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Insulin Elicits Ingestion in Decerebrate Rats

Abstract. *Insulin administered to rats reliably elicits ingestion of food. To determine whether the neural mechanisms sufficient to control insulin-elicited ingestion are located in or caudal to the forebrain, decerebrate rats were treated with insulin and ingestive responses were measured. Insulin treatment produced hypoglycemia that was comparable, in magnitude and duration, in control and decerebrate rats. Decerebrate and control rats ingested significantly more sucrose solution while hypoglycemic than while normoglycemic. In contrast, insulin did not augment the water consumption of either group. These data indicate that neural systems caudal to the forebrain are sufficient to control ingestive consummatory behavior through the integration of metabolic signals generated by insulin treatment and taste afferent input from the oropharynx.*

Feeding is elicited when metabolic fuels are insufficient to meet the energetic demands of the organism. This compensatory ingestive behavior can be triggered by insulin treatment. Receptors located in the forebrain that are responsive to decreased glucose utilization brought about by the action of large doses of insulin are thought to mediate this feeding behavior (*1*). Several lines of evidence suggest that the lateral hypothalamus (LH) is the site of these forebrain receptors. Intravenous administration of insulin alters the activity of LH neurons (*2*). Also, destruction of these putative receptors by LH lesions abolishes insulin-elicited feeding (*3*). Analogously, supplying these receptors with glucose by way of cannulas implanted in the LH blocks insulin-elicited feeding (*4*).

Recent findings, however, indicate that under special circumstances rats with LH lesions will feed in response to insulin treatment (*5*). While these data suggest that the critical receptors or pathways mediating insulin-elicited feeding are not in the LH area alone, they do not specify the neural systems sufficient to control this feeding response. These systems could reside in hypothalamic tissue not damaged by the lesions, in other forebrain tissue, or in sites caudal to the forebrain.

Evidence also suggests that neural controls of ingestion are represented at more caudal levels of the nervous system. Decerebrate rats have normal discriminative responses to taste (*6*) and there is evidence (*7, 8*) that such decerebrate rats increase their ingestion of sucrose solution in response to food deprivation. Furthermore, microinfusions of an antimetabolic analog of glucose (5-thioglucose) restricted to the fourth ventricle of intact rats elicit feeding (*9*). It remains unclear, however, whether both the energy deficit signal and the resultant compensatory food intake are mediated by hindbrain mechanisms alone, or whether hindbrain signals are relayed to the forebrain which then initiates ingestion. The purpose of the present study was to determine whether, in isolation of the forebrain, integrative mechanisms complete within the caudal brainstem are sufficient to regulate sucrose ingestion in response to the energy deficit signals evoked by insulin treatment.

Sprague-Dawley male rats (320 to 350 g) served as subjects. Brains were transected in the supracollicular plane with a hand-held spatula. Decerebration was performed in two stages with a 7-day period separating the first and second stages (*10*). Rats so treated do not spontaneously eat or drink, nor do they effectively thermoregulate. Therefore, these decerebrate rats and control rats were maintained exclusively on three 12-ml, tube-fed meals daily (*11*). Body temperature of the decerebrate rats was closely monitored and maintained between 32° and 36°C by warming or promoting evaporative cooling by wetting the fur. With the use of these procedures rats can survive transection for 1 to 2 months. The rats were housed in individual cages and maintained on a normal cycle of light and darkness.

The ingestive responses of the aphagic rats was assessed by fitting both the decerebrate (N = 12) and control (N = 20) animals with two intraoral fistulas through which taste stimuli could be delivered. Three rats from each group were also implanted with intravena cava cannulas (*12*) to facilitate blood withdrawal. The distal end of the venous cannula was anchored to the top of the skull adjacent to the intraoral fistulas. In the first experiment, control (N = 11) and decerebrate (N = 6) rats were treated with regular insulin (10 U/kg; Iletin, Lilly) or saline (0.9 percent). Subsequently, in a second experiment, six decerebrate and nine control rats were treated with 5 U of insulin per kilogram of body weight. Intake and plasma glucose testing occurred a minimum of 2 weeks after complete decerebration.

