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Short-Term Influence of Intra-Ventromedial Hypothalamic Administration of Insulin on Feeding in Normal and Diabetic Rats[1]

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HATFIELD, J. S., W. J. MILLARD AND C. J. V. SMITH. *Short-term influence of intra-ventromedial hypothalamic administration of insulin on feeding in normal and diabetic rats.* PHARMAC. BIOCHEM. BEHAV. 2(2) 223–226, 1974. – This study reports the influence of insulin on food consumption in rats as mediated by ventromedial hypothalamic glucoreceptors. Implantation of crystalline insulin in the hypothalamic ventromedial nucleus resulted in significant reduction of food intake in both normal and diabetic rats. Administration of polyleucine or polyglycine caused no change in food intake. These results are discussed in terms of the glucostatic theory of food intake regulation.

Ventromedial hypothalamus Insulin-dependent Glucoreceptors Glucostatic theory Food intake

SINCE Mayer proposed the original glucostatic theory of food intake control [7, 8, 9], numerous investigators have attempted to collect data to confirm or refute the theory. One basic premise in the original hypothesis was the presence of glucoreceptors in the hypothalamus. It has been established that there are glucoreceptor cells in the ventromedial nucleus (VMN) and the lateral hypothalamic area (LHA), both of which seem to be involved in the control of food intake [1, 2, 3, 4, 5, 6, 10 and others]. A number of investigators have demonstrated that the VMN glucoreceptors apparently require insulin in order to be influenced by circulating glucose [5, 6, 12 and others]. The fact that the LHA glucoreceptors apparently do not require insulin has recently been reported [12]. One of us (C.J.V.S.) has published a report suggesting a modification of the glucostatic theory [12]. The modification involves a physiological integration of the information from the insulin-dependent glucoreceptors of the VMN and insulin non-dependent glucoreceptors of the LHA to provide a simultaneous intrahypothalamic measure of blood glucose levels and an index of peripheral blood glucose utilization.

Among the many experiments designed to test the original as well as the modified glucostatic theory was one which postulated that the introduction of insulin into the VMN should significantly reduce the amount of food consumed by a previously fasted animal in a short-term feeding study. The rationale, according to the modified theory, is that the level of circulating insulin is minimal in an animal which has not recently fed. Thus, the amount of glucose sensed by the VMN glucoreceptor cells is less than the absolute level of blood glucose; i.e., the insulin level is the limiting factor. Under normal conditions, the circulating level of insulin increases soon after the onset of a meal, allowing more glucose to be sensed by the VMN cells. This should increase VMN activity and inhibit the LHA, thus reducing food consumption. If the level of insulin in the VMN is exogenously increased one should see an earlier inhibition of the LHA and a resulting decrease in the amount of food consumed compared to a control animal. The following experiment using both normal and diabetic rats was conducted to test the above hypothesis.

MATERIALS AND METHOD

All animals used in the experiment were young male Sprague-Dawley rats (200–300 g) housed individually in suspended wire cages. Standard rat chow pellets and water were supplied ad lib except during the testing procedure outlined below. The animal room was temperature controlled (22+1.5°C) with a 12 hr light period from 10 a.m. to 10 p.m. for the non-diabetic studies and from midnight until noon each day for the tests using the diabetic animals. In that portion of the experiment using diabetic animals, the rats were given intraperitoneal injections of alloxan monohydrate (Sigma Chemical Co.) at a dosage of 40 mg/kg body weight. The presence of diabetes was confirmed throughout the experiment using a standard urinary glucose test strip.

Guide cannulas (22 g stainless steel tubing) were bilaterally placed under sodium nembutal anesthesia (45 mg/g

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I.P.) using routine stereotaxic procedures, so that the tips were approximately 1 mm dorsal to the ventromedial nuclei. The following coordinates were used: anterior from ear bar zero (A), 6.3 mm; ±0.6 mm from the midline (L); and 7.7 mm ventral (V) from the surface of the skull. The following coordinates were used for the purposely misplaced cannulas: (1) A +6.3 mm, L ±0.6 mm, V 3.7 mm; (2) A +3.3 mm, L ±0.6 mm, V 7.7 mm; (3) A +9.3 mm, L ±0.6 mm, V 7.7 mm; (4) A 9.3 mm, L ± 1.2 mm, V 6.0 mm. Dental cement and a stainless steel bolt through the skull were used to retain the cannulas. Approximately 75,000 units of penicillin G were administered per animal after the operation.

Inner cannulas were constructed of 25 or 26 ga stainless steel tubing of such a length that when inserted into the guide cannulas the tips would rest in the dorsal boundaries of the ventromedial nuclei.

