essential step in the formation of the permanent and more complex nociceptive neuronal system in the primate spinal cord.

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Intraventricular Alloxan Eliminates Feeding Elicited by 2-Deoxyglucose

Abstract. *Evidence suggests that alloxan reacts with membrane-bound glucoreceptors and that it competes with glucose molecules for these sites. We therefore administered small quantities of alloxan into the cerebrospinal fluid of rats to determine what effect this might have on their ability to react to changes of glucose concentration. Rats treated in this manner did not eat as much as controls in response to the intraperitoneal administration of 2-deoxyglucose or to a 24-hour fast, and they became hypoglycemic significantly sooner than controls when fasted. The data suggest that the function of brain glucoreceptors is to protect the body from sudden decreases of glucose and that these glucoreceptors play little if any role in the normal regulation or maintenance of feeding, body weight, or blood glucose concentrations.*

The presence of glucoreceptive neurons within the central nervous system (CNS) has been suggested by physiological and behavioral experiments (*1*, *2*). Several functions have been attributed to these glucoreceptors, including control of normal feeding (*3*), body weight regulation (*4*), and protection against changes of blood glucose concentrations which could compromise brain functioning (*5*). Because glucose is a primary source of energy for most brain cells, it has frequently been assumed that CNS glucoreceptor cells are responsive to changes of energy availability as reflected by changes in the rate of glucose utilization (*6*, *7*).

However, a second mechanism has been postulated to exist in some cells for the recognition of changes of glucose levels. Research on the endocrine pancreas suggests that the insulin-secreting B (beta) cells initially respond to glucose molecules interacting with cell membrane receptors and only later respond to the change of intracellular metabolic activity generated by the transported glucose (*8*). There is also evidence for a membrane-bound glucoreceptor that influences secretion of glucagon from the A (alpha) cell of the pancreas (*9*). The experiments described herein suggest that the CNS glucoreceptors are similar and that their normal functions include initiation of reflexes that protect against glucoprivic challenges (during glucose deprivation) but do not include the control of normal eating or body weight regulation.

Alloxan is a toxic drug frequently used to elicit experimental diabetes mellitus in animals because large doses given systemically destroy B cells and hence limit the capacity of the organism to secrete insulin (*10*). The B cells can be protected from alloxan by the prior application of D-glucose (*11*), suggesting that alloxan molecules compete for the same receptor sites as glucose molecules (*12*). The observation that the protection against alloxan is influenced by the anomeric specificity of the glucose molecules (*13*) implies that alloxan is acting at a membrane-bound receptor [see (*8*)]. Alloxan has also been reported to reduce the capacity of the taste buds to respond to glucose, and prior treatment of the tongue with glucose protects against this response (*14*). Analogously, taste bud glucoreceptors have also been reported to be sensitive to the anomeric specificity of glucose (*15*). This suggests that membrane-bound glucoreceptors located in different organs may have similar properties.

Since there was considerable evidence for the presence of glucoreceptors in the brain (*1*-*5*), we administered small amounts of alloxan into the CNS by way of the ventricular system in an effort to determine if any responses attributed to these receptors were altered. We used female Wistar rats that were about 120 days old (375 g) at the beginning of the experiment. They were housed individually in standard hanging cages in a room with constant temperature and regular lighting conditions (12 hours of light and 12 hours of darkness; lights on at 7 a.m.) and had continuous access to water and food (Purina pellets).

Experimental rats received an injection of alloxan (40 μg in 2 μl of 0.9 percent saline) into the left lateral cerebral ventricle while they were anesthetized with Equithesin (3 ml/kg) and held in a stereotaxic instrument. The coordinates and procedures of the injection have been reported (*16*). Various control groups received either 2 μl of saline without alloxan injected into the left lateral cerebral ventricle, an intraperitoneal injection of 40 μg of alloxan, or no treatment at all. No reliable differences were observed among any of the control groups on any of the dependent variables monitored, and their data were pooled for the subsequent analyses.

Daily food intake and body weight were unaffected by the intraventricular (IVT) injection of alloxan for at least several months. These rats ate the same amount of food and gained weight at the same rate as rats in the combined control groups. Furthermore, preliminary observations indicated that the patterning of meals was also unaffected. This suggests that normal or spontaneous feeding does not rely on the integrity of whatever cells were affected by the treatment. Since no

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obvious behavioral effects were observed, any nonspecific toxic effects of the IVT alloxan were minimal. Glucose concentrations in blood obtained from nonfasting rats during the middle of the light phase were also similar for the experimental and control rats, both groups averaging around 70 mg/dl (see Fig. 1, time zero) (*17*). Therefore, under normal conditions no effect of IVT alloxan was observed.