Insulin elicits spontaneous feeding in normal rats when blood glucose levels fall below 70 mg/100 ml (*13*). To determine the optimal time for the intraoral intake tests, we monitored plasma glucose concentrations after insulin treatments. Rats were tube-fed their morning 12-ml meal and 1 hour later were injected subcutaneously with either physiological saline (0.9 percent) or regular insulin (10 or 5 U/kg in a volume of 1.0 ml/kg).

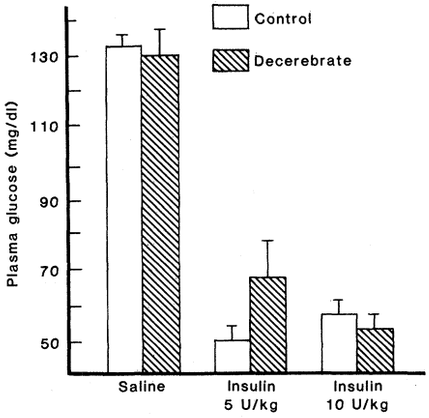

Fig. 1. Mean plasma glucose concentrations (± standard error of the mean) of control and decerebrate rats at the time of intake tests, that is, 3 hours after treatment with saline and insulin (5 and 10 U/kg).

Blood was collected from the tail vein or inferior vena cava hourly for 3 to 6 hours for subsequent plasma glucose determination (*14*). Both doses of insulin lowered the concentration of plasma glucose of control and decerebrate rats to less than 70 mg/100 ml within 3 hours after injection. On the basis of this result, we tested the rats for insulin-elicited ingestion 3 hours after insulin treatment. The rats that had received the 10 U/kg dose of insulin received either saline (0.9 percent) or another 10 U/kg dose of insulin and those that had received the 5 U/kg dose received saline or the 5 U/kg dose of insulin. Four to five days later the injection conditions were reversed. After the injection the rats were returned to their home cages. Three hours later, a blood sample was collected for plasma glucose determination. Each rat was then placed in an intake chamber, and the distal end of a length of tubing was connected to the rat's intraoral fistula. The proximal end of this tubing was attached to a syringe mounted on an infusion pump.

The rats were adapted to the intake chamber for 10 to 15 minutes before the delivery of the taste stimulus. Fluids (24° to 25°C) were infused into the oral cavity at a constant rate for control (1.1 ml/min) and decerebrate rats [0.6 ml/min (*15*)]. All rats were presented with 0.03*M* sucrose and, subsequently, 6 decerebrate and 12 control rats were given distilled water to assess the specificity of the ingestive responses. A taste stimulus (0.03*M* sucrose or distilled water) was infused into the rats' mouths until it was ejected; at that time the infusion was stopped. The stimulus could be ejected passively—by fluid dripping from the mouth; or actively—by head shakes, chin rubs, or wiping the mouth with the paws and then shaking the paws. After a 30-second interval with no infusion, the infusion pump was restarted. If the stimulus was ejected again within 30 seconds, the intake test was terminated and the amount of fluid ingested was computed. If the stimulus continued to be consumed the pump was left on and the infusion proceeded until two successive ejection responses occurred.

Histological examination of the brains of decerebrate rats revealed that in all cases the transection was complete in the supracollicular plane. Figure 1 shows the plasma glucose values of control and decerebrate rats. At the time of the intake tests plasma glucose concentrations of control and decerebrate rats treated with saline or insulin were not significantly different. Similarly, insulin treatments rapidly reduced plasma glucose concentrations. Plasma glucose levels were not differentially affected by the 5 U/kg and 10 U/kg doses of insulin 3 hours after the injection.

