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Increased Feeding in Response to Bilateral Injection of Insulin Antibodies in the VMH

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STRUBBE, J. H. AND C. G. MEIN. *Increased feeding in response to bilateral injection of insulin antibodies in the VMH.* *PHYSIOL. BEHAV.* 19(2) 309–313, 1977. – In order to investigate the role of insulin in the VMH in regulation of food intake in the rat, a specific antibody against rat insulin was injected in the VMH. The insulin antibody caused transient hyperphagia, when administered in the nighttime. This result is discussed in view of the glucostatic theory of the regulation of food intake.

Food intake VMH LH Insulin antibodies

THERE IS A fair amount of evidence in the literature [16, 17, 18, 21, 22] that variations in availability of nutrients in the blood are important determinants of feeding behavior. In particular a glucostatic theory has been worked out in detail [16, 17, 18, 22]. This theory holds in its more recent, refined version that food will be taken when the utilization of blood glucose by the organs of the body is insufficient. This rate of glucose utilization depends of course on the presence in the blood of glucose itself, but for many kinds of cells it also depends very much on adequate levels of hormones facilitating uptake of glucose. Of these hormones insulin is the most important.

More specifically it has been suggested that there are certain sensory cells, so-called glucoreceptors, the activity of which is governed by these two parameters of glucose utilization, and which inform the brain mechanisms of feeding behaviour as to the level of glucose utilization by the body [1, 4, 19, 22, 23]. In particular it has been suggested that these glucoreceptors are located in the ventromedial hypothalamus (VMH).

The results of several electrophysiological [1, 4, 22, 23] and pharmacological [7, 9, 21] studies appear to be compatible with these views. However, as will be argued in detail below, some loopholes remain to be filled before it can be definitively accepted that the VMH contains glucoreceptors in the above sense, which mediate glucostatic control of feeding behaviour in mammals under normal physiological conditions. The present study aims at closing one of these loopholes. The reasoning is as follows: if glucoreceptor activity is instrumental in preventing further feeding responses in the food sated individual, feeding behaviour should be reinstated in such an individual by localized removal of insulin from its VMH under otherwise normal physiological conditions, as it is the combined presence of glucose and insulin that appears to be required for really high glucoreceptor activity [1, 4, 22, 23].

MATERIAL AND METHOD

Animals and Maintenance

Male Wistar rats were maintained in individual Plexiglas chambers (25 × 25 × 30 cm) at a room temperature of 20°C. Lights were on from 2 a.m. till 2 p.m. Water was allowed ad lib at all times.

A standard diet providing 20% protein, 53.5% carbohydrate, 4.5% fat and 22% water, with added minerals and vitamins was available ad lib. This diet was presented in the form of a bar which could slide easily through a dispensing tube attached to one of the walls of the cage. The bar could be removed from the dispenser after a meal and weighed without disturbing the animal. Practically no food was spilled.

Fifteen male rats of about 300 g were prepared with bilateral VMH cannulas. Ten rats of another group were provided with bilateral LH cannulas.

Cannula and Injection System for Local Chemical Brain Stimulation with Small Volumes of Fluids

The cannula system was derived from the system described by Epstein [11] using a permanent guide cannula and an inner injection cannula. Guide cannulas (ID 0.35 mm, OD 0.6 mm, 23G) prepared from a disposable needle, were placed stereotactically and bilaterally just above the LH or VMH. Three screws were screwed into the skull to attach these cannulas by means of dental cement to the skull. A polythene cap was placed on the guide cannula.

Experiments were performed with an inner cannula of stainless steel tube (ID 0.1; OD 0.29 mm) and provided with a small piston made from a piece of silicon tube (ID 1 mm; OD 3 mm) and a pin (see Fig. 1). A silicon cuff (ID 0.5; OD 1 mm) on the lower end of the injection tube serves to attach this firmly to the guide cannula. Before infusion the dead space of the injection system was filled

FIG. 1. Injection system for local chemical brain stimulation.

with methyleneblue 1% in saline separated by a small air bubble from the fluid to be injected. The injection can now be made by pushing the iron piston down. The air bubble separating methylene blue from the injection fluid serves as a marker for reading off the volume administered, so that a direct visual check of the rate at which the fluid enters the brain is possible (Fig. 1).

