Activity of single neurons in the hypothalamic feeding centers: effect of glucose

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Unit activity from neurons of hypothalamic feeding and satiety mechanisms, and from adjacent hypothalamic regions was recorded in anesthetized dogs with surgically exposed hypothalamus, and in Flaxedil-immobilized cats in which a stereotaxic approach was made. Intravenous glucose or insulin, or combinations of both, were given and the changes in spike activity observed. Glucose estimations were done on blood samples taken from femoral artery and vein. In starved animals the unit activity in satiety center neurons was slower than that obtained from feeding center neurons. Frequency of spikes recorded from satiety center neurons increased and that of feeding center neurons decreased significantly after glucose was given intravenously, while spike activity from these centers showed a reverse pattern of response after intravenous insulin. No significant changes were observed from other hypothalamic and cortical neurons. Activity of neurons of the satiety center did not show a significant correlation with blood glucose level per se, but a better correlation was found between unit activity and the A-V glucose difference. It is suggested that the satiety center is activated by increased glucose utilization in the body.

hypothalamus satiety feeding neuron activity A-V glucose difference glucoreceptor

The role of the central nervous system in regulating food intake is now well recognized. Following the discovery that obesity associated with hyperplagia occurs in animals with bilateral lesions in the ventromedial nuclei of the hypothalamus (11, 19, 21), later studies (2, 3, 7) produced evidence that there are two opposing mechanisms in the hypothalamus regulating food intake, viz., a mechanism in the lateral hypothalamus which initiates feeding, and one in the medial hypothalamus which brings about satiation. Further studies by the same authors (1, 5, 6, 13) have confirmed these observations, and have demonstrated the existence of integrative cerebral influences.

Various suggestions have been advanced to explain the activation of these hypothalamic centers both in satiety after food has been eaten, and in hunger after the food has been disposed of by conversion to heat, work, or stored energy. It is believed that, in addition to stimulation arising in the digestive tract (16, 30), certain changes occurring in the milieu interieur as a result of feeding act as signals to these centers, and an extensive search is being made by many investigators to discover such changes. Mayer (26, 27) proposed the presence of glucoreceptors in the hypothalamus, sensitive to blood glucose in the measure that they can utilize it. In agreement with his proposal, we found that the electroencephalographically recorded activity of the hypothalamic satiety and feeding centers changes selectively with changes in the blood glucose content and the arteriovenous glucose difference (8, 9).

Thus presumptive evidence exists for the presence of neurons in these hypothalamic centers which respond to changes in glucose metabolism. Similar evidence has been offered already for the presence of osmoreceptors (20), thermosensitive neurons (24), and neurons sensitive to circulating hormones (14) in the hypothalamic region. More direct evidence that the firing rate of neurons in the hypothalamus may change as a result of changes in the osmotic pressure or during local heating has been presented by Cross and Green (12), and by Nakayama, Eisenman, and Hardy (29), respectively. This has encouraged us to see whether any differences in firing frequency could be detected in neurons of the satiety and feeding center as a result of procedures which alter the blood glucose and arteriovenous glucose difference.

MATERIALS AND METHODS

Animals

Two sets of animals were used for this study. In the first series 109 mongrel dogs, weighing between 8-14 kg, were used. It was possible, however, to record single neuron activity for a period of more than 1 hr in 49 ani-
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mals only. Activity from only one neuron was recorded in each animal. These animals were anesthetized, the hypothalamic region was surgically exposed, and the microelectrodes inserted under direct vision. In the second series of 47 cats, weighing between 2.6-5.3 kg, a surgical preparation was done under ether anesthesia, and subsequently microelectrodes were stereotaxically guided to the hypothalamic regions and recording was done in unanesthetized, Flaxedil-immobilized animals with local anesthesia of wound margins and pressure points. We decided to use unanesthetized preparations in the second series in order to study the activity of the neurons without any alteration in their metabolism introduced by the anesthetic, in view of the demonstrated depressive effects of barbiturates on hypothalamus in particular.

Operative Procedures

Dogs. The animals were anesthetized with intraperitoneal injection of Dial (Ciba), 0.5 ml/kg body wt. The animal's head was held on one side and the brain surgically exposed by removing the scalp, muscles, and zygoma and then opening the skull with the use of a burr. After cutting the dura, the temporal lobe was sucked out, the third cranial nerve cut, and the hypothalamic region lifted to bring it into view. The microelectrode was then guided under direct vision by a micromanipulator to the required site.

