should the possibility that nighttime death in some such patients could be related to sleep apnea.

One obvious and important conclusion can be drawn from our data. An unknown percentage of the larger number of patients complaining of chronic insomnia have profound disorders of respiratory control mechanisms (*6*). There is probably a functional association between the sleep disturbance giving rise to the complaint and the apnea. Our patients have not only nocturnally disrupted sleep, but also long periods of conscious arousals (Fig. 2). Yet, until now, their respiratory problem has been completely occult. We feel that respiratory function during sleep should be evaluated in patients who complain of chronic insomnia characterized by several conscious arousals throughout the night and early morning and who also have a short latency before onset of sleep and a history of heavy snoring.

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6. All sleep studies at the Stanford Sleep Disorders Clinic now include measurement of respiration. The three patients with sleep apnea mentioned in the text were found among an unselected group of the last 30 insomniac patients who underwent all-night sleep recordings.
7. Supported by NIH grants NS 10727 and NS 09390 and by research scientist development award MH 05804. We thank L. Hassler for technical assistance.

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Infusion of 2-Deoxy-D-Glucose into the Hepatic-Portal System Causes Eating: Evidence for Peripheral Glucoreceptors

Abstract. *Injections of 2-deoxyglucose into the hepatic-portal system of normal rabbits increased eating to a greater extent and with shorter latency than comparable injections of 2-deoxyglucose into the jugular vein or into the hepatic-portal circulation of the vagotomized rabbit. These differences suggest the existence of vagally mediated peripheral glucoreceptors important in the initiation of food intake.*

The glucostatic theory is a leading hypothesis for the explanation of the short-term control of hunger. One corollary of this theory is that central glucoreceptors signal satiety when glucose utilization is elevated (*1*). Experimental verification of the central loci of glucose-sensitive satiety neurons, however, has not been solidly established (*2*). The existence of peripheral glucose-sensitive areas has also been suggested by Russek and his colleagues (*3*) who have observed that reversible block of the vagii produces a short period of aphagia and that vagal transsection results in a temporary but longer-lasting hypophagia. Niijima (*4*) using an isolated liver preparation has shown that glucose-sensitive fibers are present in the vagus nerve. Hepatic glucoreceptors may, therefore, be important in the control of hunger.

Our experiments represent an attempt to demonstrate peripheral mechanisms that increase food consumption as a result of infusion of 2-deoxy-D-glucose (2DG) in the hepatic-portal system. Glucose utilization at the phosphohexoseisomerase level is blocked by systemic injections of 2DG, and this, in the intact animal, promotes hyperglycemia and deposition of glycogen in the liver (*5*). Cells should, therefore, become deprived of glucose after infusions of 2DG and increased eating should occur according to the glucostatic theory. Indeed, many investigators have shown that increased eating occurs in response to the administration of 2DG; however, all have attributed the effect to central "glucoprivation," although the possibility of peripheral synergism was not ruled out (*6–8*). If there are glucoreceptors in the liver which act on hunger, 2DG, by blocking glucose utilization of the cells in the capillary bed of the hepatic-portal system, should have an early and potent effect on food intake.

The initial experiment was performed with eight female (New Zealand) rabbits with cannulas permanently implanted into one of the larger collecting veins draining the duodenum and threaded as far downstream as possible. This procedure ensured that materials delivered to the cannula perfuse the liver first through the hepatic-portal system. The other end of the cannula was fixed by dental cement to the animal's skull, and the tubing was kept beneath the skin. The subjects were allowed at least 1 week for recovery by which time all animals were eating normally. At this time, infusions of 2DG and equiosmotic saline in counterbalanced order were begun. No infusions of 2DG were made within 4 days of each other because 2DG produced altered food intakes for the next 1 or 2 days. When infusions of 2DG or saline were not made, the cannulas were flushed with saline solutions containing heparin. Infusions were maintained at 1 ml/min with a Harvard Apparatus infusion pump attached to a long section of tubing ending in a needle which was inserted into the intravenous cannula. This allowed injections to be made painlessly in the unrestrained rabbit. The animals were given free access to food and were injected during the morning hours between 9:00 and 11:00 a.m. After the injection, hourly food intakes were recorded for the next 3 hours, and the latency between the beginning of injection and the initiation of feeding was measured. The definition of the initiation of a meal was the interruption for 1 minute of the beam of a photo cell mounted on the entrance to the feeding box. This was associated with at least 0.5 g of food intake. Also, daily food intakes were always recorded.

