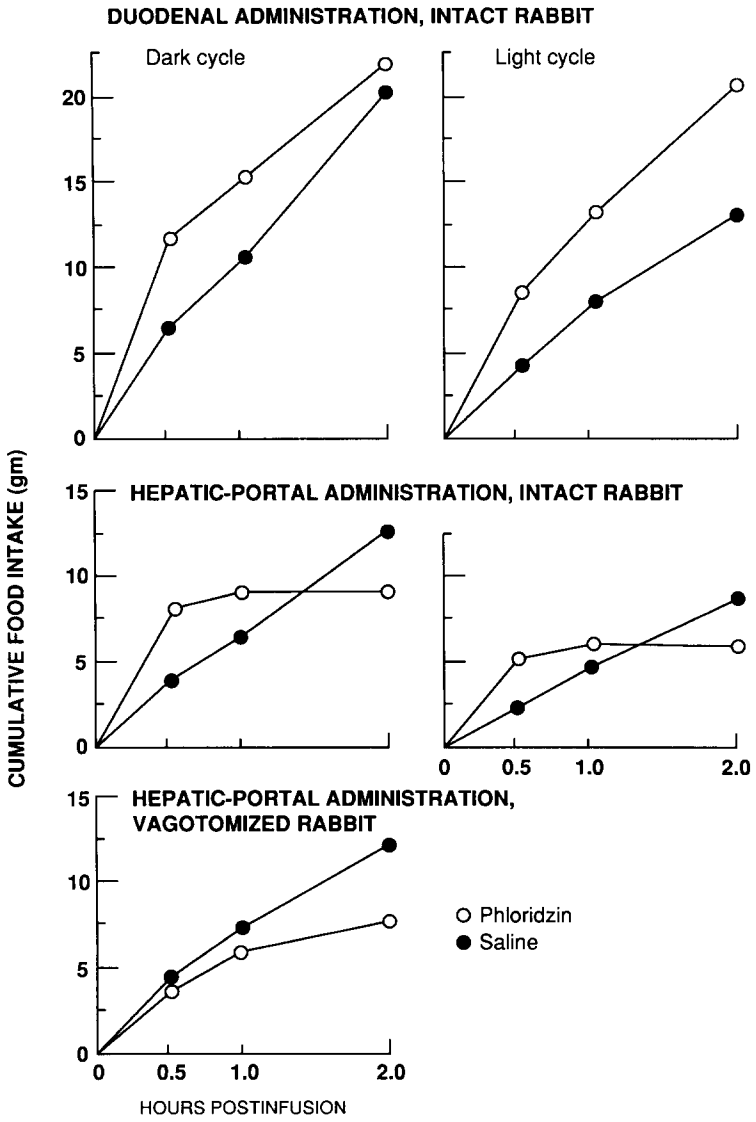

FIG. 1. Mean cumulative food intake in rabbits at 0.5, 1, and 2 h following administration of phloridzin (10 ml of 2.5×10^{-3} M solution). Phloridzin blocks glucose absorption across various tissues and initiates feeding; see text for interpretations. Food intake was significantly increased by duodenal or hepatic-portal infusion of phloridzin compared to saline controls within the first 0.5 h postinfusion in intact rabbits during both the dark and light cycles (top and middle panels). Food intake across the subsequent 1.5 h was significantly depressed following hepatic-portal phloridzin infusion but did not differ from control levels following duodenal infusion of phloridzin in intact rabbits. Mean cumulative food intake in vagotomized rabbits following hepatic-portal phloridzin infusion during either the dark or light cycle did not differ significantly from control values (bottom panel; dark cycle only reported).

for the dark cycle only are reported here. Hepatic-portal infusion of phloridzin did not significantly affect food intake measured at any time postinfusion in vagotomized rabbits.

GENERAL DISCUSSION

The first experiment demonstrated that phloridzin infusion into either the liver via the hepatic-portal vein or the duodenum of intact rabbits produced a significant increase in food intake during the first 0.5 h postinfusion. However, phloridzin infusion into the general circulation of intact rabbits via the jugular vein did not affect feeding. These latter results suggest that the feeding effects of phloridzin infusion into the duodenum or liver were specific to those peripheral loci and not merely general systemic effects. Vagotomized rabbits also did not show the hyperphagic effect of phloridzin, thus indicating

that the phloridzin effects obtained in intact rabbits were vagally mediated. These data emphasize the involvement of peripheral, vagally innervated structures in the control of short-term food intake.

Considered together, data obtained in the behavioral and blood-sampling studies of intact rabbits receiving hepatic-portal infusion of phloridzin indicate that postinfusion food intake covaries inversely with glycemic levels. That is, when glycemic levels were falling below baseline, rabbits showed a significant increase in food intake. Whereas when glycemic levels were rising, food intake was decreased. It has been reported that just a small, transient decline in blood glucose levels is sufficient to initiate a meal in lean, obese, and in untreated diabetic rats; and these data have been interpreted as suggesting that the pattern of blood glucose dynamics is important in the control of food intake (7,25,38). Additional data in rabbits suggested that the rate of decline in blood glucose levels is associated with an increase in food intake (14). Normally, one would expect decreased blood glucose levels to be associated with reduced glucose availability and utilization at some as yet unidentified receptor(s) in the body, perhaps the hepatocytes, which we believe provide the more critical signals to eat. Decreasing glucose transport may be part of a normal hunger sequence, and the basic underlying physiological mechanism(s) that ultimately control hunger may well be among the metabolic and/or hormonal sequelae that one would expect to follow a decrease in blood glucose levels (12,16).

Data obtained following duodenal infusion of phloridzin into intact rabbits did not show a systematic relationship between blood glucose levels and food intake. Glycemic measurements following phloridzin administration to the duodenum and the hepatic-portal system were in opposite directions during the first 0.5 h postinfusion, yet both of these manipulations produced an increase in food intake. At 1 and 2 h postinfusion, blood glucose levels were clearly elevated in both groups, especially the duodenal group, but the hepatic-portal group showed a suppression of food intake and the duodenal group did not. We interpret these data as indicating that, although the pattern of blood glucose dynamics can be important, glycemic level per se is not always correlated with or a determinant of food ingestion.

The present studies indicate that a) glucose transport in peripheral tissue is an important variable affecting feeding and b) an intact vagus nerve is involved in the feeding response produced by the peripheral reduction of glucose uptake in the rabbit. In addition, data collected 1 and 2 h following duodenal infusion of phloridzin suggest that normal eating can occur when the brain presumably has increased glucose availability. Although our studies do not argue against CNS feeding signals, we argue that peripheral, probably hepatic, signals are integrally involved and perhaps show primacy.

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