Glucoreceptors Controlling Feeding and Blood Glucose: Location in the Hindbrain

Abstract. *Microinfusion of 5-thioglucose into either the lateral or fourth cerebral ventricle caused increased feeding and hyperglycemia in rats when the cerebral aqueduct was unobstructed. If the aqueduct was obstructed and 5-thioglucose was infused into the fourth ventricle, increased feeding and hyperglycemia persisted, whereas feeding and hyperglycemia in response to lateral ventricle infusion were abolished. Drinking in response to infusion of angiotensin II into the lateral ventricle was not diminished by aqueduct obstruction. These results indicate that glucoreceptors that mediate feeding and hyperglycemia in response to cerebral glucoprivation are located in the caudal hindbrain and not in the hypothalamus where they have previously been sought.*

Parenteral injections of glucose analogs that inhibit cellular glucose utilization cause increased food intake in most mammals, including humans (*1, 2*). Smith and Epstein (*1*) therefore proposed the existence of a glucoprivic control of food intake. According to their hypothesis, glucoreceptors located in the brain activate feeding behavior when their glucose utilization is diminished. Miselis and Epstein (*3*) subsequently showed that intracerebroventricular (ICV) injection of the glucose analog 2-deoxy-D-glucose (2DG) caused increased feeding as well as a sympathoadrenally mediated hyperglycemia (*3*). Their findings and those of others (*4*) suggested that at least some glucoreceptors that mediate feeding and sympathoadrenal discharge reside within the brain. The precise locations of the glucoreceptors for these two phenomena are not known. The traditional assumption has been that the glucoreceptors were somewhere in the hypothalamus (*5*). Although neurons that change their firing rate in response to glucoprivation can be found at several hypothalamic loci, attempts to elicit feeding with intrahypothalamic 2DG injections have failed (*3, 5*). In addition, the claim for localization of hypothalamic glucoreceptors that mediate sympathoadrenal hyperglycemia (*6*) is questionable on technical grounds (*7*). Thus, although the hypothalamus and other forebrain structures may possess glucoreceptors, there is no good evidence that they mediate either feeding or sympathoadrenal hyperglycemia in response to glucoprivation.

The absence of compelling evidence for hypothalamic glucoreceptors mediating feeding and hyperglycemia led us to hypothesize that the brain glucoreceptors that mediate these responses to glucoprivation do not reside in the hypothalamus. Furthermore, the report of DiRocco and Grill (*8*) showing that decerebrate rats may become hyperglycemic in response to systemically injected 2DG reinforced our suspicions that at least some of the receptors that respond to glucoprivation are in the hindbrain. Therefore, we undertook experiments to determine whether feeding and hyperglycemia elicited by ICV infusions of an antimetabolic glucose analog, 5-thioglucose (5TG) (*9*), are mediated by receptors in the hindbrain as opposed to the forebrain. Specifically, we examined the feeding and hyperglycemic responses to 5TG infused by way of either the lateral or fourth cerebral ventricle both before and after occlusion of the cerebral aqueduct.

Each of 36 adult male rats was anesthetized with Metofane. Two stainless steel guide cannulae were stereotaxically implanted in each rat, the first cannula being aimed toward the cerebral aqueduct. In half of the rats, the second cannula was aimed toward the right lateral ventricle and in the other half the second cannula was aimed toward the fourth ventricle. The tips of all guide cannulae resided dorsal to the respective ventricular roofs. The ventricles were entered during experiments by inserting sharp beveled injectors that extended beyond the guide cannulae tips.