The injection tubes were long enough to perform the manipulations of injection outside the cage. Disturbance of the animal is minimized in this way. Torsion of the injection tubes by movements of the animal during the experiment is prevented by attaching the injection system to a thin thread provided with a small counterbalancing weight. In one injection tube, enough fluid ($\pm 5 \mu\text{l}$) can be stored to give several injections in succession.

Procedure

After surgery the animals were habituated to the

injection procedure during one to two weeks by means of saline injections. The localisation of the cannula tip was investigated by means of nembutal injections ($10 \mu\text{g}$ per cannula), as preliminary experiments demonstrated that in satiated rats the feeding response to nembutal (both latency and amount eaten) depends on the distance of the cannula tip from the VMH.

Only ten animals that responded to nembutal with feeding behaviour within 3 min were subsequently used in the experiments with insulin antibody. Such an experiment consisted always of three consecutive hours (Periods I, II, III). Before Period I, the animals were provided with injection tubes. The injection was given at the beginning of Period II. The response of each animal was measured once for each dose under the different conditions (see below). The various doses were given in random order. Food was available without restriction during the experiment.

The measures taken were: (a) latency between injection and the first subsequent occurrence of feeding behaviour; (b) total amount of food during Period II, which was compared with the amounts taken in Period I and Period III. Analogous observations were made in two control experiments, one in which no infusion was given at all, and another where guinea pig serum containing no anti-insulin was injected.

A specific antibody in guinea pig serum against rat insulin was diluted with 0.1% (w/v) bovine serum albumin in 0.9% (w/v) sodium chloride to 0.5; 1.0; 1.25; 1.5; 1.75 and 2 mU per ml (1 mU of antibody is that quantity which can bind approximately 1 mU of rat insulin in the immuno assay). In all experiments and controls a volume of $1 \mu\text{l}$ per cannula was given. The quantity of antibody used ranged from $0.5 \mu\text{U}$ to $2.0 \mu\text{U}$ per cannula. Because the binding percentages in vivo may well differ from those in the immuno assay, no stress should be laid on the absolute quantities administered. However, comparisons of relative quantities are certainly valid. For the controls guinea pig serum without binding properties was diluted in the same way as the antibody serum of 1 mU/ml.

Experiments were made during day and during night. During day the injection was given 2 hr after light on so that the 3 hr of observation fell in a period during which the rat normally sleeps practically all the time. During night the injection was given 2 hr before light on. This means that Periods I and II of the observation fell at a time when normally feeding behaviour would be at a relatively low level (in the final hour of the night there is some increase in food intake again).

A further experiment was made by injection of $1 \mu\text{U}$ of anti-insulin per cannula into the lateral hypothalamus. These tests were made only in the dark period.

After the experiments the brains were prepared for histological examination. The analysis consisted of sectioning through the area of cannula placement followed by microscopical examination to ascertain the location of the cannula tips [14].

RESULTS

The mean amount eaten in the different periods are presented in Fig. 2. The different loads in μU are stated in the margin of the figure. In the first column the day-time results are given. Only after $1.5 \mu\text{U}$ there is a small, nonsignificant increase of food intake. The animals slept most of the time during day and were not activated by the injection. In contrast the second column which presents the

FIG. 2. Effect of injection of antibody to insulin in the VMH and LH on the amount of food intake (Mean $\pm$ SEM). I, II, III successive one hr periods. The injection was given at the beginning of Period II.

results of the night observations shows much more feeding behaviour in all three periods. No extra food intake was observed in Period II after 0.5 μ U whereas after 0.75; 1.0; 1.25 and 1.5 μ U food intake was significantly increased compared with the control of guinea pig serum injection ($p < 0.01$; $p < 0.002$; $p < 0.002$ and $p < 0.02$, respectively, Students *t*-test). Comparison of Period II after injection with antibody with those without any injected resulted in significant increases of food intake after 1.0; 1.25 and 1.5 μ U of antibody injection. In Period III no effect of the injection on food intake was noticeable any more.

