Influences of hepatic portal receptors on hypothalamic feeding and satiety centers

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Schmitt, Mary. Influences of hepatic portal receptors on hypothalamic feeding and satiety centers. Am. J. Physiol. 225(5): 1089-1095. 1973.—Evidence for a neural pathway from the hepatic portal vein to the lateral hypothalamus was found. Injections of hypertonic saline and/or glucose into the portal vein of the rat produced changes in firing rate of certain lateral hypothalamic neurons. No responses to such injections were found in the supraoptic, paraventricular, or ventromedial nuclei. The response could be either one of increase or decrease in firing rate. Section of splanchnic nerves bilaterally or cord transection at the level of T3 eliminated these responses. Severing both vagi did not abolish but exaggerated the response. Ammonium chloride injection into the portal vein did affect hypothalamic units, but this action was not abolished by section of the spinal cord or severing afferent nerves.

The interrelated factors that maintain energy balance and monitor the food, water, and specific nutrient requirements of mammals have been extensively studied, but there is still much that is not understood. It is known that specific areas in the hypothalamus are involved in this control (1, 7, 14, 17) and that there are central and strategically placed peripheral tension receptors (15, 21), chemoreceptors (9, 16), and mechanoreceptors (26, 31) that participate in the regulation of food and water intake. The objective of the present study was to determine the possible role of another peripheral control mechanism.

A number of investigators have postulated that there are receptors that lie within the portal vein or hepatic structures. For example, a diuretic response to changes in the osmolarity of the portal vein blood has been reported by Hsberich et al. (11-13). He has suggested that some type of osmoreceptor is present in this area. Gauer (10) has likewise suggested that there is such a receptor in the hepatic portal system. The portal vein is the initial transport channel for most products of digestion, and Russek has proposed a number of receptors for this hepatic portal circuit. He and his associates (27-30) obtained evidence of a monitoring of glucose concentration, ammonium, and the final breakdown products of protein digestion and metabolism by receptors in the hepatic portal complex.

It was thought that the presence of osmoreceptors within the hepatic portal circuit might be detected by injection of hypertonic solutions into the system and recording from the supraoptic and paraventricular hypothalamic nuclei which are thought to play a role in the control of body osmolarity. Similarly, by recording from the ventromedial and lateral hypothalamic areas, it should be possible to determine whether there are glucose and proteoreceptors in the portal system capable of modifying activity in these regions of the hypothalamus known to control food intake (6, 7, 14).

The preliminary findings of this investigation were presented at the American Physiological Society meeting in August, 1971 (33).

METHODS

Mature male rats of the Long Evans strain, weighing from 250 to 300 g, were used. They were fasted, but given free access to water for 24 hr prior to the experiment. Urethan (0.6 ml/100 g body wt, 25%, weight to volume) was injected intraperitoneally to anesthetize the animals. A small (Clay Adams no. 10) polyethylene tube was inserted into the portal vein just before it branches into the various lobes of the liver. The size of this tube was such that it could lie within the vessel and be held in place by a surgical suture without impeding portal system flow, which approximates 10-15 ml/min. It was found that when the food-laden blood could not flow freely through the mesenteric veins, the gut area quickly became black with backed-up blood and the animal did not long survive.

The carotid artery was cannulated for blood pressure recording; a Statham pressure transducer and Grass recorder were used for this purpose. Taking advantage of the same ventral incision in the neck, loops of thread were placed around the vagi so that effects of vagotomy could be studied in certain experiments. The trachea was cannulated to permit adequate ventilation after the animal's head was fixed in the head holder. The stereotaxic apparatus (Baltimore Instrument Company) was so arranged that when the car plugs were positioned in the external auditory meatus and the upper incisors were fit snugly over the upper incisor bar, 5 mm above the interaural line, the coordinates of the DeGroot atlas (8) could be used for accurate placement of electrodes in the hypothalamus.

Once the head was rigidly positioned in the head holder, a midline skin incision was made and the dorsal surface of the brain was exposed in the region of the bregma. Micro electrodes could thus be introduced at several points.

In certain experiments, the splanchnic nerves were also
exposed at the point in the abdomen where they penetrate the diaphragm, and a few preganglionic fibers (those that had not yet entered the coeliac ganglion) were looped across electrodes and held in place with fine surgical thread, so that at a later time stimulation and severance could be performed.

