*Pharmacology Biochemistry & Behavior*, Vol. 27, pp. 715–721. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

# Norepinephrine Release From PVN and Lateral Hypothalamus During Perfusion With 2-DG or Insulin in the Sated and Fasted Rat

J. M. PEINADO[1] AND R. D. MYERS[2]

*Departments of Psychiatry and Pharmacology, and Center for Alcohol Studies*
*University of North Carolina, School of Medicine*
*Chapel Hill, NC 27514*

Received 8 December 1986

PEINADO, J. M. AND R. D. MYERS. *Norepinephrine release from PVN and lateral hypothalamus during perfusion with 2-DG or insulin in the sated and fasted rat.* PHARMACOL BIOCHEM BEHAV 27(4) 715–721, 1987.—Both insulin and 2-deoxy-D-glucose (2-DG) when given systemically to the rat modify the activity of noradrenergic systems in different regions of the animal's hypothalamus. The purpose of the present investigation was to ascertain whether the nutritional status of the animal would serve to influence the pattern of efflux of norepinephrine (NE) from sites in the hypothalamus perfused with either 2-DG or insulin. Permanent guide cannulae were first implanted stereotaxically above the paraventricular nucleus (PVN) or lateral hypothalamus (LH). Following recovery from surgery, each rat was either satiated with food or deprived of food for 20–22 hr with water always freely available. Then 0.1 μCi of [$^3$H]-NE was micro-injected into the intended site of perfusion in a volume of 1.0 μl. After 15 min had elapsed, the site was perfused repeatedly with an artificial CSF at a rate of 20 μl/min. At the mid-point of successive 5.0 min perfusions, either 10 μg/μl 2-DG or 4.0 mU/μl porcine insulin was incorporated into the CSF perfusate. Thereafter, an additional set of 3–4 samples of perfusate was collected. When perfused in the PVN of the satiated rat, 2-DG significantly enhanced the efflux of [$^3$H]-NE, whereas in the fasted animal insulin tended to suppress the output of the catecholamine. Conversely, at sites of perfusion in the LH, insulin evoked the release of [$^3$H]-NE when the rat was fasted, whereas 2-DG tended to induce mixed effects on the release of [$^3$H]-NE under both sated and fasted conditions. The results demonstrate that 2-DG as well as the pancreatic hormone can exert a unique action on the kinetics of noradrenergic activity in the hypothalamus which is solely dependent on the morphological site. Further, within each of the anatomical areas examined, the nutritional status of the rat, i.e., fasted or satiated, serves to determine the impact of these feeding factors on the release of NE.

Norepinephrine release Paraventricular nucleus (PVN) Lateral hypothalamus (LH) Insulin
2- Deoxy-D-glucose (2-DG) Food deprivation Satiety Noradrenergic neurons Rat Push-pull perfusion
Catecholamine neurotransmitter

FOR over a decade, evidence has continued to accumulate that the synaptic activity of noradrenergic neurons in the diencephalon, as reflected by the evoked release of norepinephrine (NE), is involved in the central control of feeding [21,22]. To illustrate, a substantial increase in the activity of NE occurs within circumscribed hypothalamic sites as the fasted rat consumes food pellets [17,29]. Further, both insulin and 2-deoxy-D-glucose (2-DG), which when given systemically act to induce spontaneous feeding, also can modify the phasic release of NE from the paraventricular nucleus (PVN) or other areas of the hypothalamus of the rat [13]. Moreover, insulin or 2-DG delivered locally to the medial or lateral hypothalamus (LH) of the rat or monkey can augment or attenuate transiently the efflux of the catecholamine from homologous anatomical sites [15,16].

In relation to the specific role played by NE in the mediation of the feeding response induced by central glucoprivation [24], there is incomplete agreement principally because of differences in several experimental findings. Nevertheless, it is likely that insulin given systemically to the fasted or satiated rat serves to rapidly signal the brain to reduce the overall activity of NE in the hypothalamus [27]. Further, from an anatomical perspective, insulin binding in the normal rat is lower in receptors obtained from the lateral hypothalamus than in medial hypothalamic tissue [18]; however, the prolonged restriction of food reduces the binding of insu-

[1]Present address: Department of Physiology and Biophysics, University of Granada, Granada, Spain 18003.
[2]Requests for reprints should be addressed to Professor R. D. Myers, Director, Bowles Biomedical Research Laboratory, Medical Research Bldg. A 218H, University of North Carolina, Chapel Hill, NC 27514.