Mostly the animals began to eat within half an hour after the injection, depending on the dose (Fig. 3). The latency of feeding is much longer here than after nembutal

FIG. 3. Effect on latency of the injection of antibody to insulin in the VMH and LH (individual cases).

injection (1–3 min). There was no significant indication that the latency score was an individual characteristic of rats, in the sense that an individual tended to score high (or low) for all doses.

The third column of Fig. 2 presents the results obtained after injections in the lateral hypothalamus. No significant influence on amount or latency of food intake is observed here (see also Fig. 3).

Histological examination revealed as coordinates for the VMH cannula tips A 5.8 to 6.2; L 0.2 to 0.5; V -1.0 to -3.0 and for the LH cannula tips A 4.2 to 6.0; L 1.1 to 2.1; V -2.0 to -3.0.

DISCUSSION

Over the past twenty years it has often been asserted, firstly, that regulation of (caloric) food intake operates largely through a negative feedback control in which utilization of blood glucose by the organs of the body is the critical variable [16, 17, 18, 19, 22], and, secondly, that the task of monitoring glucose utilization is entrusted to receptor cells, situated in the VMH and/or LH, which are sensitive to glucose and/or insulin concentrations in the blood. On what grounds are these assertions based?

There are convincing electrophysiological reports that the unit activity of certain cells in VMH and LH is influenced by the glucose and insulin levels [1, 4, 22]. However, these reports contain no proof that these same cells are involved in nutritional homeostasis.

Another line of evidence is based on the hyperphagia seen after injury to the VMH caused by administration of goldthioglucose (GTG) [2, 6, 7, 8, 9, 10, 15, 20, 25, 27]. It has been suggested that VMH glucoreceptors are unable to distinguish between GTG and glucose, take up GTG and then pay the penalty for their mistake [19]. This view is strengthened by the fact that a normal plasma insulin level is needed for the entrance of GTG into the VMH neurons as appears from the work of Debons [7, 8, 10].

Since glucose utilization in many organs of the body depends on blood levels of both glucose and insulin, glucoreceptors involved in the glucostatic control of food intake should be sensitive to both of these parameters. One may therefore predict that if these glucoreceptors are located in the VMH, local removal of insulin from that area in food satiated animals will reinstate feeding behaviour. This prediction was tested in the present experiment.

The results show that feeding is activated in the nighttime when adequate amounts of insulin-antibody were injected in the VMH compared with control injections with serum without insulin antibody. After control injections in the VMH with guinea pig serum without antibody, food intake in Period II is nonsignificantly inhibited (compare Figs. 2a and 2i). Therefore the increase of food intake in Period II after injection of antibody into the VMH in the night must undoubtedly be attributed to local removal of insulin by the antibody. The relatively long latency in some cases (see Fig. 3) may be explained by the slow diffusion of the big antibody molecule.

That the same result was not obtained in the daytime, is probably due to interaction from other behaviour systems, resulting in inhibition of feeding behaviour despite low local glucose utilization. Further, the general motivation to eat is higher during night than during day.

It has been reported [30] that besides the VMH it is especially the LH which contains insulin sensitive cells, unlike those in the rest of the brain [5]. Therefore the lateral hypothalamus was chosen for control injection with insulin antibodies. Injection of serum either with or without antibody into the LH leaves food intake unaffected. To this extent the anti-insulin effect appears to be specific to the VMH.

