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BRIEF COMMUNICATION

Noradrenergic Feeding System in Monkey Hypothalamus is Altered by Localized Perfusion of Glucose, Insulin, 2-DG and Eating[1,2]

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MCQUEEN, A., S. ARMSTRONG, G. SINGER AND R. D. MYERS. *Noradrenergic feeding system in monkey hypothalamus is altered by localized perfusion of glucose, insulin, 2-DG and eating.* PHARMAC. BIOCHEM. BEHAV. 5(4) 491–494, 1976. – Hypothalamic sites in the monkey were labelled by micro-injections of ^{3}H-NE and successive push-pull perfusions were carried out at a rate of 25 μl/min. When the monkey was fed, ^{3}H-NE within the perifornical region increased. When 2-deoxy-D- glucose (2-DG) was added to the perfusate, ^{3}H-NE release was also enhanced, whereas insulin perfused at the same rate caused a delayed increase in catecholamine levels as reflected by increased radioactivity. Glucose supressed the release of ^{3}H-NE, suggesting overall that the noradrenergic feeding system in the hypothalamus of the monkey is modulated by the regional level of glucose as well as the local concentration of insulin.

Hypothalamus Feeding Glucose ^{3}H-norepinephrine Noradrenergic mechanisms Insulin 2-deoxy-D-glucose Push-pull perfusion

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THE THEORY that noradrenergic pathways in the brain-stem mediate feeding behavior is based principally on pharmacological studies. When norepinephrine (NE) is microinjected into specific areas of the hypothalamus of the rat, monkey and other species, the previously satiated animal consumes food [1, 7, 17], the magnitude of feeding depending on the dose of NE [3, 14, 25]. Such NE-induced feeding is attenuated if the hypothalamic locus or sites along the ventricle are pretreated with an alpha adrenergic antagonist, but eating is enhanced by substances such as desmethyl-imipramine which prevents re-uptake of endogenous NE [2, 10, 26, 27].

At present, only three pieces of direct physiological evidence support the view that endogenous catecholamines in the brain stem are involved in feeding: (1) the release of a catecholamine-like factor from the diencephalon of a food deprived monkey [31]; (2) the enhanced release of ^{3}H-NE from the medial hypothalamus which is evoked when the fasted rat feeds [12]; and (3) changes in catecholamine content in the hypothalamus as a result of food deprivation [5,6].

More recently, basic questions have been directed towards the problem of the precise function of the noradrenergic pathways in the diencephalon. For example, what activates the catecholaminergic system of neurons? What is the role of these synapses in regions such as the

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ventromedial and lateral hypothalamic areas, classically implicated as comprising major convergent systems for hunger and satiety?

In the present experiments, we have confirmed that eating releases NE [12] and also shown that insulin, glucose and its competitive analogue, 2-DG, all affect the activity of noradrenergic neurons, as reflected by changes in the release of ^{3}H-NE within the hypothalamus of the fed or fasted monkey.

METHOD

Following procedures described previously [20], an array of four 18 ga push-pull guide tubes was implanted stereotaxically in two monkeys, under aseptic conditions, above sites in the perifornical region of the hypothalamus. On post-operative recovery, each monkey was kept in a primate restraining chair and maintained on ad lib water and a twice-daily feeding regimen until its intake of food had stabilized.

To label NE stores in the monkey's hypothalamus [22], 25 μCi NE-7-^{3}H (Amersham Searle Ltd.) were microinjected in a 1 μl volume at a predetermined depth 7–10 mm below the tip of the guide tube. The aliquot of isotope was drawn into a 28 ga needle and after the injector was lowered into the guide, the injection was made over a 60 sec interval. The needle was kept in place for 45 sec after the injection to permit dispersion of the solution. After a period of 30 min had elapsed, concentric push-pull cannulae [18] were lowered bilaterally to the same depth at which the ^{3}H-NE was infused. The push-pull pump was switched on and a perfusion was carried out for 5 min at a rate of 25 μl/min.

Each sample was expelled into collecting vials and a 50 μl aliquot was pipetted into a scintillation vial containing 10 ml of Brays solution [12]. The activity of each sample of perfusate was counted for 10 min on a Packard 3320 Tri-Carb liquid scintillation spectrometer. Successive perfusions were carried out at 20 min intervals. During the fourth perfusion, the animal was either fed biscuits composed of rat chow, or, alternatively, one of the following solutions was substituted for the control CSF solution [19] used as the perfusion medium: glucose (2.75, 5.5 or 10% w/v); 2 deoxy-d-glucose (2-DG, 50 or 100 μl) in CSF; and bovine insulin (50 or 100 mU/25μl). A total of seven successive samples of perfusate were collected before the washout in radioactivity had reached asymptote, and at least 24 hr elapsed between each experiment. To determine the sites of push-pull perfusion at the termination of the experiments, each animal was given an overdose of sodium pentobarbital; the brain was perfused with formalin, then sectioned and stained according to standard histological procedure.

RESULTS AND DISCUSSION

The proportional method of Hall and Turner [8] was employed in the analysis of the data as follows: the radioactivity in the third sample, that is the one immediately preceding the episode of feeding, (or the addition to the perfusate of one of the above-mentioned compounds) was taken as representative of 100% radioactivity. Then the DPM (disintegrations per minute) value in each perfusate was calculated as the proportion of this value.