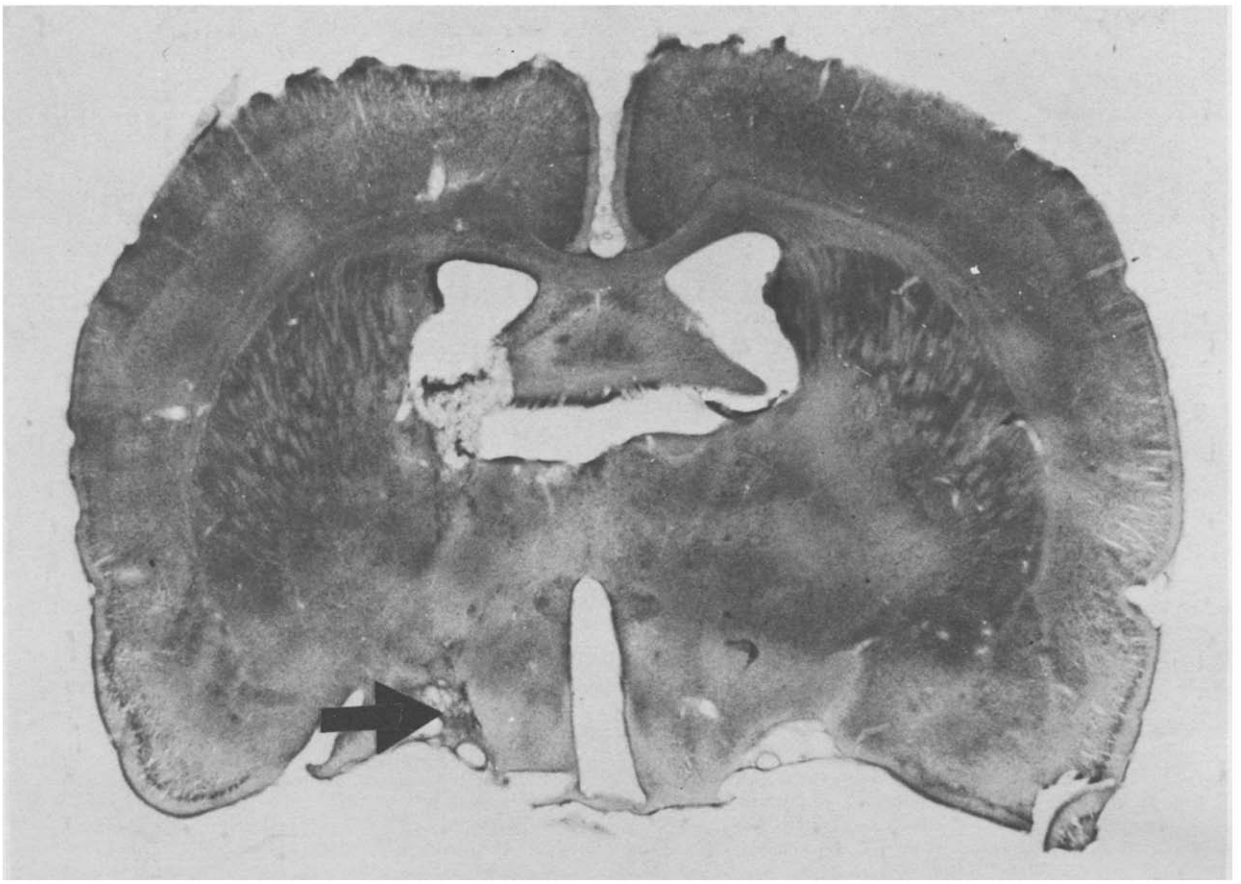

FIG. 1. Histological section in the coronal plane depicting site of perfusion (arrow) in the lateral hypothalamic area of the rat.

lin only in fractions obtained from the medial area of the rat's hypothalamus [18]. In this connnection, the ventromedial portion of the hypothalamus also is apparently involved in the peripheral elevation of circulating levels of insulin as correlated with the presentation of a food stimulus [28].