Overall, the decerebrate rats ingested less sucrose and water than control rats (Table 1). Regardless of the lower volumes ingested by the decerebrate rats, their sucrose intake significantly increased after the 5 U/kg dose of insulin, $t(5) = 3.82$, $P < .05$, and the 10 U/kg dose, $t(5) = 4.9$, $P < .01$. Sucrose ingestion by control rats also increased after the 5 and 10 U/kg doses; $t(8) = 3.82$, $P < .01$; $t(10) = 2.3$, $P < .05$, respectively. Although insulin treatment (5 U/kg and 10 U/kg) increased sucrose consumption, water intake was not differentially affected by insulin or saline in either decerebrate or control rats.

The present results demonstrate that forebrain neural mechanisms are neither essential for the detection of energy deficit signals nor for the production of compensatory ingestive behaviors. Rather, neural systems caudal to the forebrain are sufficient to control ingestion of an orally presented food in response to the energy deficit signals evoked by insulin treatment. Although the caudal brainstem is sufficient to effect behavioral changes in response to insulin treatment, the afferent limb of this response is unknown. Detection of energy deficit signals may take place in the periphery, caudal brainstem, or both.

These data do not exclude the importance of forebrain structures in controlling feeding behavior. As noted above, decerebrate rats never eat spontaneously. Although caudal brainstem mechanisms are sufficient to control insulin-elicited ingestive responses, it appears that interaction with forebrain systems is required to effect appetitive behavior. Also, forebrain mechanisms could be involved in regulating ingestive consummatory responses in intact rats. These suggestions are consistent with hierarchical or distributed systems models of the neural control of behavior (*16*).

Table 1. Sucrose and water ingestion (mean ± standard error of the mean) of control and decerebrate rats treated with saline and insulin (5 and 10 U/kg).

Treatment	Decerebrate	Control
Sucrose		
Saline	3.2 ± 1.0	8.1 ± 1.1
Insulin (5 U/kg)	6.7 ± 1.4	11.3 ± 1.2
Change	3.5 ± 0.9*	3.2 ± 0.8†
Water		
Saline	2.5 ± 1.2	4.7 ± 1.0
Insulin (5 U/kg)	1.7 ± 0.7	5.8 ± 1.0
Change	−0.9 ± 0.9	0.7 ± 1.2
Sucrose		
Saline	2.2 ± 0.5	11.6 ± 2.9
Insulin (10 U/kg)	5.5 ± 0.7	15.8 ± 2.2
Change	3.3 ± 0.7†	3.8 ± 2.1*
Water		
Saline	1.1 ± 0.2	8.9 ± 1.7
Insulin (10 U/kg)	1.0 ± 0.2	6.3 ± 2.5
Change	−0.2 ± 0.1	−2.6 ± 0.9

*$P < .05$. †$P < .01$; paired t-test comparisons, significantly different from saline of that group.

Taste is an important determinant of ingestive responses (*17*). The decerebrate and control rats in the present experiments increased their ingestion of sucrose, but not water, in response to the energetic emergency posed by the insulin treatment (*18*). Since the rats discriminated between these solutions, the enhanced ingestion of sucrose after insulin treatment did not reflect a general facilitation of fluid intake. It appears that the combination of internal insulin-elicited energy deficit signals together with specific types of taste signals was necessary to enhance intake. Caudal brainstem mechanisms alone are sufficient to integrate these signals in the control of ingestive consummatory responses.

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15. The stimulus was infused at a slower rate for decerebrate rats than for intact rats to allow for their slower rate of ingestion and correlated rhythmic cycle of mouth movements (5.1 cycle/sec, compared with 6.6 cycle/sec in intact rats) and of tongue protrusions (6.3 cycle/sec, compared with 8.8 cycles in intact rats).
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Leaf Color Used by Cabbage Root Flies to Distinguish Among Host Plants

Abstract. *In experiments in which spectrophotometric reflectance patterns of real leaves were mimicked with mixtures of artists' pigments, leaf color was shown to be a character used by cabbage root flies, before landing on leaves, to discriminate among the host plant cultivars radish, green cabbage, and red cabbage. It may be possible to take advantage of factors that affect leaf color, such as epicuticular bloom, pubescence, and masking of chlorophyll by other pigments, to decrease the attraction of certain pest insects to plants.*