Cats. Under ether anesthesia the neck was opened, the trachea was cannulated, and the animal was placed on artificial respiration. The femoral vessels were then exposed on one side and indwelling polyethylene tubes left in them for taking blood samples as well as for intravenous infusion of glucose-saline. All wound edges in the neck and thigh and all pressure points were infiltrated with 2% Procaine solution in saline, and the wounds and cut surfaces were thoroughly and repeatedly smeared with Procaine solution. Procaine infiltrations were made in the vicinity of the supraorbital and infraorbital nerves, and into the tissues anterior and posterior to the ear bars, before the animal's head was fixed in a Johnson stereotaxic instrument. The skull was exposed on its dorsal aspect and tiny burr holes made in it. The wound edges here were also infiltrated, and cut surfaces were smeared with Procaine. The smearing of all the cut surfaces with Procaine solution was often repeated, and the infiltrations were repeated after about hourly intervals. Intravenous Flaxedil was given in the dosage of 4 mg/kg body wt. Ether was stopped and the animal allowed to recover. The microelectrode was passed through the burr hole and guided stereotaxically to the appropriate hypothalamic area. The recording procedures were started when the effect of ether was over.

Recording

Steel microelectrodes, with their tips etched to 1-2μ and the remaining electrode insulated with polystyrene, were used for recording the extracellular unit activity. (We are grateful to Prof. J. D. Green of the University of California, Los Angeles, for his advice in the preparation of electrodes and the recording.) The activity picked up by these electrodes was fed into a Grass preamplifier, through a cathode-follower input probe, and displayed on a Dumont oscilloscope. Photographs were made with a Grass camera. The unit activity was recorded, in different animals, from neurons in the satiety center, in the feeding center, and in adjacent hypothalamic regions, as well as in frontal and parietal cerebral cortex. The cortical sites served as controls. The number of units utilized is given below in RESULTS.

Changes in Glucose Levels

After a unit from any one of these regions had been stable for over 1/2 hr, intravenous infusion of 20-25 ml of 10% glucose in saline was given. The unit activity was observed for more than an hour after this, and records made at frequent intervals. Samples of arterial and venous blood were obtained just before the intravenous glucose was given, and at 15-min intervals thereafter. The glucose content of these samples was determined by the Folin-Wu method (17). The arteriovenous glucose
FIG. 2. Unit activity recorded from a neuron in the feeding center of a cat before giving glucose (A), and at intervals of 5 (B), 10 (C), 15 (D), 20 (E), 25 (F), 30 (G), 35 (H), 40 (I), 50 (J), and 60 (K) min after intravenous glucose infusion. The activity was decreased with the increase in blood glucose.

difference for the leg was calculated to provide a measure of glucose utilization.

In five animals, 20-25 ml NaCl solution of the same osmolarity as the glucose solution (0.9 osmoles) was given intravenously, after the effects of glucose infusion were over, to determine that the observed effects were due to glucose and not to changes in osmolarity.

In five cats (5 neurons) used for this study, intravenous insulin (Unichem Laboratories), 20 units, was given instead of glucose and the same procedure was repeated. In nine other cats (9 neurons) intravenous glucose was given first, and then 1-5 hr later intravenous insulin was given to compare their effects in the same animal. Also in eight cats (8 neurons) insulin was given first and later on glucose was given. In two cats (2 neurons) the recording was first done from the satiety region and the effect of intravenous glucose was studied. Two to three hours later in the same animal recording was made from the feeding region and again intravenous glucose was given.

Disposal of Animals and Locating Microelectrodes

At the end of the recording the unanesthetized animals were again anesthetized with ether, and a small deposit of iron was made at the site of the unit recorded by passing a direct current of 3 ma for 30 sec through the microelectrode. The brain was then perfused through the carotid artery, first with a freshly made 1-2% solution of potassium ferrocyanide (to give the Prussian blue reaction with iron deposit), and later with 10% formalin. The brain was removed, serially sectioned, and studied for the presence of iron deposit. The marker lesions were large enough to be detected visually. In the dogs where the microelectrodes were guided under direct vision, most of these were confirmed to be placed in the anticipated areas. In the cats, stereotaxic coordinates provided accurate guides to microelectrode positions.