Recently, it was found unexpectedly that two other peptides, cholecystokinin (CCK) and neurotensin (NT), which are thought to be involved in the central mechanism underlying satiety, also act directly on the hypothalamus to enhance or reduce differentially the efflux of NE [23]. However, the functional effect of both peptides depends entirely upon the anatomical site within the hypothalamus, lateral or medial, as well as on the state of hunger of the rat, i.e., fasted or satiated [10]. The present investigation was undertaken, therefore, to determine whether a reciprocal relationship exists similarly within the lateral or medial hypothalamus following an insulin or 2-DG challenge, which in turn is contingent upon the nutritional status of the animal. In these experiments, the rat was either satiated or food-deprived before either insulin or 2-DG was perfused within the PVN or LH at sites labelled earlier with radioactive NE.

## METHOD

Sprague-Dawley rats of either sex, weighing 272±6.0 g were housed in individual cages in a laboratory room maintained at a temperature of 21–23°C. The room was illuminated on a reversed 12 hr cycle with red fluorescent lighting on from 1030–2230 hr. Food and water were always freely available except in those experiments in which the rat was food-deprived for a period of 20–22 hr. In each case, the animal was provided with a standard ration of Purina rat chow ad lib for an interval of two hr after each series of perfusions.

### *Surgery*

Under equithesin anesthesia (2.0 ml/kg), a single 20 ga stainless steel push-pull guide tube was implanted stereotaxically [20] in each of 13 rats to rest just dorsal to the region of tissue surrounding either the paraventricular nucleus (PVN) or, alternatively, the lateral hypothalamus (LH). The stereotaxic coordinates ranged from: AP 5.5 to 7.5; Lat 0.5 to 2.5 and Hor −1.0 to 3.0. After the cannula was lowered into position, cranioplastic cement was packed around the pedestal and four bone screws which had been inserted in the calvarium [20].

### *Perfusion Procedure*

Following 7–10 days post-operative recovery, each rat having a cannula placed dorsal to the PVN was screened to identify sites which were reactive to NE [12]. In each case NE (±arterenol hydrochloride, Sigma) in a dose of 1.0 $\mu$g/$\mu$l was micro-injected, at progressively deeper sites in a volume of 1.0 $\mu$l over a period of 1.0 min. An anatomical site was considered to be positively reactive when the animal consumed food with a latency of less than 5.0 min and over an interval of greater than 2.0 min. Subsequently, a set of successive push-pull perfusions was undertaken at that precise depth, with each animal not only serving as its own control but also tested ordinarily under both sated and fasted conditions.

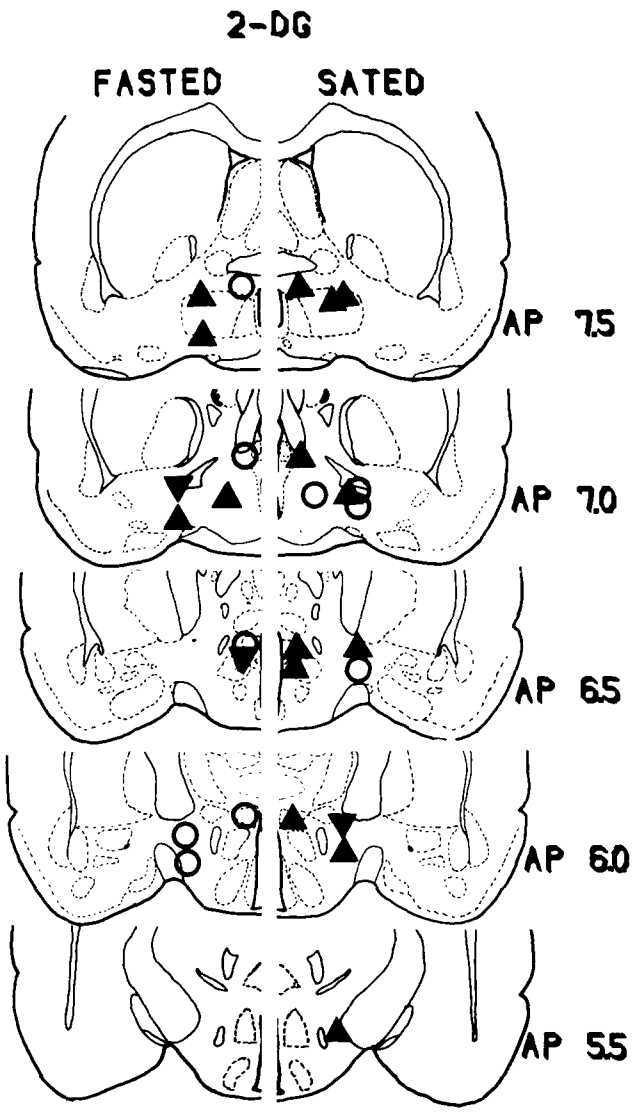


FIG. 2. Anatomical mapping of $^3$H-NE-labelled sites between AP 7.5 and AP 5.5 in medial and lateral hypothalamic areas perfused with 10 $\mu$g/$\mu$l 2-DG. At the time of perfusion, animals were either sated (right) or food-deprived (left). Nature of $^3$H-NE release denoted as follows: ▲=increase; ▼=decrease; ○=no change.