Several weeks later we challenged both the experimental and control rats when they were in the nonfasted state with an intraperitoneal injection of 2-deoxyglucose (150 mg/kg), an agent known to elicit feeding by normal rats (*18, 19*). The injections were given during the light phase. Cumulative food intake was measured at 30-minute intervals for 3 hours and again at 24 hours. As shown in Fig. 2, the combined control rats had consumed an average of 4 g of food by 2 hours after the injections, but the rats that had previously received IVT alloxan had consumed an average of less than 1 g. The difference of food intake reached significance (two-tailed t-test, $P < .05$) by 1.5 hours after injection and was significant at each sampling time for the duration of the 3-hour test; and, although no longer statistically significant, the trend remained at the 24-hour measurement. Therefore, although the spontaneous food intake of IVT-alloxan rats was normal, feeding in response to the administration of a low dose of 2-deoxyglucose was essentially absent. The IVT-alloxan rats simply did not respond to this glucoprivic challenge.

In another group of rats which had received identical amounts of alloxan (IVT) or control treatment, a dose of 300 mg of 2-deoxyglucose per kilogram had a similar effect: controls consumed an average of 9.8 ± 1.5 g over the 3-hour interval after injection, whereas the IVT-alloxan rats consumed 4.1 ± 0.8($P <$ .05). In addition, in a separate experiment, the simultaneous injection of caffeine (50 mg/kg) and 2-deoxyglucose (300 mg/kg) also caused an analogous differential increase of food intake in IVT-alloxan rats over a 3-hour period. This combined drug treatment reportedly increases the sensitivity of rats to 2-deoxyglucose (*20*). Therefore, these data suggest that there may be some residual increment of feeding because of the excitation of other, perhaps peripheral, sites sensitive to low glucose concentrations.

In another series of experiments, we withheld food to impose a more natural method of reducing functional glucose levels and stimulating food intake. Blood

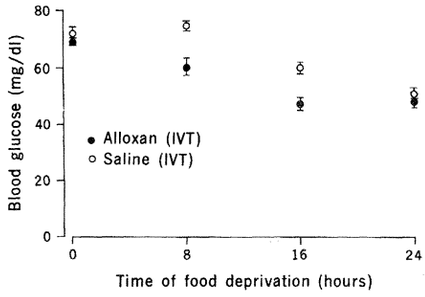

Fig. 1. Mean concentrations of glucose in the blood of rats which received either alloxan ($N = 9$) or saline ($N = 9$) as a function of hours of food deprivation. Symbols represent the means ± standard errors of the means.

glucose concentrations were determined every 8 hours for 24 hours (Fig. 1). Although both the experimental and control rats had essentially equal concentrations of glucose prior to fasting (time zero), those in the IVT-alloxan group became hypoglycemic significantly sooner than control rats. The differences of glucose concentrations attained were significant (two-tailed t-test, $P < .05$) after both 8 and 16 hours of fasting. By 24 hours without food, the difference had disappeared, both groups being comparably hypoglycemic. These results suggest that brain glucoreceptors may normally provide an important initial defense against ensuing hypoglycemia and that, if these receptors have been compromised by alloxan, the initial decline of glucose during a fast proceeds unchecked. However, glucose concentrations did not decrease below 45 mg/dl and were maintained at that level even though the animals continued to be fasted. This suggests that a second, probably peripheral, defense protects against a progressively worsening hypoglycemia.

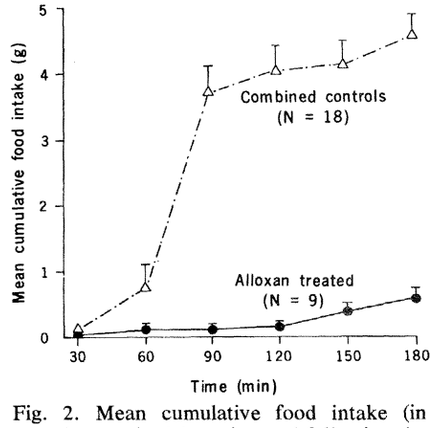

Fig. 2. Mean cumulative food intake (in grams) over the 3-hour interval following the intraperitoneal administration of 2-deoxyglucose (150 mg/kg). Symbols represent the mean and 1 standard error of the mean.

We also measured food intake after 24 hours of food deprivation in both the light and the dark phases of the 24-hour light cycle. In both instances there was a significantly greater food intake by the control rats over the 2 hours following food presentation relative to that of the IVT-alloxan rats (*21*).