RESULTS

In dogs, where the skull was open and the brain exposed, a large number of units could not be held stable for periods sufficiently long to complete the experimental procedures; these units, therefore, are not included in the results. Successful recording for sufficiently long periods of more than an hour could be done in 49 such animals (49 units), and in these the effect of intravenous glucose could be studied. In the cats it was easier to hold some units for periods as long as 3-6 hr. Only the units surviving initial testing were exposed to experimental procedures.

Characteristics of Spike Activity

Spikes recorded from all the hypothalamic regions, medial as well as lateral, showed similar characteristics and were indistinguishable from each other. In dogs 24 units were recorded from the satiety center, 10 from the feeding center, and 7 from other hypothalamic areas. In cats 22 units were recorded from the satiety center, 17 from the feeding center, and 10 from other hypothalamic areas. All these animals were starved for about 18-20 hr, and we observed that in this hunger state in a majority of animals the frequency of spontaneous discharge was slower by about one-half in units of satiety center as compared with those of feeding center (for comparison see Figs. 4, 5). Also, although the spontaneous spike activity in hypothalamic neurons was generally low, the firing rates in hypothalamic neurons of the dogs
were generally higher (varying from about 1 in 10 sec to 10/sec) than firing rates in cats (varying from about 1 in 20 sec to 2/sec). Species characteristics, and the fact that the dogs were anesthetized with Dial while the cats were only Flaxedil immobilized, may have been responsible for this difference in rates. The amplitude of these extracellular spikes varied greatly, but it was generally between 0.3–5 mv; in only a few was it higher than 5 mv.

Responses to Glucose Infusion

Twenty-four units recorded from the medial satiety center of 24 dogs were tested with glucose infusion, and 22 responded by a variable increase in their spike frequency. Two showed no apparent change. Eleven units recorded from the satiety center region of 11 cats were exposed to glucose infusion, and all of these responded with a variable increase in their spike response. Figure 1 is a representative recording from such a unit in the satiety center of a cat.

Similarly, 10 units picked up in ten dogs from the lateral feeding center were tested with glucose infusion, and 9 of these responded by a decrease in their spike activity. One did not show any obvious change. Five units of the feeding center in five cats were tested with glucose infusion and all showed a decrease in their spike response. Figure 2 is a representative record from such a unit in the feeding center of a cat. Figure 3 illustrates the frequencies of spikes from a unit in the satiety center and a unit in the feeding center of two cats with the changes in the arterial glucose level and the arteriovenous glucose difference. It is apparent that the intravenous infusion of glucose altered the unit activity of these neurons in an inverse manner.

In dogs, activity of 7 units was recorded from other scattered areas in the hypothalamus, both anterior and posterior to the region of the satiety and feeding centers. Six of these units showed no response to glucose infusion, while one neuron in the medial part of the anterior hypothalamus increased its spike frequency after glucose infusion. In dogs 6 units were also recorded from cortical regions and 2 from thalamus. Seven of these did not re-
FIG. 5. Mean frequencies of unit discharges, with their standard errors, recorded from the satiety center (22 units), the feeding center (9 units), and the control hypothalamic regions (6 units) from all the dogs, correlated with changes in blood sugar values.

SATTIETY CENTER

FEEDING CENTER

CONTROL

FIG. 5. Mean frequencies of unit discharges, with their standard errors, recorded from the satiety center (22 units), the feeding center (9 units), and the control hypothalamic regions (6 units) from all the dogs, correlated with changes in blood sugar values.

respond to glucose, but the spike frequency of one unit located in the orbitomesial cortex decreased after glucose. Similarly, in cats 9 units recorded from other hypothalamic regions were exposed to glucose and 8 of these did not show any change in frequency. One unit located in the medial part of anterior hypothalamus responded to glucose by an increase in its frequency.

Figures 4 and 5 represent the averages with standard errors of spike frequencies of all the units from the satiety, feeding, and other hypothalamic regions (controls) of cats and dogs exposed to glucose infusion, together with the average values of arterial blood glucose and the arteriovenous glucose difference. All these figures bring out clearly that within 5–15 min after glucose infusion the spike activity of satiety center neurons increased. This activity was then sustained at a high level for a variable period of 5–1 hr and then gradually started declining. The increase in the activity appeared to be maximum about half an hour after glucose infusion. One hour after infusion, either the activity had returned to its original level, or was still somewhat higher, but gradually declining. The decrease in the spike activity of feeding center neurons also generally followed a similar pattern.

In three cats (3 units) intravenous glucose infusion was repeated nearly 2 hr after the first infusion. The direction of change in the spike activity was similar to the response obtained after the first infusion, but the magnitude of response was less marked.