Each anatomical site of perfusion was radio-labelled with 0.1 $\mu$Ci of DL-(7-$^3$H) Norepinephrine HCl (Amersham; specific activity 17.6 Ci/mMol) infused over a period of 1.0 min in a volume of 1.0 $\mu$l according to standard procedures [22]. Following an interval of 15.0 min, the labelled site then was perfused with an artificial cerebrospinal fluid (CSF) containing $Na^+$ 127.7 mM; $K^+$ 2.6 mM; $Ca^{++}$ 1.3 mM; $Mg^{++}$ 0.9 mM and $Cl^-$ 134.6 mM [10]. A standard concentric push-pull cannula system [20], consisting of an outer 23 ga needle and inner 28 ga tube, was connected by PE tubing to a Harvard infusion-withdrawal pump. To prevent degradation of the amine, the CSF was adjusted to pH 4.0 with 0.01 mg/ml of ascorbic acid [16]. Immediately prior to each sequence of perfusions the CSF was passed through a 0.22 $\mu$m Swinnex filter (Millipore) into a pyrogen-free vial. In each experiment, 7–8 perfusions were undertaken sequentially, with each sample collected for a period lasting 5.0 min and an interval of 5.0 min elapsing between successive perfusions [14].

After the level of radioactivity in a series of perfusates had begun to stabilize, usually after the third perfusion, either 4.0 mU/$\mu$l porcine insulin (Squibb) or 10.0 $\mu$g/$\mu$l 2-DG (Sigma) were added to the perfusion fluid. These concentrations were selected on the basis of their efficacy in earlier experiments in which a significant change in the activity of NE was evoked at circumscribed sites in the hypothalamus [15,16]. An aliquot of 80.0 $\mu$l of each sample of perfusate was transferred to a scintillation vial containing 4.0 ml of PCS fluor solution (Amersham/Searle). The samples were then counted for 10.0 min in a Tracor Mark III liquid scintillation spectrometer as described previously [22].

*Histology and Data Analysis*

At the end of the experiments, the rat was perfused transcardially with 0.9% NaCl, followed by 10% buffered formalin. Each brain was placed on a cryostat, sectioned at 40 microns in the coronal plane, and stained with cresyl violet according to standard histological procedures [30]. The location of each perfusion site was verified subsequently under light microscopy and then reconstructed on standard anatomical maps [10,23]. A representative histological section of a perfusion site in the LH of the rat is presented in Fig. 1.

The statistical analysis of the data was undertaken according to the standard Hall-Turner method [8], which is used to determine the proportional change in $^3$H-NE activity from the base-line level of radioactivity contained in the third sample of the perfusion sequence. Statistical comparisons were made using a Student $t$-test between the means obtained for the control washout set of perfusions and the means obtained for a given structure, LH or PVN, as well as for the two compounds perfused, 2-DG or insulin.

## RESULTS

When applied directly to the hypothalamus of the unrestrained rat, insulin and 2-DG evoked differential effects on $^3$H-NE activity. However, the magnitude of effect and the direction of change produced by these substances depended not only on the nutritional status of the rat but also on the anatomical site of perfusion.

*Anatomical Analysis*

At loci of perfusion within the PVN and contiguous tissue, 2-DG in the satiated rat consistently evoked an increase in the efflux or $^3$H-NE. As shown in Fig. 2. (RIGHT), sites encompassing the PVN from AP 6.0 through AP 7.0 as well as those in medial and mid-pre-optic areas were uniformly reactive to the local presence of 2-DG. On the other hand, when the same sites were perfused after the individual rat was deprived of food for 20–22 hr, 2-DG failed to augment the release of $^3$H-NE; in one case, 2-DG perfused within AP 6.5 inhibited the efflux of this catecholamine. Perfusion of the LH with 2-DG generally elicited a series of mixed effects on the kinetics of $^3$H-NE release either under the fasted or sated condition. As illustrated in Fig. 2. (LEFT), within three of four sites at AP 7.0 and AP 7.5, 2-DG enhanced $^3$H-NE output in the fasted rat whereas within AP 6.0, 2-DG was without a clear-cut effect.