The infusion of a 7.5 percent (weight to volume) solution of 2DG (250 mg per kilogram of weight) caused eating in all eight animals within a range of 6 to 14 minutes after the beginning of

the injection. The mean latency was 9.2 ± 0.56 minutes between the initiation of the injection and eating. In all, 21 infusions of 2DG were given, and, in 18 of these cases, the animals were eating before the end of the infusion. In all instances, the injection was continued until the subject had received the full dose. The average volume injected was 10 ml. In 21 infusions, in the same eight subjects, with an equiosmotic solution of saline [1.28 percent (weight to volume)], only one infusion resulted in eating while the solution was being infused, and the mean latency until eating was 64 minutes. Further, the amounts of food ingested after administration of 2DG were significantly greater than after administration of saline. The mean hourly food consumption for the 3 hours after 2DG was 10.5 g/hour, as compared to only 3.5 g/hour for the equiosmotic saline solution ($P < .01$).

A second experiment was carried out in six animals with cannulas inserted in the jugular vein to test the effects of a more general systemic infusion of 2DG. The subjects were injected exactly as the animals with the hepatic-portal cannula, and the same measurements were made. Animals infused with 2DG in the jugular vein showed a mean latency of 70 minutes between the beginning of the injection and the initiation of feeding. In all, 16 infusions were made in the six animals and only 4 of these infusions were accompanied by eating during the injection. The mean hourly food intake for the 3 hours after 2DG infusions into the jugular vein was 6.9 g/hour, and this amount was significantly greater than the 3.4 g/hour consumed after infusions of equiosmotic saline in the jugular vein ($P < .05$). The jugular vein infusions, however, were accompanied by increases in food consumption largely during the second and third hour after injection of 2DG.

A third experiment involved the infusion of 2DG in the hepatic-portal system of rabbits that had been bilaterally vagotomized subdiaphragmatically. Vagotomizing the rabbit is not without complications, and a 30 percent mortality was found, probably because of extreme hypophagia after the operation. Vagotomy is also known to affect stomach emptying (*9*) and, for these reasons, three animals were maintained on a liquid diet (Slenderway, composed of 1.35 percent fat, 5.2 percent protein, and 11.6 percent carbohydrate) and three on a standard rabbit-pellet food

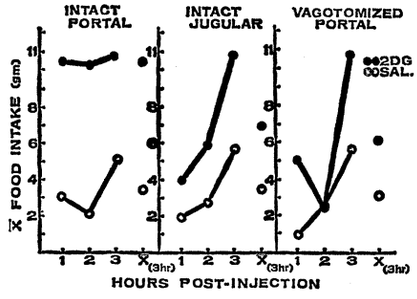

Fig. 1. Hourly food ingestion after infusion of a 7.5 percent (weight to volume) or 1.28 percent saline solution in the rabbit. Data points on the right of each portion of the figure represent the group-mean hourly intake over the 3-hour measurement period. The number of subjects and infusions and critical significance levels are given in the text.