In five animals (5 units from satiety region) a saline solution of the same osmolality as the glucose-saline solution was given by intravenous infusion after the effect of the previous glucose infusion was over. This did not produce any significant change in the spike activity, emphasizing that the original response was due to glucose and not to osmotic changes induced by the infusion.

In two cats a unit from the satiety center was first
recorded and the effect of glucose was noted. Later in the same animal another unit from the feeding center was recorded and a second infusion of glucose was given about 2–3 hr after the first. The responses of these units presented the pattern of inverse relationship described above.

Responses to Insulin

Responses to insulin were studied in cats only. Two units from the satiety center were tested with intravenous insulin injection, and both of these responded with a transitory increase followed by a prolonged decrease in the spike frequency. On the other hand, 2 units from the feeding region so tested showed an increase in the firing rate. One unit from the anterior hypothalamus did not respond after insulin injection. Figures 6–8 demonstrate these effects on the spike activity recorded from satiety and feeding neurons.

Combinations of glucose and insulin infusions were also given. Four units from the satiety region and four from the feeding region were first exposed to glucose infusion, to be followed later by insulin. These injections produced the expected responses (Fig. 9), with the difference that the insulin response was not so pronounced. This may be attributed to the already high blood glucose level at the time the insulin was given. Similarly, 2 units from the satiety center and 4 from the feeding center were first tested with insulin and later on with glucose infusion. Again, expected responses were obtained (Fig. 10) from five of these; one unit from the feeding center did not show any response either to insulin or glucose. Three units from other hypothalamic areas were exposed to combinations of glucose and insulin, but did not show any response.

It is interesting to note that immediately after insulin for about 5–10 min, the frequency of spikes from the satiety center neurons was increased before a decrease in activity was registered. This possibly has relation to the action of glucagon in the insulin, or perhaps to an initial transient increase in glucose utilization after insulin injection, followed by hypoglycemia and a decrease in glucose utilization (31).

Correlation of Spike Activity With Changes in Glucose

The frequency of spike activity changed with alterations which glucose infusion or insulin injection produced.
in the arterial blood glucose level and in the arteriovenous glucose difference. The figures showing averages of changes in the unit activity, as well as the figures showing changes in the activity of individual units, reveal that it took some minutes after infusion of glucose before the frequency of spikes changed. Although glucose estimations were not carried out during this initial period, we suppose that the arterial glucose level rose immediately after the infusion, but that the arteriovenous glucose difference would take some time before it increased. Later, after 45–60 min, the spike frequencies tended to return gradually toward the preglucose infusion levels. The arterial blood glucose was still quite high at this time, but the A-V difference had started to decrease gradually. It may not be possible on the basis of these results to rule out a direct correlation between spike activity of hypothalamic centers and arterial blood glucose level, yet it appears that this correlation

FIG. 9. Spike frequency of a unit from the satiety center and a unit from the feeding center of two different cats exposed first to intravenous glucose infusion and later to intravenous injection of insulin.

FIG. 10. Spike frequency of a unit from the satiety center and a unit from the feeding center of two different cats tested first with intravenous insulin injection and later with intravenous glucose infusion.
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is more direct with the level of A-V glucose difference. Following insulin injection a similar situation exists (see above).

DISCUSSION

The facts which stand out from our results are that individual neurons in hypothalamic "satiety" and "feeding" centers exhibited spontaneous activity, and changed their activity following intravenous injections of glucose or insulin. Changes shown by these neurons were not exhibited by neurons which were explored under similar experimental conditions in the adjacent hypothalamic areas, nor in certain cortical regions (except in 2 units from anterior hypothalamus and one unit from orbitomesial cortex; the anterior hypothalamic units may have been responding to changes in osmolarity). Also the changes in activity of satiety and feeding center neurons were inversely related.