The perfusion of insulin within the PVN or the more rostral but adjacent medial pre-optic region of the sated animal

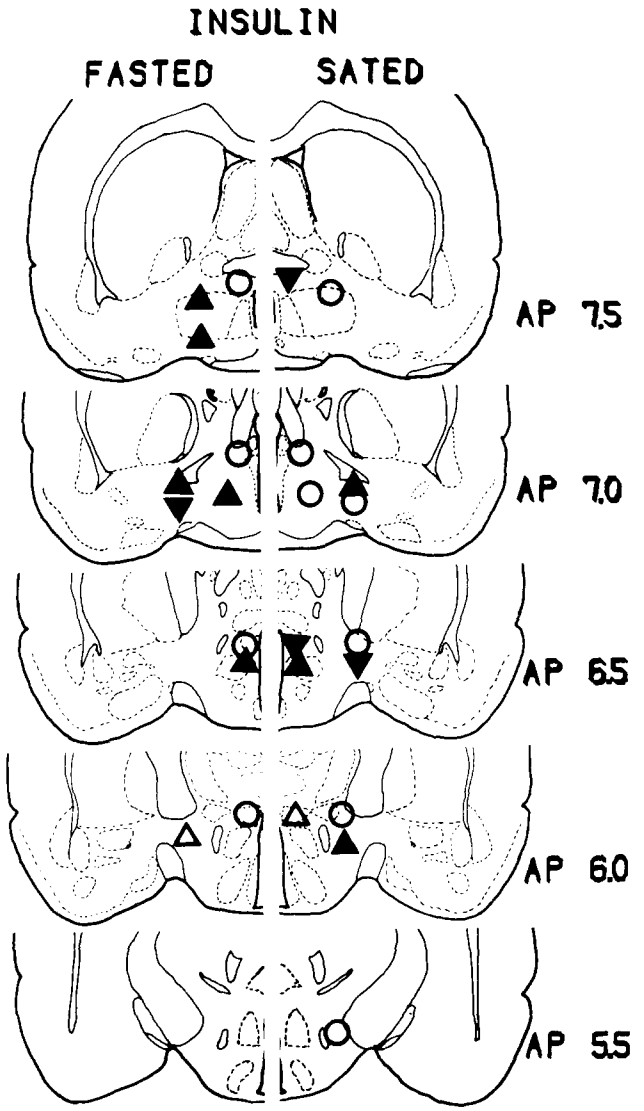


FIG. 3. Anatomical mapping of $^3$H-NE-labelled sites between AP 7.5 and AP 5.5 in medial and lateral hypothalamic areas perfused with 4.0 mU/$\mu$l porcine insulin. At the time of perfusion, animals were either sated (right) or food-deprived (left). Nature of $^3$H-NE release denoted as follows: ▲=increase; △=delayed increase; ▼=decrease; ○=no change.

produced mixed effects on the activity of $^3$H-NE in anatomical sites extending from AP 6.0 through AP 7.5. As illustrated in Fig. 3 (RIGHT), these effects included an enhanced, reduced or delayed release of $^3$H-NE or no effect on the catecholamine's activity. Similarly, when the same rats were fasted, insulin exerted no effect in three of four coronal planes in which a perfusion series was undertaken (Fig. 3, LEFT). However, within the LH and the lateral aspect of the pre-optic area extending from AP 6.0 through AP 7.5, insulin evoked an immediate release or delayed augmentation of $^3$H-NE output in the food-deprived rat (Fig. 3, LEFT) at all but one of the loci of perfusion (AP 7.0). However, when insulin was perfused in the lateral area of the fully sated animal, this peptide hormone failed to induce consistent alterations in the efflux of $^3$H-NE within the eight sites of perfusion (Fig. 3, RIGHT).