(2.0 percent fat, 16.0 percent protein, and 60.5 percent carbohydrate). In all, six animals were successfully vagotomized and eventually showed stable food intakes. Vagotomy was verified by measuring stomach contractions after cervical vagus stimulation. In that the vagii were cut proximal and liver afferents enter distal to the stomach, the lack of effect of vagal stimulation on stomach contractions must mean the liver was also deprived of vagal innervation. Those animals on solid diets were deficient in stomach emptying and had, when killed after 24 hours of food deprivation, greater volumes of food in the stomach than did nonvagotomized animals. In contrast, the vagotomized rabbits showed very little difference in the volume of stomach contents in comparison to intact rabbits when the diet was liquid food. As the results from the animals on solid and liquid food were much the same in terms of latency until eating and the amount ingested, the data of the two were combined as follows. Liquid food volumes were divided by 3.5 to convert them to the protein value of the solid food. Animals matched this 3.5 conversion factor closely in that the intake of food to which they had free access was roughly 3.5 times as much liquid food as solid. The effect of infusions of 2DG into the hepatic-portal system of the vagotomized animals was to produce a significant increase in food intake over the control saline injection. The mean hourly intake following 2DG infusion over the 3 hours was 6.1 g/hour while that following saline infusion was 3.1 g/hour ($P < .05$). The difference in the latency to eat, however, was not significantly different from that with control saline injections.

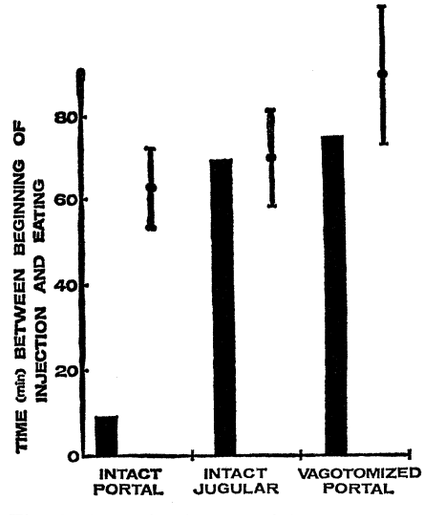

Fig. 2. Latencies between initiation of infusion of 2DG and beginning of eating are given by the bar graph. Data points represent latencies and standard error of the means when equiosmotic saline infusions were made. The standard error of the mean is not plotted for the 2DG infusions, because in the intact, portally infused subjects, it is too small to appear—less than 1 minute. Critical significance levels appear in the text.

Figure 1 shows the patterns of food intake over the 3-hour observation period following infusions of 2DG or control saline in the three types of preparations that were used. Vagotomized animals, injected via the hepatic-portal system with 2DG, showed a decrement in food intake in the second hour and a marked increase in food intake in the third hour after the infusion. We have no explanation, at present, for this decrement; however, we believe that it is not a chance occurrence because the pattern appears regardless of whether the rabbit is on solid or liquid food. Animals perfused in the jugular vein showed an increasing amount of food intake over the 3-hour period after injection. Intact animals with hepatic-portal infusions showed constant, increased amounts of food intake over the 3-hour period after infusion.

The most interesting observations are the comparisons between the three types of preparations with respect to the amount of food intake and the latencies to eating following 2DG infusions. On both measures, the jugular vein infusions and the hepatic-portal infusions in the vagotomized rabbits are not significantly different. In contrast, the mean hourly intake after 2DG infusions into the hepatic-portal circulation of the intact rabbit is significantly greater than either of the other two preparations

($P < .01$). The mean latency to eat following 2DG is also significantly shorter in the intact hepatic portally infused rabbit than in the other two preparations ($P < .01$ in both comparisons). The latencies are shown in Fig. 2.