Of course one might formulate and test an analogous prediction for the effect of local removal or administration of glucose in the VMH. Although to some extent studies with intracranial injection of 2 deoxy-D-glucose (2-DG) appear to confirm that intracerebral glucopenia by 2-DG results in an increased feeding tendency [3,21] this finding is not borne out when injections are made in VMH and LH regions. In neither of these cases was an increase of food intake observed and moreover injection of 2-DG into VMH resulted in a decreased food intake. In addition the inhibition of intake was even more pronounced in the latter case after a more concentrated dose was given [21]. This would indicate that 2-DG may have either a specific or a disturbing and irritative action in that area. After 2-DG injection in the VMH of rabbits no increase of feeding tendency was observed [13]. It seems likely that 2-DG though a substance which interacts with glucose utilization

in different tissues may have some toxic side effects. Therefore a more physiological approach is needed to solve these problems. Insulin placed via intracerebral cannulae directly in the VMH reduced via a short term influence food intake of alloxinized diabetic rats as well as that of normal rats [12]. Therefore the increase of food intake after local insulin removal is in line with the latter facts. However, so far there is little evidence that glucostatic short-term regulation of food intake occurs under normal physiological circumstances. So far there is strong evidence that under conditions of dangerous hypoglycemia [29], an extremely low rate of glucose utilization in diabetes [24], or after systemic 2-DG injections [28] glucostatic triggering of feeding behaviour may act as an emergency mechanism.

Similarly, the insulin-antibody used in the present study may have lowered insulin in the VMH to a level below the normal physiological range, in which case we may be dealing here with an emergency mechanism rather than the normal control of food intake. However, there is no proof that the slight hypoglycemia and low insulin level seen under conditions of mild food deprivation (e.g., 24 hr) takes part in the control of feeding. Furthermore, intravenous infusions of glucose, resulting in continuously high insulin and glucose levels and so in a high rate of glucose utilization do not affect timing or size of meals [26,31].

In view of these facts, what precisely is the contribution of glucostatic control to the regulation of food intake under normal ad lib conditions remains a problem needing further investigation. Whatever the answer may be, the data here presented indicate that lack of blood insulin sensed by receptors in the VMH may compel the rat to ingest more food than it would normally do under otherwise the same conditions.

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REFERENCES

1. Anand, B. K. Central chemo-sensitive mechanisms related to feeding. In: *Handbook of Physiology. Alimentary Canal, Vol. I: Control of Food and Water Intake*. Washington, D.C.: American Physiological Society, 1967, 249–270.
2. Arees, E. A., B. I. Veltman and J. Mayer. Hypothalamic blood flow following goldthioglucose induced lesions. *Expl Neurol* 25: 410–415, 1970.
3. Balagura, S. and M. Kanner. Hypothalamic sensitivity to 2-deoxy-D-glucose: Effects on feeding behavior. *Physiol. Behav.* 7: 251–255, 1971.
4. Chhina, G. S., B. K. Anand, B. Singh and P. S. Rao. Effects of glucose on hypothalamic feeding centres in deafferented animals. *Am. J. Physiol.* 221: 662–667, 1971.
5. Crone, C. Facilitated transfer of glucose from blood into brain tissues. *J. Physiol.* 181: 103–113, 1965.
6. Debons, A. F., L. Silver, E. P. Cronkite, H. A. Johnson, G. Brecher, D. Tenzer and I. L. Schwartz. Localisation of gold in mouse brain in reaction to goldthioglucose obesity. *Am. J. Physiol.* 202: 743–750, 1962.
7. Debons, A. F., I. Krinsky, H. J. Likuski, A. From and R. J. Cloutier. Goldthioglucose damage to the satiety center: Inhibition in diabetes. *Am. J. Physiol.* 214: 652–658, 1968.
8. Debons, A. F., I. Krinsky, A. From and R. J. Cloutier. Rapid effects of insulin on the hypothalamic satiety center. *Am. J. Physiol.* 217: 1114–1118, 1969.
9. Debons, A. F., I. Krinsky, A. From and R. J. Cloutier. Site of action of goldthioglucose in the hypothalamic satiety center. *Am. J. Physiol.* 219: 1397, 1970.
10. Debons, A. F., I. Krinsky and A. From. A direct action of insulin on the hypothalamic satiety center. *Am. J. Physiol.* 219: 938–943, 1970.
11. Epstein, A. N. Reciprocal changes in feeding behaviour by intrahypothalamic chemical injections. *Am. J. Physiol.* 109: 969–974, 1960.
12. 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: 223–226, 1974.
13. Gonzales, M. F. and D. Novin. Feeding induced by intracranial and intravenously administered 2-deoxy-D-glucose. *Physiol. Psychol.* 2: 326–330, 1974.
14. Groot, J. de. The rat forebrain in stereotaxic coordinates. *Verhandel. Koninkl. Ned. Akad. Wetenschap, afd. Natuurk., Sect. III: Deel LII*, no. 4, 1967.