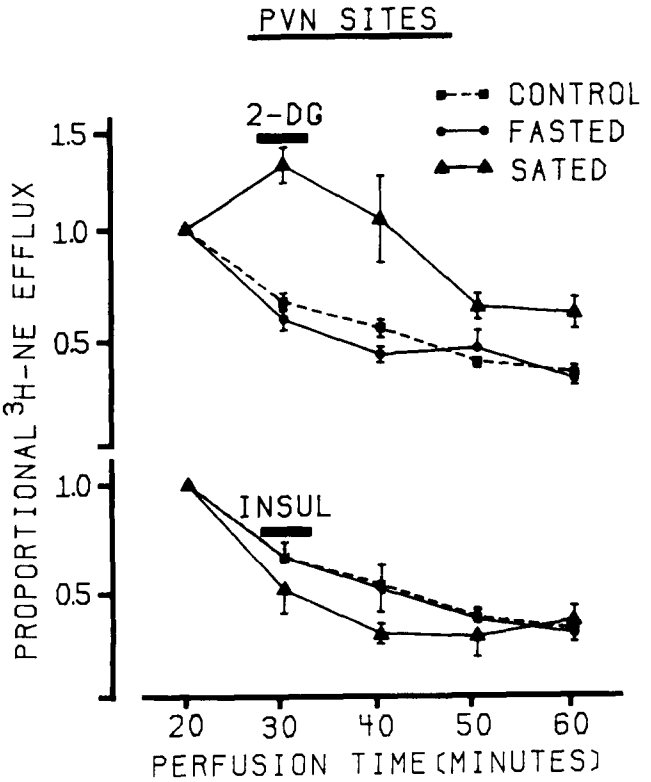


FIG. 4. Mean (±S.E.) proportional efflux of 3H-NE efflux across time within paraventricular nucleus of hypothalamus (PVN) either of sated or food-deprived rats. Either 4.0 mU/$\mu$l insulin (INSUL) or 10.0 $\mu$g/$\mu$l 2-DG was perfused at the 30 min interval (denoted by bar). During the control perfusions, only the CSF vehicle was perfused at the same flow rate and conditions.

*2-DG and Insulin in PVN*

A composite analysis of the temporal characteristics of both 2-DG and insulin on $^3$H-NE activity within the PVN and adjacent tissue is presented in Fig. 4. When the rats were satiated (n=6), 2-DG caused an intense and substantial efflux of $^3$H-NE from this medial hypothalamic region, $t(11)=3.04$, $p<0.05$. There were no significant changes, however, when the same sites were perfused after the animals had been deprived of food (Fig. 4, TOP). As portrayed in Fig. 4 (BOTTOM), insulin perfused in the PVN of the fasted rats (n=5) failed to alter the pattern of declining radioactivity characteristic of the control washout perfusion [14,17]. Although insulin tended to suppress the output of $^3$H-NE during the satiated condition, this effect was not statistically significant, $t(9)=0.57$, $p>0.05$.

*2-DG and Insulin in LH*

In accord with the mixed effect produced by 2-DG perfused within sites of the LH, as depicted in Fig. 2, the temporal pattern of $^3$H-NE release revealed an elevation in the activity of $^3$H-NE during both the sated and fasted conditions. Figure 5 illustrates the somewhat prolonged output of $^3$H-NE activity induced by 2-DG. Conversely, a relatively sharp but variable enhancement in the efflux of $^3$H-NE was produced by insulin in the fasted rat, as the peptide was perfused in this lateral area. Moreover, this release was sustained significantly to an even higher level, $t(5)=2.85$,