The increments in food consumption after 2DG injections into the jugular vein or into the hepatic-portal system of the vagotomized rabbit correspond with those reported by others. Houpt and Hance (*8*) observed a 90 percent increase and Smith and Epstein (*6*) observed a 104 percent increase in eating in animals not deprived and administered 2DG peripherally. Also, Balagura and Kanner reported an increase of only 79 percent after administration of 2DG directly into the lateral hypothalamus of the rat (*2*). Our observations showed 2DG producing an increase of 103 or 106 percent, respectively, in the vagotomized rabbit with administration into the portal system and after administration into the jugular vein in the intact rabbit. In contrast, the mean increase in food intake that follows 2DG in the intact, portally infused subject was 209 percent. In addition, the latencies we observed are with one exception the shortest that have been reported (*10*). Thus, the results of our experiments suggest the existence of hepatic glucose utilization receptors that are innervated by the vagus nerves and that are important in the initiation of feeding. While the hyperglycemia that is known to result from 2DG injections might eventually cause increased insulin secretion, the latency to eat after insulin administration is quite lengthy (*6*). Although we have not yet looked at the changes in glucose or hormone concentrations after 2DG administration, the short latency feeding response observed suggests that these are probably not involved.

Nothing in our experiments argues against the existence of central glucoreceptors. Moreover, the fact that 2DG in the vagotomized animal still increased food intake supports the notion of extrahepatic receptors or at least receptors not innervated by the vagus nerves. However, our results suggest that there are, in addition, peripheral receptors which in response to decreased glucose utilization promote a state of hunger followed by immediate food ingestion.

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Glutamate Uptake by the Isolated Toad Brain

Abstract. *The isolated toad brain accumulates* L-*glutamate against strong concentration gradients until a tissue-to-medium concentration ratio of about 3000 : 1 is attained. The accumulated glutamate does not equilibrate with most of the endogenous tissue glutamate but is converted rapidly to glutamine and released into the medium. This mechanism may be involved in the preservation of low extracellular levels of cerebral glutamate.*

L-Glutamate, at concentrations of 2 to 20 m*M*, is one of the most abundant amino acids in tissues of the nervous system (*1*). Considerable evidence suggests that glutamate may be an excitatory neurotransmitter (*2–4*). An essential requirement of any synaptic neurotransmitter is that it must be rapidly inactivated subsequent to its release, by destruction or removal from the synaptic cleft (*5*). Removal of a transmitter from the synaptic cleft may be mediated by re-uptake into the presynaptic terminal, uptake into the postsynaptic cell, or uptake into surrounding glial tissue. It is reasonable to assume that a substance should be maintained at very low levels in the extracellular fluid if it is to function efficiently as a transmitter.

The present study shows that the isolated toad brain has the capacity to remove glutamate rapidly from the extracellular environment against enormous concentration gradients. This capacity exceeds that shown for mammalian brain slices (*6*) and glial cell preparations (*7*).

Toads (*Bufo boreas*) weighing 25 to 50 g were anesthetized by chilling them in crushed ice for 20 minutes. The brain of each toad was exposed by removing the upper portion of the cranium; the spinal cord was ligated and the brain was excised. The dura was removed and the brain was blotted and transferred to a tared weighing bottle containing an oxygenated, ice-cold Ringer-like medium composed of NaCl (100 m*M*), $NaHCO_3$ (25 m*M*), KCl (2 m*M*), KH_2PO_4 (3 m*M*), $MgCl_2$ (1 m*M*), Na_2SO_4 (1 m*M*), $CaCl_2$ (1 m*M*), glucose (10 m*M*), and urea (10 m*M*). The final *p*H of the medium was 7.3. The brain was weighed and transferred to a test tube containing 2 ml of the medium to which L-glutamate (0.04 m*M* or 0.10 m*M*) and L-[U-^{14}C]glutamate (0.02 μc, New England Nuclear Corp., 100 mc/mmole) were added previously. The brain weights ranged from 55 to 85 mg with a mean weight of 69.7 mg. The brains were incubated at 25°C for 10 to 120 minutes. During incubation, a mixture of 95 percent O_2, 5 percent CO_2 was bubbled into the medium to oxygenate and mix the system. After incubation each brain was rinsed twice with cold medium (containing no glutamate), blotted, weighed, and homogenized in 1 ml of cold 3 percent perchloric acid, and the tissue extract was separated by centrifugation. The content and specific radioactivity of glutamate and glutamine in the tissue extract and the incubation medium were measured by methods described elsewhere (*8*).