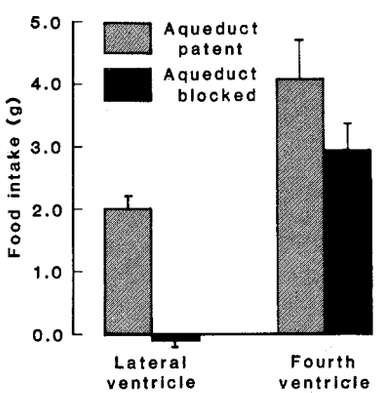

Fig. 1. Increase in food intake in response to infusion of 5TG into the lateral or fourth ventricle before and after obstruction of the cerebral aqueduct. The values reported represent the mean amounts eaten above any intake which occurred after control infusion. For rats with lateral ventricle cannulae, control intake was 0.6 ± 0.2 g before aqueduct obstruction and 0.5 ± 0.3 after obstruction. Rats with fourth ventricle cannulae had control intakes of 0.2 ± 0.2 g before aqueduct obstruction and 0.7 ± 0.3 g after obstruction. There were no significant differences between control intakes before and after obstruction. Infusion of 5TG into the fourth ventricle elicited significantly more food intake than did infusion into the lateral ventricle when the aqueduct was open ($P < .05$). After aqueduct obstruction the increased feeding in response to infusion into the lateral ventricle was virtually abolished whereas feeding in response to infusion into the fourth ventricle was not significantly diminished ($P > .1$).

The rats were given free access to food and were tested when they were satiated. Injections of 5TG were made into the lateral or fourth ventricle by means of a microsyringe connected to the appropriate injector via polyethylene 10 tubing. When we wanted to obstruct the cerebral aqueduct irreversibly, we inserted a sharpened injector into the aqueduct cannula and injected 10 μl of charcoal-pigmented silicone grease (Dow Corning) while the rat was lightly anesthetized with Metofane. After all experiments were completed, each rat was injected with 5 μl of red ink by way of the lateral or fourth ventricular cannulae. The rats were then deeply anesthetized and the brain fixed by Formalin perfusion. Subsequently, the brains were bisected midsaggitally and examined under the dissecting microscope to verify patency of the ventricular cannulae, the position of the aqueduct obstruction, and the extent of ink diffusion.

After the rats had recovered from surgery, they were first tested for feeding and hyperglycemic responses to 90 μg of 5TG in 3 μl of 0.9 percent NaCl (*10*) infused via the lateral or fourth ventricle cannulae. After ICV infusion, the rats were returned to their home cages in the absence of food. Blood samples (50 μl) for glucose determination were taken from the tip of the tail at 30, 15, and 5 minutes prior to and at 0, 15, 30, 60, 120, and 180 minutes after infusion. After the last blood sample was obtained, food was returned and food intake was monitored for 2 hours. All rats in which ink injection demonstrated successful ventricular infusion displayed significantly greater increases in blood glucose concentrations after they had received 5TG than after they had received the NaCl control infusion. Furthermore, all rats with successful placements ate significantly more food after 5TG infusion than after NaCl. Rats that were lightly anesthetized prior to receiving NaCl or 5TG infusions were used as controls for the anesthetization necessary for aqueduct obstruction. Since the levels of hyperglycemia and food intake in these rats were

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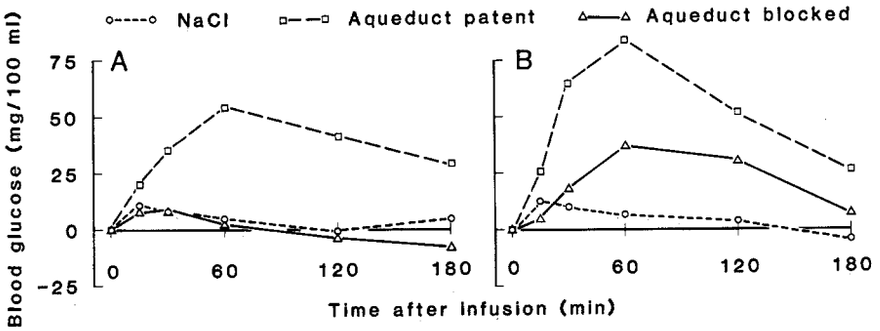

Fig. 2. Increase in blood glucose concentrations elicited by infusion of 5TG into the lateral (A) or fourth ventricle (B) before and after aqueduct obstruction. Values represent the mean increase in blood glucose over preinfusion concentrations. In rats with lateral ventricle cannulae, resting glucose concentrations were 81.3 ± 1.7 mg/100 ml before aqueduct obstruction and 76.0 ± 3.5 mg/100 ml after obstruction. Rats with fourth ventricle cannulae had resting concentrations of 84.0 ± 1.2 mg/100 ml before aqueduct obstruction and 89.3 ± 2.4 mg/100 ml after obstruction. Aqueduct obstruction abolished hyperglycemia in response to infusion of 5TG into the lateral ventricle ($P > .5$), and diminished but did not abolish ($P < .05$) hyperglycemia in response to infusion into the fourth ventricle.

no different from nonanesthetized rats, we combined the results for these two control groups.