Insl-X-coated, stecl microelectrodes (modified method of Zeballos et al. (36)) were introduced into various parts of the hypothalamus for recordings of unit activities as well as into various areas of the thalamus and cortex used as control sites. The signal picked up by each recording electrode was fed into a Tektronix 122, condenser-coupled preamplifier and displayed on a type 532 oscilloscope. The output of the preamplifier was continuously monitored by a pulse-counting device designed in our department, which made it possible to record the number of spikes occurring per unit time. The output from this counter was fed into one channel of a Grass polygraph, used also to record individual pulses and blood pressure. An audio-monitor was employed to facilitate recognition of cellular activity.

When stimulation of the splanchnic nerve was performed, a type 502A oscilloscope, with a Grass C4F camera, was used to record the effects of stimulation and the shape of evoked potentials in the hypothalamic region.

Cannulas were also inserted in the jugular or a tail vein so that comparisons could be made between effects produced by injecting materials into the general circulation and effects produced by injecting these substances directly into the hepatic circulation.

The exact positions of the recording electrodes were determined by the Prussian blue spot method at the end of each experiment (4). Frozen sections of the brain, 75 μ thickness, were made and stained with thionin. This method showed localization of the cell from which recordings had been made by a distinct green mark against a background of blue-violet-stained neurons.

Substances injected were hypertonic NaCl (5% or 0.85 m), ammonium chloride of the same molarity, and glucose, 2 times the molarity of the salts (1.7 m). Physiological saline, water, and sucrose were used for comparisons and controls. Each intraportal and intravenous injection was 0.25 ml in volume, delivered over a 5-set time interval. A minimum of 5 min was allowed between any two injections.

RESULTS

Spontaneous firing. The only hypothalamic area showing any response to portal vein injections was that region of the lateral hypothalamicus adjacent to and extending about 1 mm anterior and posterior to the ventromedial nuclei. Various cells which did not respond to materials injected were admixed with responsive cells in this area so that a generalized artifactual reaction could be ruled out. Specific sensitivities were demonstrable.

Most cells of the lateral hypothalamicus had a very distinctive pattern of spontaneous discharge. Figure 1 shows the shape of a typical action potential as well as the pattern of spontaneous firing of a lateral hypothalamic neuron which responded to portal injection of glucose or saline.

The rate of firing shown here was typical for the late afternoon period.

The lateral hypothalamic neurons responding to portal injections showed that activity varied with the time of day. A moderately fast, regular rate of from 10 to 25 spikes/sec was typical of the morning hours. During the midday period, the cells were most frequently completely silent. A gradually increasing rate of discharge developed in mid-afternoon and reached a peak of from 30 to 40 spikes/sec in the early evening hours. Considerable attention has been given to this fluctuation in the discharge rate in another article (32).

The fact of major importance to the testing carried out, however, was that the rate of cell discharge within 0.5-1 hr was relatively constant. The effect of portal injections of various chemicals could therefore be assessed with a reasonable degree of accuracy. Whenever a cell was found, the firing pattern was monitored for 5-10 min and only when it showed a regular rate of discharge, as in Fig. 1C, were portal injections carried out and the change was observed. Occasionally cells showing very irregular rates of discharge were found in the lateral hypothalamicus. Such irregularities rendered these cells unsuitable for assay of the effect of portal injection and were not used in this study. It was also necessary to hold a cell for a significant period before and after injection, but this too was not always possible. Testing was carried out on 195 cells quite successfully, and results reported were based on the response of this population.

Responses to glucose. Figure 2 shows examples of neuron responses to glucose injections into the portal vein. The rate of cellular discharges was increased in some cells and decreased in others by the same procedure. This principle of opposite effects applied not only to different cells in a
given experiment, but also to the same cell at various times of the day. Whether it was excited or inhibited depended on how the injection coincided with the pattern of “spontaneous discharges” (32).

Neurons that responded to portal injection of glucose were most concentrated in the lateral hypothalamus but extended through the zona inserta into a part of the ventral thalamus (Fig. 3). Such cells were scattered among others that did not respond to glucose. In the lateral hypothalamus glucose-responsive cells comprised approximately 49% of all successfully tested neurons. Strangely enough, no glucose-responsive cells could be found within the ventromedial nuclei.

