The Forebrain Is Not Essential for Sympathoadrenal Hyperglycemic Response to Glucoprivation

Abstract. *The reduction of glycolysis by hypoglycemia or the glucose analog 2-deoxy-D-glucose (2DG) stimulates compensatory sympathetic alterations of metabolism. Considerable attention has been focused on the hypothalamus as the probable locus of requisite metabolic signal detection. We report, however, that unanesthetized chronically decerebrate rats are capable of exhibiting sympathoadrenal hyperglycemia in response to the metabolic challenge presented by 2DG. This finding demonstrates that the forebrain is not necessary for glucoprivic stimulation of this reflex. Since cervical cord transection has been shown to eliminate hyperglycemia induced by 2DG, we conclude that the caudal brainstem contains an essential part of the neural mechanism which both detects metabolic need and ameliorates that need through the release of stored fuels.*

Deficits in metabolic fuels produce compensatory ingestive and metabolic responses that maintain adequate concentrations of plasma glucose (*1-3*). Attempts to define the neural organization of energy homeostasis have generally focused on the hypothalamus as the locus of elements sensitive to signals of metabolic need (*4-6*). Recently, however, several lines of evidence have converged to call attention to the importance of extrahypothalamic mechanisms for energy homeostasis (*7, 8*). Since considerable uncertainty persists concerning the afferent dimension of metabolic homeostasis, our strategy has been to examine a metabolic reflex whose efferent mechanisms are well specified in order to localize its mediating afferents.

An example of such a reflex is the sympathoadrenal stimulation of hyperglycemia during "glucoprivation," the competitive inhibition of glycolysis by the glucose analog 2-deoxy-D-glucose (2DG) (*2, 9*). Like starvation and the induction of hypoglycemia by insulin, 2DG leads to the sympathetic activation of adrenal catecholamine secretion (*10-12*). Epinephrine acts on the liver, pancreas, and adipose tissue to increase plasma glucose concentration (*13, 14*). In addition, the release of norepinephrine from sympathetic nerve endings in the liver, adipose tissue, and pancreas also increases plasma glucose concentration (*14-16*). These responses are neurogenic since they are eliminated by transections of the spinal cord (*12, 17-19*). Furthermore, barbiturate anesthesia interferes with 2DG-induced hyperglycemia (*20*).

The notion that receptor neurons within the central nervous system provide the afferent limb of the sympathoadrenal reflex was first proposed by Cannon (*11*). Considerable attention has been focused on the hypothalamus as the probable locus of metabolic signal detection by studies showing that electrical stimulation of that region increased adrenal catecholamine secretion and blood glucose concentration (*6, 21*). Electrophysiological demonstrations of hypothalamic chemosensitivity to insulin, glucose, and free fatty acids (*5*) have tended to support the notion of the hypothalamus as the site of receptors for energy homeostasis. More specifically, several studies have concluded that the hypothalamus is the location of sensory elements for the sympathoadrenal reflex (*22, 23*).

Conversely, other studies have suggested that hypothalamic receptors are not necessary for the stimulation of adrenal catecholamine secretion during metabolic stress. Insulin-induced hypoglycemia has been reported to trigger the sympathoadrenal reflex in acute decerebrate sheep and dogs (*17, 18*). However, acute decerebration is itself frequently followed by elevated systemic concentrations of epinephrine and glucose (*17, 18, 24*). This observation has been cited as a problem in interpreting results obtained from the acute decerebrate preparation (*22*). Owing to that criticism and the report of evidence to the contrary (*22*), the conclusion that the forebrain is not necessary for the neurogenic stimulation of adrenal catecholamine secretion has either not been generally accepted (*3, 25*) or has been specifically denied (*22*).