After testing the feeding and hyperglycemic responses to ICV 5TG with the mesencephalic aqueduct open, we repeated the experiments in the same rats with the aqueduct obstructed. Rats always appeared fully recovered from the obstruction procedure within 10 minutes and were clinically indistinguishable from rats that did not receive anesthesia or aqueduct obstruction. 5-Thioglucose was infused into the lateral or fourth ventricle 30 minutes later at time zero and measurements were made as described above. Figure 1 shows the effect of aqueduct obstruction on feeding elicited by 5TG infusion into either the lateral or fourth cerebral ventricle. Rats that received 5TG infusions into the fourth ventricle ($N = 10$) prior to aqueduct obstruction ate 4.1 ± 0.6 g more than they ate after control infusion ($P < .001$). This amount was significantly larger ($P < .05$) than that consumed by rats infused via the lateral ventricle (2.0 ± 0.2 g) ($N = 12$). This result may indicate that 5TG infused into the fourth ventricle was placed closer to the putative glucoreceptors than that which was infused into the lateral ventricle. Obstruction of the cerebral aqueduct totally abolished feeding (−0.05 ± 0.3 g) elicited by lateral ventricle infusion of 5TG. However, rats given 5TG via the fourth ventricle after aqueduct obstruction still ate 2.9 ± 0.4 g. This amount was significantly more than they ate after control infusion ($P < .001$) but did not differ ($P > .1$) from the intake elicited by infusion of 5TG into the fourth ventricle before aqueduct obstruction. The single rat that ate in response to 5TG infused into the lateral ventricle after aqueduct obstruction was subsequently found to have a misplaced silicone plug that allowed ink and presumably 5TG to flow into the fourth ventricle. We interpret these results to mean that the brain glucoreceptors that mediate feeding in response to cerebral glucoprivation are in the hindbrain.

Figure 2 shows the hyperglycemic response to infusion of 5TG into the lateral or fourth ventricle before and after aqueduct obstruction. Peak blood glucose concentrations were achieved at 60 minutes when the 5TG was infused into either the fourth (84.2 ± 15.0 mg/100 ml) or lateral (53.2 ± 10.8 mg/100 ml) ventricles with the aqueducts open. However, infusion into the fourth ventricle appeared to produce a more rapid increase in blood glucose than did infusion into the lateral ventricle. Furthermore, infusion into the fourth ventricle resulted in significantly higher peak blood glucose values ($P < .05$) than did lateral ventricle infusion. When the mesencephalic aqueduct was obstructed, the hyperglycemic response to lateral ventricle 5TG infusion was abolished. Aqueduct obstruction did not abolish the response to fourth ventricle 5TG infusion, although the peak increase in blood glucose (37.8 ± 10.5 mg/100 ml) was significantly ($P < .05$) reduced.

We believe these data indicate that the receptors that mediate the hyperglycemic response to glucoprivation are located caudal to the forebrain. We do not know why the hyperglycemic response to fourth ventricular 5TG was diminished after aqueduct obstruction, but there are two possible explanations. First, the silicone plug that usually extended into the cranial portion of the fourth ventricle may have covered some of the glucoreceptors that mediate the hyperglycemic response thereby interfering with their function. Second, the forebrain ventricles, while perhaps not containing receptors that mediate sympathoadrenal hyperglycemia, may contain receptors that mediate a permissive or potentiating neuroendocrine response. Glucoprivation increases adrenocorticotropic hormone and growth hormone secretion (*11*), both of which could potentiate the hyperglycemic effect of sympathoadrenal discharge. It is possible that receptors that mediate these pituitary responses reside in the forebrain and are, therefore, not stimulated when 5TG is infused into the fourth ventricle after aqueduct obstruction.