Sucrose of the same osmolarity as glucose was used routinely for comparison. On no occasion did any hypothalamic cell respond to portal injections of this sugar.

Responses to hypertonic saline. As shown in Fig. 4, the results of portal injections of hypertonic saline were very similar to those obtained with glucose. Fig. 4, B and C, illustrates responses of two different neurons in the same experiment. Isotonic saline injections produced no change in the rate of firing (Fig 4A). Again these responsive cells were located in the lateral hypothalamus, interspersed with nonresponsive cells and extended into the same regions in which glucose “sensitive” cells were found (Fig 3). No cells located in the supraoptic or paraventricular nuclei were found to respond to portal injections of hypertonic saline.

An important point to be emphasized here is that the same osmolar concentrations of glucose and saline were used. Some cells were responsive to injections of both; others were responsive to one or the other, but not to both. Also, certain cells were responsive to both injections, but in opposite ways. This is depicted in Fig. 5, which shows in one cell a significant increase in firing rate with portal injection of saline and a decrease in firing rate with injection of glucose. Therefore, it is difficult to consider osmolarity as the determining parameter in these responses. The fact that the injection of sucrose of the same osmolarity as glucose did not affect the hypothalamic neurons, as stated previously, also supports this statement.

Figure 6 compares the proportion of cell groups which responded to glucose and/or hypertonic saline injections. Twenty-five of the 195 cells successfully tested and which responded to portal vein injections were located in the zona inserta and ventral thalamus. These neurons had discharge patterns quite different from those of the lateral hypothalamic cells. Responsive and nonresponsive neurons were intermingled, but cells selectively responsive to the different solutions were often found to lie very close together. In compiling these data, an increase or a decrease in rate of firing by 20% or below the preinjection control level for an average of 60 sec was taken as an indication that recording was from a “responsive cell.” All cells giving less than this change of firing rate were classified as nonresponsive. The basis for this decision was that variations in firing rates during control periods never attained 20% either before or after recovery from an evoked response. It is apparent from Fig. 6B that approximately two-thirds of the cells tested responded to glucose or hypertonic saline or to both, whereas one-third did not respond to either stimulus.

In some experiments, effects of injections of glucose and hypertonic saline into the portal vein were compared with effects of similar injections into the general circulation via the jugular or a tail vein. The changes produced by these latter injections on hypothalamic neurons were insignificant, if they occurred at all. This led to the conclusion that glucose and hypertonic saline introduced into the portal circulation most probably affect hypothalamic neurons through neural pathways from receptors in the portal system or the liver, rather than through a direct action of these chemicals reaching the hypothalamus by way of the circulation.
FIG. 5. Response of hypothalamic cell to portal injection of both sodium chloride and glucose. Dotted line represents effect of saline on firing rate; heavy line denotes glucose response. Increase or decrease is a percentage comparison with 60-sec preinjection firing rate of cell.

Neural pathways between portal circulation and the hypothalamus. To determine what neural pathways are involved in these hypothalamic responses described, the neural connections from the liver to the higher centers were severed. Since response of a hypothalamic neuron to the portal injection of glucose or hypertonic saline varied, depending on the time of day or fluctuations in its daily rhythm (32), all experiments described here were done in the mid-afternoon when cellular activity is high, and the responses obtained were reproducible and similar in magnitude.

Fig. 7, A and B, shows the effects of bilateral vagotomy, taken from a polygraph recording. Bilateral vagotomy did not destroy the response to glucose injection, but rather attenuated it. Therefore, if anything, the vagus exerts a modulating effect on this reaction; it certainly is not essential to the mediating of impulses from the portal system receptors.