The purpose of our experiment was to examine the capacity of unanesthetized chronically decerebrate rats that had survived transection for an average of 47 days to exhibit sympathoadrenal hyperglycemia in response to 2DG. Increased posttransection time would eliminate transient effects of acute decerebration on glucostasis and permit an unclouded examination of the glucostatic capacity of the caudal brainstem isolated from the

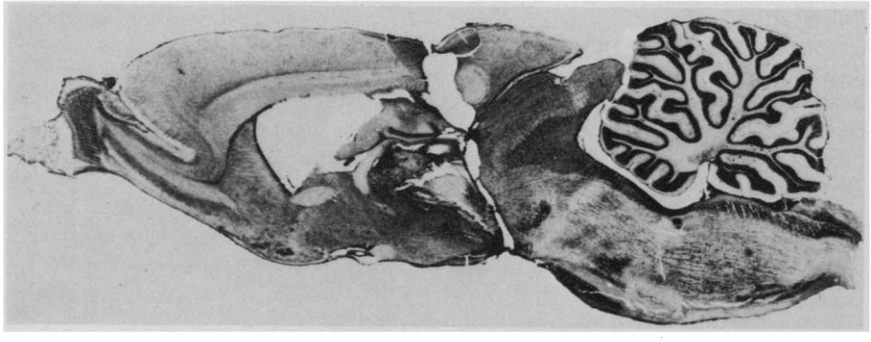

Fig. 1. Sagittal section of a representative decerebrate brain stained with cresyl violet. The supracollicular plane of section was similar in all of the decerebrate rats.

Table 1. Plasma glucose concentrations (mg/100 ml) measured 0, 30, 60, and 120 minutes after the peripheral administration of 2DG (200 mg/kg) and physiological saline (0.85 percent) to chronically decerebrate and control rats. Also shown is the area between each rat's 2DG and saline curves (mg/100 ml) (minute). Values of *U* and *P* are presented for the comparison between groups at the times indicated.

	2DG				Saline				Area (mg/100 ml) × (minute)
	0	30	60	120	0	30	60	120	
Decerebrates									
Mean	134	171	244	239	135	140	145	138	+7,805
Standard error	7	15	14	34	5	4	4	3	1,425
Controls									
Mean	157	204	294	293	152	150	153	148	+11,457
Standard error	6	14	27	35	4	4	3	3	2,227
Mann-Whitney U test									
U	4	27	38	36	4	29	34	31	32
P	>.05	<.1	>.1	>.1	>.05	<.1	>.1	<.1	>.1

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neural influence of the forebrain. This experiment demonstrates that the forebrain is not necessary for glucoprivic stimulation of sympathoadrenal hyperglycemia.

Transection at the supracollicular level was performed in two stages with the use of a hand-held spatula (*26*). The completeness of transections was verified histologically in all cases. A representative sagittal section is shown in Fig. 1. Despite the loss of the forebrain, the chronically decerebrate rat shares a number of behavioral similarities with full surgical controls, including the maintenance of a righted posture, effective grooming, and coordinated locomotion when stimulated (*27*). Decerebrates require the same time to empty the stomach (*28*) and gain weight at the same rate as pair-fed controls (*27*). The decerebrate does not effectively thermoregulate, never feeds or drinks spontaneously, but does consume orally administered fluids (*26*). Rectal temperature was recorded three to five times daily, and body temperature was maintained between 34° and 37.5°C by warming or evaporative cooling. Decerebrates and their controls were tube-fed three 12-ml meals consisting of equal parts of sweetened condensed milk and water (with multiple vitamin supplement) each day. Rats were housed individually and subject to a 12:12 hour day:night cycle (the light was on from 0600 to 1800 hours).

All testing began around noon, 3 hours after the morning tube feeding (between 0900 and 1000 hours). Tail blood was taken from unanesthetized restrained rats 30, 60, and 120 minutes after the isotonic injection of 2DG (200 mg per kilogram of body weight, injected intraperitoneally) or physiological saline. In some cases blood was sampled before injection. On any given test day approximately half of the rats from each group (decerebrate and control) received the experimental treatment while the other half received a saline control injection (administered to match volume and site of experimental injection). On the next test day (a minimum of 3 days later) treatments were reversed. Tail blood was taken into heparinized capillary tubes (Natelson, Scientific Products) and kept in crushed ice after collection. Plasma was obtained after centrifugation. Plasma glucose concentrations were determined with a glucose analyzer (Beckman).