15. Likuski, H. J., A. F. Debons and R. J. Cloutier. Inhibition of goldthioglucose-induced hypothalamic obesity of glucose analogues. *Am. J. Physiol.* **212**: 669–676, 1967.
16. Mayer, J. Genetic, traumatic and environmental factors in the etiology of obesity. *Physiol. Rev.* **33**: 472, 1953.
17. Mayer, J. Regulation of energy intake and body weight, the glucostatic theory and the lipostatic hypothesis. *Ann. N.Y. Acad. Sci.* **63**: 15–43, 1955.
18. Mayer, J. Some aspects of the problem of regulation of food intake and obesity. *New Engl. J. Med.* **274**: 610–616, 662–672, 722–731, 1966.
19. Mayer, J. and E. A. Arees. Ventromedial glucoreceptor system. *Fedn. Proc.* **27**: 1345–1348, 1968.
20. Mayer, J. and N. B. Marshall. Specificity of goldthioglucose for ventromedial hypothalamic lesions and hyperphagia. *Nature* **178**: 1399–1400, 1956.
21. Miselis, R. R. and A. N. Epstein. Feeding induced by intracerebro ventricular 2-deoxy-D-glucose in the rat. *Am. J. Physiol.* **229**: 1438–1447, 1975.
22. Oomura, Y. Central mechanism of feeding. *Adv. Biophys.* **5**: 65–135, 1973.
23. Oomura, Y., T. Ono, H. Ooyama and M. Wayner. Glucose and osmosensitive neurons of rat hypothalamus. *Nature* **222**: 282–284, 1969.
24. Panksepp, J. and M. Ritter. Mathematical analysis of energy regulatory patterns of normal and diabetic rats. *J. comp. physiol. Psychol.* **89**: 1019–1028, 1975.
25. Ruiter, L. de and P. R. Wiepkema. The goldthioglucose (GTG) syndrome in mice. *Psychiat. Neurol. Neurochir.* **72**: 455–480, 1969.
26. Scharrer, E., D. W. Thomas and J. Mayer. Absence of effect of intra-aortal glucose infusions upon spontaneous meals of rats. *Pflügers Arch.* **351**: 315–322, 1974.
27. Smith, C. J. V. Hypothalamic glucoreceptors. The influence of goldthioglucose implants in the ventromedial and lateral hypothalamic areas of normal and diabetic rats. *Physiol. Behav.* **9**: 391–396, 1972.
28. Smith, G. P., J. Gibbs, A. J. Strohmayer and P. E. Stokes. Threshold doses of 2-deoxy-D-glucose for hyperglycemia and feeding in rats and monkeys. *Am. J. Physiol.* **222**: 77–81, 1972.
29. Steffens, A. B. The influence of insulin injections and infusions on eating and blood glucose level in the rat. *Physiol. Behav.* **4**: 823–828, 1969.
30. Storlien, L. H., W. P. Bellingham and G. M. Martin. Localization of CNS glucoregulatory insulin receptors within the ventromedial hypothalamus. *Brain Res.* **96**: 156–160, 1975.
31. Strubbe, J. H., A. B. Steffens and L. de Ruiter. Plasma insulin and the time pattern of feeding in the rat. *Physiol. Behav.* **18**: 81–86, 1977.