It is tempting to suppose that such changes in activity may have been due to the presence of a "glucoreceptor" mechanism in the hypothalamic centers. A number of experimental observations suggest this. It was first shown that the injection of gold thioglucose (25, 28) destroys cells in the satiety center region, whereas gold linked to radicals other than glucose does not. Glucose injections into the cerebral ventricles depress food intake (18). Respiration studies in Warburg manometers on tiny slices of hypothalamus containing these regions (10) have further demonstrated that in fed animals the satiety center region takes up more glucose and oxygen. The greater uptake of P32 in the hypothalamic feeding region of hungry rats (15) has also been interpreted by Mayer (27) as confirming the existence of glucoreceptors there. Then the satiety and feeding center regions were demonstrated to show selective changes in EEG activity as a result of experimentally produced hyperglycemia and hypoglycemia (8), and such changes were correlated with arteriovenous glucose differences in fed and hungry animals (9). We have now demonstrated that experimentally produced hyper- or hypoglycemia, or changes in A-V glucose difference, alter the frequency of unit spikes of the neurons situated in these two regions, while no such changes are observed in neurons situated in other hypothalamic and cortical areas. One must add, however, that the greatest change in unit activity does not occur at the time of maximal change in blood sugar level, and that the activity is correlated better with the increase in A-V glucose difference rather than with the hyperglycemia as already discussed under RESULTS. The changes observed after insulin injection corroborate these results.

In our present studies the arteriovenous glucose differences obtained from the femoral vessels should give the levels of glucose utilization in the leg, provided that the rate of blood flow is constant. As the experiments were performed either under general anesthesia or under Flaxedil with the muscles relaxed, the A-V differences in the leg were expected to provide indices of glucose utilization generally in the periphery. It thus appears that the activity of the satiety center neurons varies with the level of glucose utilization at the periphery. The evidence does not rule out the possibility that hyperglycemia, as such, provides the stimulus for these neurons. Under the experimental conditions used the level of glucose utilization in the leg may or may not provide index of glucose utilization in neurons, specifically the neurons of the satiety center. It is not possible to tell whether glucose utilization by the central nervous system, or even for that matter by the hypothalamic centers, varies in the same way as utilization at the periphery.

The changes in spike activity of the satiety neurons, therefore, could be either the direct effect of glucose utilization by the cells, or the indirect effect, in some way, of glucose utilization at the periphery. Respiratory studies (10) do suggest an increased utilization of glucose by the satiety region in fed animals.

The method of unit recording also does not reveal whether the neurons were directly responsive to changes in their glucose utilization or whether they were activated synaptically from other regions. The fact that the unit activity recorded from hypothalamic regions adjacent to the satiety and feeding centers did not show any significant change with glucose suggests, at least, that the evidence is more in favor of a direct response.

Another suggestion emerging from these observations is that the activity of satiety neurons may be linked in some way to insulin. After insulin injection the activity of satiety neurons was increased for a short period. Later, when hypoglycemia occurred, this activity was decreased. Similarly, after glucose infusion the activity of these neurons did not change for the first few minutes. By the time the activity increased an increased insulin secretion would have been produced by the hyperglycemia. It is possible, therefore, that glucose utilization as affected by insulin may provide the specific stimulus for these neurons.

The osmotic changes of hypertonic glucose infusion were not responsible for changes in spike activity as hypertonic saline infusions of the same osmolarity did not produce any such changes.

Our evidence suggests, therefore, that the activity of neurons in the satiety center increases at the time of an increased glucose utilization in the body, possibly as some function of their own level of glucose utilization. Neurons in the feeding center, on the other hand, show an inverse relationship. It is also worth noting that in the fasted animals the rate of spontaneous activity recorded from feeding center neurons was generally higher than the spontaneous rate of satiety neurons. This emphasizes that in the hunger state the feeding center is more active; and, when after glucose infusion the satiety cells are activated, the activity of feeding cells is decreased to the resting level of activity of the satiety and other hypothalamic neurons. It is not clear whether the slight decrease in activity of feeding center neurons is a direct effect of increased A-V glucose difference or whether these are indirectly "inhibited" as a result of...
increased activity of satiety neurons. Experimental evidence has been provided previously (3) for the inhibition of the feeding center by the activation of the satiety center, possibly through lateral projections. Various suggestions had been put forward to explain how the inhibition of the feeding center by the activation of the satiety center, possibly through lateral projections. Various evidence has been provided previously (3) for the increased activity of satiety neurons. Experimental studies suggest that these centers. The present studies suggest that one such change is the increased utilization of glucose as a result of feeding. Our results do not preclude the possibility of other changes produced by feeding, which may also stimulate these hypothalamic neurons. Some of these other changes suggested are the specific dynamic action of food increasing the heat stress of the body (32), the concentration of certain circulating metabolites influenced by the fat in the depots (20); and the water concentration or its shifts among the compartments of the body (23). All these changes within the body occur some time after a meal has been taken. Immediately after eating, afferents from the gastrointestinal tract (30) bring about activation of the satiety center.

REFERENCES