Average plasma glucose concentrations of 11 control and 10 decerebrate rats after the peripheral administration of saline and 2DG are shown in Fig. 2. The

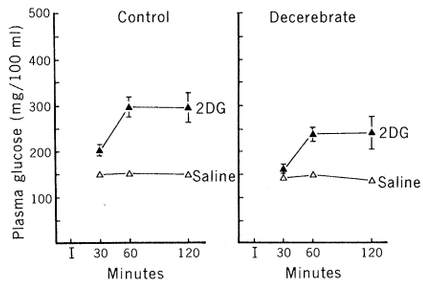

Fig. 2. Plasma glucose concentrations of decerebrate and control rats 30, 60 and 120 minutes after intraperitoneal injection (*I*) of 2DG and physiological saline.

area between each rat's 2DG and saline curves over the interval between 30 and 120 minutes was determined by subtracting the area under its saline curve from that under its 2DG curve (with the exception of 21 CD). The resultant area was taken as the measure of the overall effect of 2DG for each rat (*29*). In every case plasma glucose concentration was greater after the administration of 2DG than after saline, as indicated by the positive signs of the areas between each rat's 2DG and saline curves (*29*). The binomial probability that this results from chance is $P = .002$ for decerebrates and $P = .0005$ for controls. Clearly these chronically decerebrate rats retained the capacity to increase plasma glucose concentration in response to 2DG. The apparent difference between decerebrates and controls at any of the sampling points or for the overall effect was not significant by the Mann-Whitney U test for unpaired measures (*30*). The average plasma glucose concentration of decerebrates was slightly lower than that of control rats before the administration of either saline or 2DG. Specific values of U and P may be found in Table 1.

The plasma glucose concentrations of decerebrate rats were statistically equivalent to those of tube-fed control rats before treatment with 2DG and increased to hyperglycemic levels thereafter. This finding demonstrates that chronically decerebrate rats are capable of exhibiting sympathoadrenal hyperglycemia in response to the metabolic challenge presented by 2DG. Afferent systems contained within the caudal brainstem, or reporting to it from the periphery, are therefore sufficient for the stimulation of this reflex (*31*). In contrast to decerebration, cervical transections of the spinal cord eliminate this response (*15, 17*). We conclude that the caudal brainstem and its descending projections to sympathetic motor neurons of the spinal cord are necessary for the mediation of this aspect of metabolic homeostasis, whereas the forebrain is not. It appears, therefore, that the perception of metabolic need and compensatory neurogenic mobilization of stored fuels does not require the participation of the hypothalamus. This demonstration of metabolic homeostasis in chronically decerebrate rats is consistent with the finding that this same preparation demonstrates hunger and satiation (the ingested volume of an orally administered sucrose solution increased as a function of time since last tube feeding) (*8*). Taken together, these two pieces of evidence suggest that, although the forebrain may be instrumental in the mediation of energy homeostasis, complete sensorimotor aspects of this control are represented within more caudal neural levels.

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28. H. J. Grill, unpublished observations.
29. Owing to inhomogeneity of variance we used areas (instead of analysis of variance) as an index of the effect of 2DG over time. We determined least-error estimates of areas geometrically in a manner analogous to integration by the trapezoidal rule [M. C. Gemignani, *Calculus and Statistics* (Addison-Wesley, Reading, Mass., 1970), p. 169].