The fact that aqueduct obstruction caused rats with infusion of 5TG into the fourth ventricle to increase their food intake suggests that failure to respond to lateral ventricle infusion was not due to nonspecific behavioral suppression. Nevertheless, in order to test the ability of rats with aqueduct obstructions to respond to a behavioral stimulus that is mediated by forebrain receptors (*12*), we examined the drinking behavior of rats given infusions of angiotensin II (AII) (15 ng in 3 μl of 0.9 percent NaCl) into the lateral ventricle prior to and after aqueduct obstruction.

The design of the experiments was the same as that described for feeding in response to 5TG, except that drinking in response to AII was tested in the absence of food. Infusion of AII into the lateral ventricle caused significant increases in drinking (5.2 ± 1.4 ml above control) that were not diminished after aqueduct obstruction (6.8 ± 2.1 ml above control). These results show that rats with aqueduct obstruction can respond behaviorally to a stimulus that depends on receptors in the forebrain. In contrast to the drinking response to infusions of AII in the lateral ventricle, infusions of AII into the fourth ventricle failed to elicit drinking even prior to aqueduct obstruction. This observation is in accordance with that of Hoffman and Phillips (*13*), who were also unable to elicit drinking with fourth ventricle AII infusions. Since the receptive areas for AII, the subfornical organ and the anterior-ventral third ventricle are forebrain structures (*12*), we did not test rats for drinking elicited by infusions of AII into the fourth ventricle after aqueduct obstruction.

The results of our experiments do not rule out the participation of nonbrain glucoreceptors in feeding or hyperglycemia (*14*). Nor does our work disprove the existence or reduce the potential importance of putative hypothalamic glucore-

ceptors. However, our results clearly show that hypothalamic glucoreceptors are not responsible for feeding and hyperglycemia elicited by brain glucoprivation. The cerebral receptors that mediate glucoprivic feeding and sympathoadrenal discharge are located in the hindbrain.

ROBERT C. RITTER
PETER G. SLUSSER
STEVEN STONE

Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman 99163, and *WOI Regional Program in Veterinary Medicine, University of Idaho, Moscow 83843*

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7. The current belief that the glucoreceptors that mediate sympathoadrenal discharge are in the hypothalamus stems mainly from Himsworth (*6*). In his experiments, injections (3 μl) of local anesthetic (lidocaine) were made bilaterally into the lateral hypothalamus of pentobarbital-anesthetized rats. Lidocaine-injected rats displayed a smaller increase in blood glucose than did controls in response to intraperitoneal injection of 3-*O*-methyl glucose. Himsworth concluded that he had anesthetized the hypothalamic glucoreceptors. However, it is also possible that he simply anesthetized neurons required in the mobilization of the effector response. Furthermore, there is no assurance that the local anesthetic used did not diffuse, via the ventricles, to exert its effect in other brain regions. Finally, considering that the injection volume was 3 μl on each side, the lidocaine would almost certainly have diffused into the ventromedial area. Anesthetization of ventromedial region is known to release insulin and such an effect could mask the sympathoadrenal activation by enhancing removal of mobilized glucose from the blood.
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9. 5-Thioglucose is an analog of glucose in which sulfur is substituted for the pyranose ring oxygen [M. Chen and R. L. Whistler, *Arch. Biochem.* **169**, 392 (1975)]. Recently we demonstrated that 5TG causes dose-dependent increases in feeding and sympathoadrenal hyperglycemia when administered systemically or by ICV cannulae. We also found that 5TG elicits feeding at 2.5 percent of the molar dose required of 2DG when infused by ICV cannulae [R. C. Ritter and P. G. Slusser, *Am. J. Physiol.* **238**, E141 (1980); P. G. Slusser and R. C. Ritter, *Brain Res.* **202**, 474 (1980)].
10. The concentration of 5TG in the infusate was only 154 m*M*. The 5TG with the 0.9 percent NaCl solution was, therefore, about one and one-half times the osmotic concentration of cerebrospinal fluid. In other experiments, we have infused nonglucoprivic osmotically active substances (0.25*M* fructose or 0.125 to 1.20*M* glucose in 0.9 percent NaCl) into the lateral or fourth ventricles. Such infusions have consistently failed to elicit increased feeding. Furthermore, Miselis and Epstein (*3*) have obtained feeding in response to lateral ventricle infusions of 3.0*M* 2DG. They also found that ICV infusion of equiosmotic glucose, sucrose, or urea failed to elicit feeding. Thus, it seems clear that the feeding elicited by intracranial administration of glucose analogs is a specific effect and is not dependent on osmotic properties of the infusate.
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Evidence for Extensive Overlap of Sporophytic and Gametophytic Gene Expression in *Lycopersicon esculentum*