The general testing procedure used was to fast the nondiabetic animals for 12 or 24 hr and the diabetics for 12 hr, except in the misplaced cannula study where both the nondiabetic and the diabetic groups were fasted for 12 hr. After the period of fasting the inner cannulas, empty for control baseline studies or loaded with a test material, would be inserted and the animal given a measured amount of rat chow pellets. In some of the studies a 15 min period was allowed for diffusion of the compound before the food was given (Table 1). Food consumption was then recorded at the end of 1 hr. Uneaten food and spillage were recorded to the nearest half gram. Water was available at all times during both the fasting and testing periods. Testing was initiated at the start of the dark period of the lighting cycle.

The compounds used to pack the cannulas were porcine insulin (Schwartz–Mann), bovine insulin, polyleucine (MW 11,000) or polyglycine (MW 6,000) (Sigma Chemical Co.). The polyamino acid compounds were used as a control in addition to the empty cannulas. These two compounds were chosen because of their similarity to insulin in molecular weight and other chemical properties.

The design of the testing procedure allowed for alternating baseline (empty cannulas) and compound testing with at least one 24 hr rest period between tests. The number of animals used and the total number of tests are given in Table 1. For statistical analysis the baseline values were compared with the immediately following test values using a paired comparison *t* test procedure [13]. The values for food consumption are reported by calculating the mean and standard error of all values in the above comparison *t* test procedure.

To load the test material into the cannulas the finely ground compounds were placed on a glass slide and the cannulas pressed into the material a total of 20 times. The outer surface was wiped clean prior to cannula placement. At the conclusion of the test the cannulas were checked for leaching of the test material. In the few cases where it appeared that the test material had not leached at all, the data for that particular test animal was disregarded.

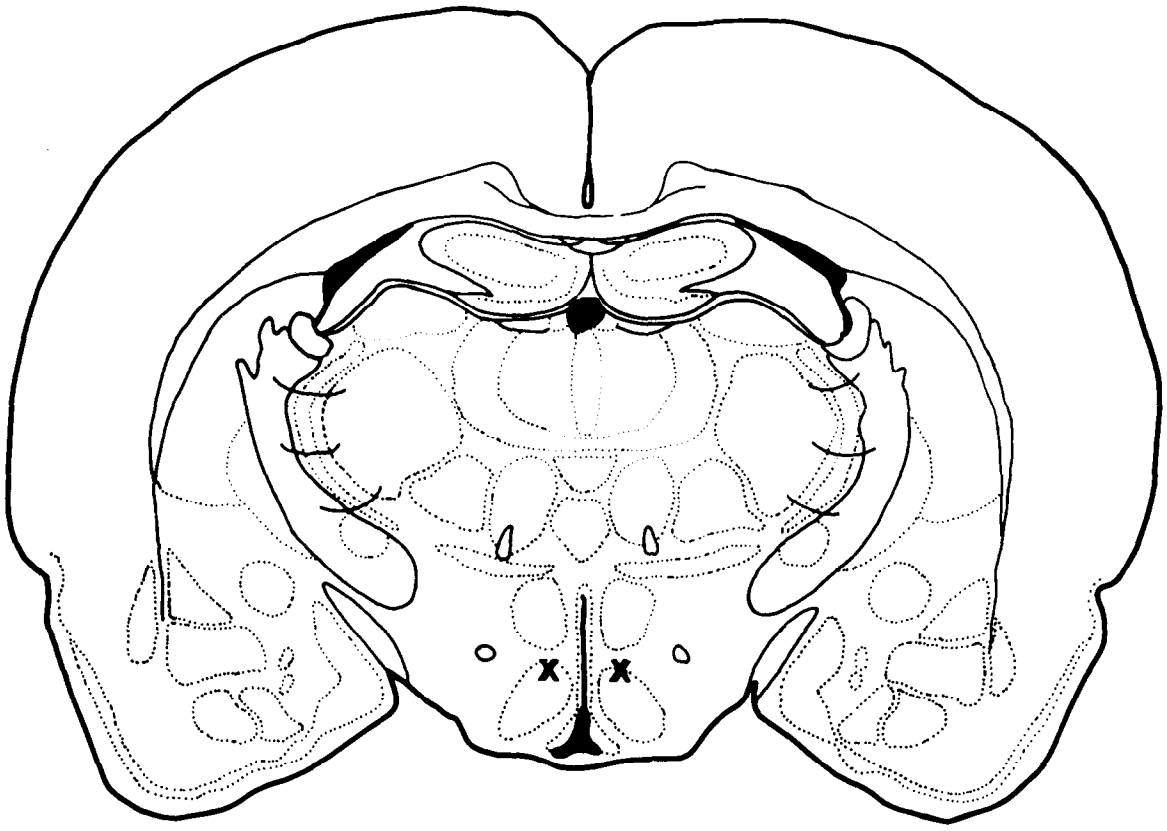

FIG. 1. Representative section showing approximate placement of inner cannula tips in the majority of animals (X).