In order to eliminate other possible afferent pathways from the liver, spinal transection was next performed at the level of T5 in a number of experiments. After sufficient time was allowed for blood pressure recovery, portal injections were again attempted. Although the vagi were intact, no response was obtained to this procedure after spinal transection. Figure 8 shows one such experiment. It should be noted that the rate of cell firing did not change after the spinal transection. The blood pressure was slightly

FIG. 6. A comparison of number of hypothalamic cells responsive to portal injections of either or both glucose and sodium chloride solutions. Bar graph shows compilation, from all experiments, of number of cells reacting. Top bar indicates cells that responded only to NaCl, next only to glucose. Third bar indicates cells that responded to both chemicals but in opposite directions. Fourth bar shows number of cells that responded to both chemicals in the same direction. Circle graph shows, on a percentage basis, relative fraction of each type of response elicited in comparison with total number of hypothalamic cells tested.
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Fig. 7. A and B: effects of bilateral vagotomy (A—before and B—after nerve transection) on hypothalamic unit responses. Note prolonged effect following severance of these nerves. Pulse-counting device counted spikes per 5-set interval. C: effects of stimulation of splanchnic nerve on glucose-responsive hypothalamic cell (10 v, 3-msec duration, at 1/set).

Fig. 8. Effect of spinal transection at Tg on response of hypothalamic cells to glucose injected into portal vein. Blood pressure from carotid artery is shown at top. A: before transaction. B: soon after transaction and 30 min later. Blood pressure was allowed to stabilize before portal injections of glucose were again attempted. C: glucose injection was repeated after B.

DISCUSSION

Reference to the concept of liver "glucoreceptors" is becoming more prevalent in the literature. It was first introduced by Russek (28) and Russek and Morgane (30) on the basis of behavioral studies. Initially they found that intraperitoneal injection of glucose solutions caused anorexia in hungry animals. In 1968, workers in the same laboratory, using anesthetized animals, studied the blood levels of glucose following such intraperitoneal injections (27). They found that the arterial-portal venous difference, normally positive, was reversed after this intraperitoneal injection, and they concluded that a high portal concentration of glucose relative to the arterial level is a potent signal of satiety. This suggested to them the presence of glucoreceptors in the liver.

Anand's studies (1) also emphasized the arteriovenous difference as an indicator of tissue utilization of glucose and the degree of body need for new fuel.

The only previous recordings of nerve fiber activity originating from receptors located in the portal system were reported by Niijima (22). The correlation which he observed between flow of glucose through the liver and changes in rate of discharges from afferent fibers leading therefrom was considered to be evidence that a neural pathway from a hepatic glucoreceptor does exist. Up until now, however, there have been no reports in the literature of cellular responses within brain centers which might be indicative of such peripheral receptor actions.

In the work reported here, certain cells within the lateral hypothalamus were found to respond to the portal injection of glucose solution, but not to sucrose of the same molarity. The effect was abolished by bilateral splanchnic nerve section or spinal cord section at the level of Tg. This elevated and to the same degree by the injection as before the transection.

To determine the role of splanchnics, direct stimulation of the greater splanchnic nerve on the ipsilateral side to the recording was attempted, once a responsive cell was located. Fig. 7C indicates the increase in firing rate of the hypothalamic cell under study when this nerve was stimulated; 10-v shocks at a 1/sec rate were used. Severance of both splanchnics at the end of this procedure abolished, as did cord section, all further hypothalamic cell response to portal vein injections.

Effects of NH₄Cl injections. It was mentioned in the introduction that Russek proposed very sensitive "proteoreceptors" in this area, since millimolar quantities of NH₄Cl could cause anorexia in hungry animals for significant lengths of time. In numerous experiments, this chemical was injected into the portal vein in the same quantities as the other chemicals. A number (40%) of hypothalamic cells tested responded by either an increase or a decrease in firing rate. Some of these cells were also responsive to either or both sodium chloride and glucose; some were not. The responses were frequently opposite in character from those evoked by the other stimulants.

Most important, however, was the fact that in no case did severance of the splanchnics of vagi and cord transection change the magnitude of the response to NH₄Cl. The reaction therefore did not appear to be neurally mediated. NH₄ ions are very toxic to the brain if not handled by the liver. A small quantity of NH₄Cl injected intraportally as in Russek's experiments (29) produced no response in hypothalamic cells. Thus, the effect of this chemical when injected at the same molarities as employed with glucose and saline was evidently due to its transport in the circulatory system and a generalized toxic action at this high concentration. Furthermore, NH₄Cl had the same stimulating effect on responsive hypothalamic neurons when injected into the jugular and tail vein as when infused into the portal system. Our procedures produced no evidence of a special ammonium ion-sensitive portal receptor.
result indicates the existence of a peripheral neural input which is activated by portal injections of this particular sugar (glucose). The location of these receptors may be in the liver or along the portal vein itself, but no attempt has been made in this work or in any published works, to date, to specify the exact localization of the receptor elements. All the authors cited obtained effects when they injected through the portal vein, not into the hepatic artery, but they all refer, ambiguously to "hepatic" osmo-, glucose-, or proteoreceptors. This question remains to be clarified.