Compared with the substantial progress that has been made in identifying specific sexual attractants of herbivorous insects, little progress has been made in identification of specific attractive chemical or visual components of plants (*1, 2*). Several reports suggest, but do not directly prove, that certain insect herbivores are able to discriminate among plant species or cultivars partly on the basis of one or more leaf color attributes (hue, saturation, or intensity) (*3–8*). Direct proof is lacking because no studies have been done of herbivore response to artificial leaves having spectral reflectance properties equivalent to those of real leaves. By analogy, direct proof of insect response to chemical stimuli from host plants may be lacking because of failure to identify and synthesize the chemical stimuli.

The host range of the cabbage root fly *Delia radicum* includes more than 40 species of wild and cultivated Cruciferae of diverse morphologies (*9*). Although plant odor attracts gravid females to patches of host plants (*10*), visual as well as olfactory stimuli determine whether insects will land on individual plants (*11*). We coated leaf-shaped pieces of cardboard with mixtures of artists' pigments (*12*) to mimic spectrophotometrically the reflectance patterns of real leaves. Our results provide strong evidence that leaf color is a character used by cabbage root flies, before landing, to discriminate among three types of host plants—radish, green cabbage, and red cabbage. We suggest that foliage bearing epicuticular bloom (such as green and red cabbage) or red-colored foliage may be less detectable by (or less attractive to) certain insect herbivores than green foliage.

All but one of our tests were conducted in a cage (125 by 125 by 100 cm tall), constructed of white Terylene netting, in a greenhouse illuminated solely by skylight. Single real leaves or oval-shaped artificially pigmented leaves, approximately 64 cm^2, were placed, adaxial surface up at an angle of 45°, into black plastic pots (8 by 8 cm) filled with soil. If the real leaves were smaller than 64 cm^2, two or more were attached with double-sided tape to a 64-cm^2 clear cellulose acetate leaf to give the appearance of a single large leaf. Testing was completed within 2½ hours of removal of real leaves from plants. Colors for the artificial leaves were produced by mixing oil pigments (*13*). Reflectance from 350 to 650 nm (the visible spectrum of the insect) was measured against a MgO standard with a spectrophotometer (Pye-Unicam ST 1800). For testing, leaf-containing pots were placed 25 cm apart on the soil-covered floor of the cage, which contained 150 to 300 gravid laboratory-cultured females (*14*), 5 to 7 days old when introduced, that had been reared under diapause conditions. We counted each female that landed on a leaf and removed her immediately from further testing.

More females landed on real leaves of mature radish plants (*Raphanus sativus* cv. Cherry Belle) than on real leaves of mature green cabbage plants (*Brassica oleracea* var. *capitata* cv. Avon Coronet), which had a distinct layer of epicuticular bloom. The latter received more landings than real leaves of mature red cabbage plants (*B. oleracea* var. *capitata* cv. Mammoth Red Rock), which likewise had distinct bloom, or than clear cellulose acetate leaves (experiment 1 in Table 1). Landings on the artificial mimics of radish, green cabbage, and red cabbage leaves of mature plants were similar in proportion to those on real leaves (experiment 2 in Table 1). Spectral reflectance curves of the artificial leaves closely approximated those of their real-leaf counterparts (Fig. 1A) (*15*). The curves reveal a distinct peak of reflectance from radish leaves at 500 to 600 nm. In contrast, although there was a slight peak of reflectance at 500 to 600 nm from green cabbage leaves, this was coupled with more intense reflectance than that from radish at all wavelengths from 350 to 650 nm. There was no reflectance peak at 500 to 600 nm from red cabbage leaves. In two-choice tests (eight replicates per treatment), direct comparison of landings on mature real versus artificial leaves, respectively, showed the following: radish (41 versus 39), green cabbage (27 versus 26), red cabbage (18 versus 21).

Confirmation that females discriminated among leaves of mature host plants on the basis of color was obtained in a test conducted in a large field cage (6 by 6 by 2 m tall) in which sticky (coated with