Area (mg/100 ml) (minutes)	
Decerebrates	Controls
1,200	1,620
4,905	3,405
4,545	6,165
6,975	8,460
7,965	10,440
9,045	10,920
9,465	12,585
11,220	12,600
14,925	16,095
*	20,220
	23,525

*120-minute sample broken in centrifugation

30. S. Siegel, *Nonparametric Statistics* (McGraw-Hill, New York, 1956), p. 116.
31. On the basis of our observations alone, we cannot determine whether glucoprivation is detected by receptors in the viscera, caudal brainstem, or both. Recent findings appear to rule out an essential contribution of peripheral receptors to the sympathoadrenal reflex [E. M. Stricker, N. Rowland, C. F. Saller, M. I. Friedman, *Science* **196**, 79 (1977)]. Adrenal catecholamine output during insulin-induced hypoglycemia was diminished by the intravenous injections of mannose or β-hydroxybutyrate, which can serve as alternate metabolic substrates for the brain. Injections of fructose, however, which does not cross the blood-brain barrier, were not effective. It appears, therefore, that if the necessary receptors for the stimulation of this reflex are contained within the central nervous system, they must reside in the caudal brainstem between the high mesencephalic transections in our subjects and the cervical cord transections used by others.
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Structure of Physiologically Identified X and Y Cells in the Cat's Lateral Geniculate Nucleus

Abstract. *Horseradish peroxidase injected into 18 single, physiologically identified geniculate X and Y cells permitted a detailed morphological correlate to be determined for the physiological properties of each neuron. Class 1 morphological characteristics were associated with Y cells, class 3 with X cells, and class 2 structural traits were seen in both physiological types.*

One of the major goals of neuroscience is to identify the structural basis of function at the single cell level. Recently, this has been approached in many neural loci by intracellularly injecting dyes, such as fluorescent markers or horseradish peroxidase (HRP), into physiologically identified cells (*1*). The HRP seems to diffuse throughout the cell into the finest processes, and this permits a detailed morphological view of the cell comparable to that possible with Golgi impregnation (Fig. 1).

We have begun to use this technique to relate physiological and morphological classes in the laminated portion of the cat's lateral geniculate nucleus. Previous morphological studies of this nucleus, based mostly on Golgi impregnation of cells, have identified three main classes of cells found throughout the laminae (*2*): class 1 cells are characterized by the largest somata and thick, fairly straight dendrites with occasional spinelike appendages; class 2 cells, by intermediate soma sizes, fine and somewhat curved dendrites, and frequent clusters of specializations appended at or near dendritic branch points; and class 3 cells, by small somata, very fine, wavy, and tortuous dendrites, and frequent clusters of complex stalked appendages along these dendrites (*3*). Physiological studies have identified the following main cell types distributed in all laminae (*3-5*): X cells are relay cells with slowly conducting retinal afferents and slowly conducting axons to cortex, fairly linear spatial summation, small receptive fields, and often tonic responses to stimuli of appropriate standing contrast; Y cells are relay cells with fast-conducting retinal afferents and fast-conducting axons to cortex, nonlinear spatial summation, relatively large receptive fields, and usually phasic responses; the rare interneurons (*6*) have not been extensively studied but many have receptive field properties similar to the X and Y cells that project axons to the visual cortex (*7*). Our preliminary data suggest that most Y cells are of class 1 and that the morphology of most X cells is intermediate between classes 2 and 3 (*8*).

We collected data from normal adult cats. The physiological preparation, anesthesia, and recording techniques were identical to those described extensively in previous reports (*5, 9*), with minor exceptions noted here. We used fine micropipettes, filled with 2 to 3 percent HRP (Sigma type VI) in 0.2*M* KCl buffered with 0.05*M* tris at *p*H 8.6. The tips were beveled to a size < 0.5 μm and an impedance at 200 Hz of 100 to 200 megohms. In most experiments, it was necessary to remove a portion of the overlying cortex 5 mm in diameter and 4 to 8 mm deep to reach the lateral geniculate nucleus with these electrode tips intact (*10*), but occasionally tips reached the nucleus successfully without cortical extirpation (*11*). The electrode was inserted into the brain through a hydraulically sealed chamber.

Once a geniculate cell was isolated extracellularly, its physiological properties, including latency to optic chiasm stimulation and receptive field characteristics, were studied; the neuron was duly identified as an X or Y cell (*12*). If the cell was clearly identified as one of these types, we advanced the electrode in 1-μm steps until it impaled the neuron. Figure 2A illustrates some of the criteria for intracellular recording, which include

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