Figure 1 illustrates the results of a set of experiments in which the site of perfusion of the monkey's hypothalamus

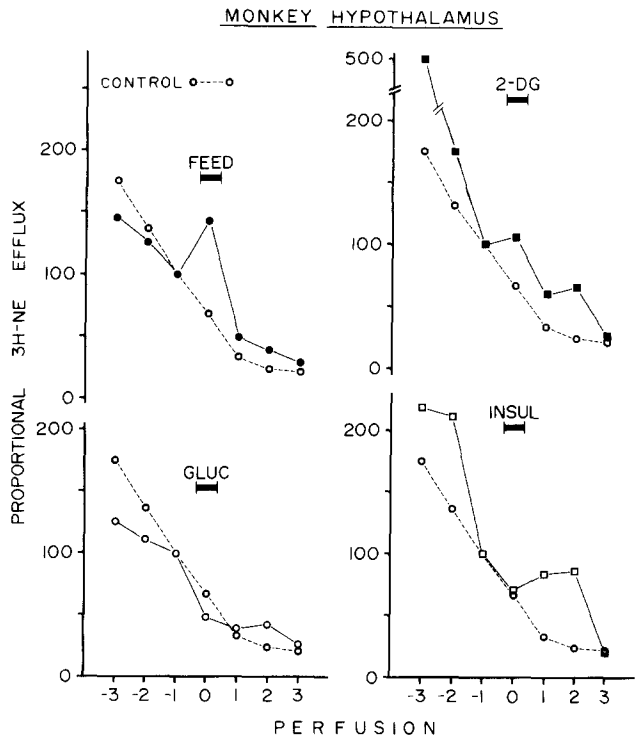

FIG. 1. Proportional efflux (see text) of ^{3}H-norepinephrine (NE) from a push-pull perfusion site in the perifornical region of the monkey's hypothalamus. A control washout curve of ^{3}H activity is plotted in each experiment (o---o). Upper left: monkey ate biscuits during the 0 perfusion. Upper right: 50 μg/min of 2-DG was perfused during the 0 interval (⊢—⊣). Lower left: 5.5% glucose was perfused during the 0 interval (⊢—⊣). Lower right: 100 mU/min of insulin was perfused during the 0 interval (⊢—⊣).

was verified to be adjacent to the descending columns of the fornix at AP 15.0. In each experiment the feeding or the addition of the compound to the perfusate always occurred during the fourth perfusion, as denoted at ZERO by the black bar on the figure. At this zero point, the fasted animal was fed (left top) or given either 5.5% glucose (left bottom) or 50 μg/min 2-DG (right top) or 100 mU insulin (right bottom) as an addition to the perfusion medium. The pattern of ^{3}H-NE activity under the four experimental conditions is contrasted with the control curve which depicts the typical washout of radioactivity at the same site when there is no experimental manipulation [13]. As the monkey was eating (left top) the efflux of ^{3}H-NE was enhanced. The perfusion at 50 μg/min of 2-DG also caused an immediate enhancement in ^{3}H-NE release even though the monkey was not allowed to eat (right top). On the other hand, the perfusion of 5.5% glucose suppressed the release of the catecholamine from this same perifornical locus (left bottom). Insulin added to the push-pull perfusion solution evoked yet another pattern of ^{3}H-NE efflux. During its perfusion in a concentration of 100 mU/min for 5 min, insulin exerted no immediate effect on the kinetics of NE release; however, during the two following perfusions, 20 and 40 min later, the activity of ^{3}H-NE was augmented. This delayed action of insulin is illustrated in Fig. 1 (right bottom).

These experiments provide the first direct in vivo evidence that the noradrenergic feeding system has a physiological basis in addition to its pharmacological

significance as shown in earlier studies [10,21]. That is, insulin, glucose and its antagonist, 2-DG, all act differentially on neurons that have taken up radioactive NE, as reflected by the changes in the ^{3}H-NE activity in the perfusates. Of special significance is the fact that the sites at which ^{3}H-NE release is altered by these compounds are homologous to those at which feeding causes an enhanced catecholamine release [24].

Our results confirm those obtained with the rat in which feeding or lever pressing for food pellets augments the release of hypothalamic NE and its metabolites from perfusion loci identified along the midline, anterior and perifornical regions [12,13]. The findings suggest that cells sensitive to glucose [4, 11, 16, 23, 24] and to insulin [9, 28, 29] in the lateral or ventromedial regions of the hypothalamus could either contain NE in their nerve endings or, on the other hand, synapse upon other neurons containing this monamine. Further, 2-DG which causes feeding presumably by mimicing local signals of glucoprivation [15,30] produces a release of the catecholamine similar to that following eating.

In a preliminary set of as yet unpublished experiments with the monkey and the rat, we have noted also an opposite efflux pattern of ^{3}H-NE from one push-pull cannula site positioned contralaterally in the hypothalamus. This occurred with feeding as well as during the perfusions with glucose, insulin and 2-DG. Support is thus offered for the concept that NE synapses could modulate both excitatory and inhibitory mechanisms [10] in different parts of this structure. Thus, the act of feeding, the presence of excess glucose or a local deficit of the sugar can serve to alter the endogenous activity of NE. Our results favour the view that the titre of plasma nutrients could modify directly the synaptic response pattern of noradrenergic neurons in the hypothalamus in a complementary manner [21].

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