Weighing a series of cannulas before and after loading indicated that each cannula contained approximately 20 μg of insulin.

At the termination of the experiment the animals were sacrificed with an overdose of anesthetic and the brain removed and fixed in buffered formalin. Gross examination of each brain indicated the area of cannula placement. The data used in the final analysis was obtained only from animals whose cannula implants were directed toward the VMN (except in the purposely misplaced cannula groups). Fig. 1 illustrates a typical cannula placement.

RESULTS AND DISCUSSION

The data from Table 1 indicates that when the level of insulin was exogenously increased in the VMN of normal (non-diabetic) rats, a significant reduction in food intake results during the first hr when the results were compared with baseline (empty cannula) values. Using bovine insulin, 24 hr of starvation and no delay after implantation the reduction was significant ($p<0.02$). With porcine insulin, a 12 hr starvation period and 15 min delay the difference was highly significant ($p<0.001$). The results were essentially the same when diabetic animals were used. Insulin cannulation significantly reduced food consumption compared to the baseline tests (12 hr starvation – no delay $p<0.02$; 15 min delay $p<0.001$). There was no significant difference between the baseline values and either polyamino acid cannulation. In addition, there were no significant differences noted with the intentionally misplaced cannulas.

A recent report has indicated that the direct application of a glucose solution into the ventromedial area of fed animals does not result in any significant alteration in food intake if the testing is done within several hours after application [11].

Balagura and Kanner [1] have shown that crystalline glucose implanted in the dorsomedial nucleus of a small number of animals significantly increased short term food consumption in deprived but not in ad lib fed animals. No data was given for ventromedial glucose placement.

In addition, Panksepp and Nace [11] have reported that microinfusion of a mixture of insulin and glucose into the VMN has little effect on feeding when testing is done soon after infusion.

Unpublished data from this laboratory obtained using cannulas containing a mixture of crystalline insulin and glucose has shown essentially the same results: No significant effect on food consumption was observed during a short duration feeding test. In both of the above experiments the amount of glucose reaching the VMN glucoreceptor cells

TABLE 1

THE INFLUENCE OF CANNULATION OF THE VENTROMEDIAL NUCLEUS WITH INSULIN OR POLYAMINO ACIDS ON THE FOOD CONSUMPTION OF NORMAL AND DIABETIC RATS

Experimental Group	Animals Tested	Length of Time Starved (hr)	Delay-Minutes	Compound Tested	Food Consumption (g) Baseline	Test	Statistics (*t*-test baseline vs test)
1	Normal VMN Cannulated	24	0	Bovine Insulin	8.2 ± 0.3† (43/15)*	6.3 ± 0.3 (43/15)	$p<0.02$ (42)‡
2	Normal VMN Cannulated	12	15	Porcine Insulin	4.9 ± 0.2 (47/14)	4.0 ± 0.2 (47/14)	$p<0.001$ (46)
3	Diabetic VMN Cannulated	12	0	Bovine Insulin	5.5 ± 0.3 (34/8)	4.5 ± 0.3 (34/8)	$p<0.01$ (33)
4	Diabetic VMN Cannulated	12	15	Bovine Insulin	6.7 ± 0.3 (47/23)	5.2 ± 0.3 (47/23)	$p<0.001$ (46)
5	Diabetic VMN Cannulated	12	15	Poly Leucine	7.0 ± 0.3 (29/15)	6.8 ± 0.3 (29/15)	N.S. (28)
6	Diabetic VMN Cannulated	12	15	Poly Glycine	6.7 ± 0.4 (29/16)	6.8 ± 0.3 (29/16)	N.S. (28)
7	Normal Misplaced Cannulas	12	15	Bovine Insulin	6.4 ± 0.4 (31/7)	5.6 ± 0.4 (31/7)	N.S. (30)
8	Diabetic Misplaced Cannulas	12	15	Bovine Insulin	8.0 ± 0.8 (11/5)	7.5 ± 1.0 (11/5)	N.S. (10)

*(Number of trials/Number of animals involved) †Mean ± S.E.M. ‡Degrees of freedom used in the analysis

may be so much higher than physiological levels as to overload the receptor mechanism, which by some indications could be a finely tuned system temporarily inactivated by surges of excess glucose. However, excess insulin, as used in this study, would have little effect on the system because the molecule (glucose) thought to actually activate the receptor is present only in physiological concentrations.

These data provide supportive evidence for a portion of the modified glucostatic theory; i.e., insulin and not glucose is the limiting factor as far as the regulatory activity of the VMN glucoreceptor cells are concerned.

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