Abstract. *Male gametophyte (pollen) isozyme profiles were compared with those of the sporophyte for nine enzyme systems. Sixty percent of the structural genes coding for these enzymes in the sporophyte were also found to be expressed by the gametophyte. All the genes tested were found to be expressed after meiosis, apparently transcribed and translated in the haploid gametophytes.*

Compared to the sporophyte, the gametophytes of higher plants have been the subject of relatively little genetic and physiological research. This is especially true of genetic research. A basic question about gametophyte-sporophyte relations in higher plants concerns the extent to which the same genes are active in the two phases. Interest in this question has been stimulated by a recent proposal (*1*) that selection among haploid male gametophytes (pollen grains) might have a positive, correlated effect on the sporophytic generation resulting from selection of genes expressed in both stages. With such a scheme, higher plants, particularly angiosperms, would enjoy a unique mode of evolution in which adaptive advances could be made at minimal cost by selection at the haploid gametophytic stage (*1*). Furthermore, the plant breeder could select with great effectiveness in the haploid generation. The hypothesis is supported by experiments that demonstrate correlations in specific fitness parameters between sporophytic and gametophytic generations (*2*).

A fundamental requirement of this selection model is that a portion of the genes expressed by the sporophyte also be expressed by the haploid pollen genome. Previous studies have revealed gametophytic control of such characters as pollen composition and dimension (*3*), self-incompatibility of the gametophytic type (*4*), pollen tube growth rate (*5*), selective fertilization (*6*), genic lethality (*7*), and specific proteins (*8*). The fact that even small chromosomal deletions are not transmitted through the pollen suggests that the number of genes functioning in the gametophyte is large (*9*). Other studies, particularly on isozymes, demonstrate that some of the genes expressed by the haploid pollen genome are also expressed at one or more stages in the sporophyte (*10*); however, there have been no direct estimates of what proportion of the sporophytic genome is expressed in the male gametophyte.

The tomato, *Lycopersicon esculentum*, was selected for our investigations because many of its enzyme systems have been genetically analyzed. This information provides a basis for estimating the overlap of sporophytic and gametophytic genes as well as the portion of gametophytic genes expressed after meiosis. The strategy was to use starch gel electrophoresis, coupled with histochemical staining techniques for specific enzymes, to generate isozyme profiles for sporophytic and gametophytic tissues. These data, combined with previously published genetic data for the individual isozymes, were used to estimate gene overlap.

For dimeric enzymes present in pollen, it is possible to determine whether the corresponding genes are expressed after meiosis. The test involves obtaining plants known to be heterozygous at the locus in question. Extracts from diploid, sporophytic tissue of such plants display on the gel two homodimeric bands and an intermediate heterodimeric band revealing enzymes composed of one fast

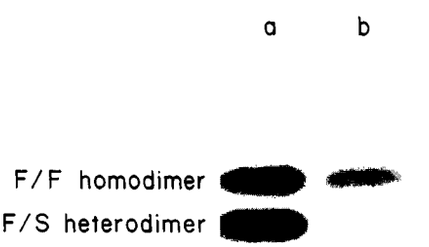

Fig. 1. Zymograms of F_1 heterozygote: (a) leaf tissue (sporophyte) and (b) pollen.

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