Analysis of the brains of the rats which had received IVT alloxan revealed no obvious pathology in the region of the ventral hypothalamus under light microscopy. This region has been implicated as a site for CNS glucoreception (*1-4, 22*). We have not examined other likely sites such as the circumventricular organs, nor has a systematic analysis of the ventral hypothalamus been completed.

Our finding that rats injected with IVT alloxan show no obvious disruption of spontaneous feeding, weight regulation, or blood glucose concentrations, except under certain conditions, implies that the CNS glucoreceptors normally play little if any role in the maintenance of these responses. However, when functional glucose concentrations are lowered either by the intraperitoneal administration of 2-deoxyglucose or by imposed fasting, rats that had previously received IVT alloxan did not respond to these glucoprivic challenges as normal rats do. They ate less when given 2-deoxyglucose or a prolonged fast and became hypoglycemic significantly sooner when fasted.

These results indicate that CNS glucoreceptors are part of a glucoprivic emergency system that functions when glucose levels are decreased, and that alternative systems may exist for the maintenance of blood glucose concentrations. The results also suggest that CNS glucoreceptors may be similar to those of the pancreas and tongue and that they are probably associated with the cell membrane. Oomura and his colleagues recently reached a similar conclusion on the basis of electrophysiological observations of single cells in the hypothalamus (*23*).

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Short-Term Memory: The "Storage" Component of Human Brain Responses Predicts Recall

Abstract. *An evoked potential component with a poststimulus peak at about 250 milliseconds is related to the storage of information in short-term memory. This storage component was found in an investigation of brain potentials in relation to a number and letter comparison task. In replications of this experiment at three different light intensities spaced 1.0 log unit apart, the component had essentially the same waveform and pattern of scores. The memory storage interpretation was confirmed in a behavioral experiment that probed short-term memory. Recall was predicted by the magnitude of the storage component.*

A critical ingredient in most human information processing is the short-term storage of incoming information so that it can be integrated with other incoming information. Various memory processes have been proposed as retainers of information for various lengths of time (*1*), for example, a sensory register, a short-term store, and a long-term store. We now present electrophysiological and behavioral evidence for a neural process related to storage in short-term memory. A latent component of electrically recorded brain responses [evoked potentials (EP's)] with a poststimulus peak about 250 msec was found to be related to the storage of stimulus information for later use in tests comparing numbers and letters.

We discovered the storage component of the EP and tentatively interpreted it as being associated with information storage in an experiment in which the latent components and component scores of the brain responses from 12 subjects were obtained by a varimaxed principal components analysis (*2*). The storage component, which is the focus of this report, was one of eight orthogonal EP components obtained from that analysis.

The generality of the storage component and its independence of the physical characteristics of the stimuli were tested in further experiments (*3*). The same stimuli and procedures were used except that the intensity of all stimuli within a run of 102 trials was ten times greater, the same, or one-tenth that of the original experiment (*4*).

Two numbers and two letters were flashed individually in random order at intervals of 3/4 second preceded and followed by a blank flash. The subject's task was to compare the two numbers on number-relevant runs, the letters being irrelevant to the task. On the other half of the runs, the numbers were irrelevant and the task was to compare the two letters (*5*).

The stimulus processing demanded by the task depended on a number of factors, including whether (i) number or letter stimuli were task-relevant, (ii) the number or letter class of stimulus could be anticipated, and (iii) the character was the first or second relevant stimulus of the pair to be compared. For the first relevant stimulus in each trial, the information had to be stored by the subject until the second relevant stimulus occurred, after which the comparison could be made. While the subject was performing the letter or number comparison tasks, electrical brain activity [electroencephalogram (EEG)] was recorded from scalp electrodes (*6*).

By averaging the brain activity evoked by stimuli for similar conditions, averaged EP's were obtained for 16 conditions: relevant and irrelevant numbers and letters at four intratrial positions. From trial to trial, the first number (or letter) occurred in intratrial positions 1, 2, or 3 and the second in intratrial positions 2, 3, or 4. To simplify interpretations, certain EEG data were discarded, so the EP's for intratrial positions 1 and 2 were based only on the first number and letter stimuli presented within each trial, whereas the EP's for intratrial positions 3 and 4 were based only on the second number and letter stimuli. For each of the three intensities, EP's were collected in the same manner, and each of the three sets of data was analyzed separately (*7*). Latent components and component scores of each of the data matrices were computed according to varimaxed principal components analysis (*2*, *8*).

In all three data sets, an EP component emerged that was strikingly similar to the storage component previously found (*2*) with regard to both waveform and relative magnitude for the 16 conditions (Fig. 1, A to C). The waveforms of these components are similar in all four sets of data, reaching their maximum about 250 msec after the stimulus. The coefficients of factorial similarity among

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