The morphological studies of Wolf and Sutin (35) have shown that there are interconnections between the regions where cells responding to portal vein injections were found. Following a lesion in the lateral hypothalamus, degeneration of cells occurred in areas of the zona inserta and surrounding regions shown in Fig. 3 to contain cells that were affected by these injections. Also, lesions in the ventral thalamus produced degeneration in the lateral hypothalamus. The fact that responsive neurons were found only in these related lateral areas and not in ventromedial nuclei is noteworthy. Numerous investigators have reported reciprocal relationships between activities of the lateral areas and the ventromedial nuclei of the hypothalamus. Anand (1), Oomura, et al. (24), and Brooks, Koizumi, and Zeballos (5) all have reported reciprocal actions, using similar recording techniques in cats. On the other hand, the degeneration studies of Wolf and Sutin (35) showed that in rats lesions in the lateral hypothalamus and lesions in the ventral thalamus failed to produce degenerations in the ventromedial nuclei. There may be species differences in interrelationships, but thus far evidence indicates that portal receptors activate only the lateral hypothalamus directly.

The response of hypothalamic cells to portal injection of glucose could be either an increase or a decrease in firing rate. Cells in close proximity could show the opposite effect. Oomura et al. (25) demonstrated the same phenomenon by iontophoretic application of glucose through microinjections. He found that cells in the lateral hypothalamus could respond by either an increase or a decrease in rate of firing, whereas cells in the ventromedial nuclei always responded by an increase in discharge rate. These observed variations of response of cells within the lateral hypothalamus might be explained if one assumes that these stimuli may activate interneurons located in this region, which can exert inhibitory or facilitatory influence on cells directly concerned with food intake.

Changes in the composition of the immediate milieu of cells could have opposite effects on these cells either by direct action or through activation of related neurons. In the present study, it was found that the same cell might respond differently at different times, that is, depending on the time in the diurnal cycle at which the injection was given. This will be discussed at length in another report (32).

Since hypertonic NaCl injection into the portal vein likewise influenced the activity of hypothalamic cells which were located approximately in the same area as those influenced by glucose injection, the existence and role of a possible "osmoreceptor" in this portal vein-hepatic complex need to be discussed. Haberich and co-workers (11-13) and Ohm and Haberich (23) obtained evidence suggesting that there are osmoreceptors in the immediate region supplied with blood by the hepatic portal vein. These receptors were thought to influence the brain of the state of osmolarity of the blood more rapidly and accurately than could be done by the mechanism of central osmoreception described by Verney (34). The regulation of ADH release and water and salt excretion were thought to be altered by action of these receptors and this same neural pathway. Gauer (10), in a survey of volume-regulating mechanisms, emphasized the likelihood that such a source of osmolar information was present. However, up to this time, no one has satisfactorily demonstrated, either anatomically or physiologically, the presence of these receptors.

Lesions in the lateral hypothalamus not only lead to complete aphagia but also to complete adipsia (17). Cort (6) contends that food intake is intimately tied up with water intake, and Brobeck (3) suggests that cells that respond to changes in water concentration in body fluids, so-called osmoreceptors, are also involved in regulation of feeding. A "drinking center" first described by Anderson and McCann (2) was found to be closely related to or in the same area as the feeding center (18-20).

The work cited does indicate an interrelationship between glucose and hypertonic sodium chloride as a central stimulant. It is possible, therefore, that cells responsive to both types of solutions, hypertonic glucose and sodium chloride, may participate in regulation of nutritional homeostasis.

Whether or not the portal circuit contains receptors to other blood-borne compounds remains to be determined. Ammonium ions do cause firing of hypothalamic cells affected by portal injections of glucose and sodium chloride but not in low concentrations. Ammonium ions appear to exert their effects through a generalized or toxic action but definitely not through selective portal NH4Cl receptors.

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