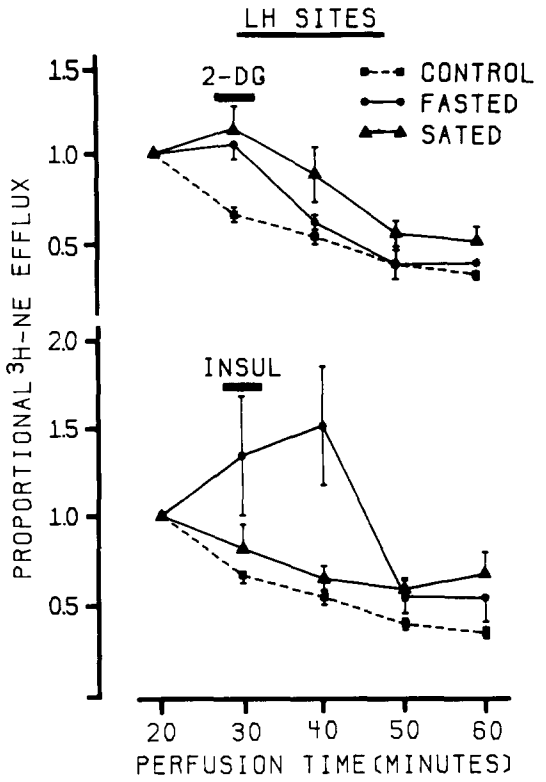


FIG. 5. Mean (±S.E.) proportional efflux of $^3$H-NE efflux across time within lateral hypothalamus (LH) either of sated or food-deprived rats. Either 4.0 mU/μl insulin (INSUL) or 10.0 μg/μl 2-DG was perfused at the 30 min interval (denoted by bar). During the control perfusions, only the CSF vehicle was perfused at the same flow rate and conditions.

$p<0.05$, following the discontinuation of the perfusion, i.e., at the 40 min sample. Under the satiated condition, however, no significant alteration in $^3$H-NE efflux occurred during or after the localized perfusion with insulin.

## DISCUSSION

That a functional interaction exists between insulin and glucose in tissue of the brain as well as in the periphery is well known [24,31]. For example, it was shown recently that insulin produces a time- and dose-dependent excitation of 2-DG uptake in cultured glial cells obtained from the brain of the rat, which implies that a specific class of insulin receptors might mediate the utilization of glucose in brain tissue [5]. Further, it is apparent that there are functional differences between insulin receptors within neuronal and glial elements of the brain of the rat [13]. In addition, insulin-like immunoreactivity is differentially distributed anatomically in the rat's brain with particularly higher levels found in the paraventricular, supraoptic , arcuate and other hypothalamic nuclei, as well as in the lateral hypothalamus [2].

In contrast to these *in vitro* studies, the present results have demonstrated that in the intact and freely moving animal highly specific effects are exerted on the hypothalamus by 2-DG and insulin. Of special interest is the observation that the changes induced by 2-DG and insulin are differentiated on the basis of both the metabolic or nutritional status of the animal as well as the anatomical region of the hypothalamus examined [3]. In view of previous findings, however, these results are not necessarily unexpected. A glucoprivic stimulus seems to exert little or no effect on the turnover of NE in fractions of hypothalamic and other tissue obtained postmortem in the brain of the rat [24]. Nevertheless, considerable evidence does exist to suggest that a glucoprivic challenge introduced systemically or directly within the hypothalamic tissue of the intact and conscious animal does lead to a clear-cut change in the activity of the catecholamine systems [15,16].

Theoretically, it would appear that the state of hunger or satiety in the animal can bias or otherwise modulate a distinct population of neurons in both lateral and medial hypothalamic regions [2,9]. In fact, the metabolic and/or nutritional status of the animal, or even a specific foodstuff, can functionally modify the milieu of the neurons to the extent that a compound which impinges upon the feeding-satiety mechanism will differentially alter the local synaptic activity of these neurons [10,25]. With regard to specific hypothalamic mechanisms, one of several processes may be influenced by the presence of insulin, or 2-DG, or both substances. These would include: (1) the enhancement by 2-DG or insulin of the presynaptic release of the neurotransmitter into the synaptic cleft; (2) a local action of these substances on membranes of catecholaminergic-containing neurons; (3) an alteration in the selective pre- or postsynaptic uptake of the catecholamine; (4) a direct effect on the pre- or postsynaptic receptors, and (5) modification by 2-DG or insulin of the metabolism of the neurotransmitter in the nerve terminal itself [15, 21, 23].

If one considers that NE is an inhibitory neurotransmitter in the medial hypothalamus [10], then its presynaptic release would inhibit satiety neurons which are located presumably within the PVN [11]. As a consequence, when NE is applied locally to this structure, the rat would begin to eat immediately since the satiety mechanism is blocked [11]. In accord with this concept is the pattern of the noradrenergic response to 2-DG when it is applied directly to the PVN of the sated or fasted rat. This pattern corresponds physiologically to that which is envisaged to occur under normal conditions. In the satiated animal, NE release from neurons in the PVN would achieve presumably a steady state level since a deficit in glucose availability does not exist and, thus, there is no signal for hunger. Consequently 2-DG applied locally would produce conceivably a locally circumscribed condition of glucoprivation, a subsequent signal of hunger, and a resultant release of NE [21]. In turn, the satiety neurons would be inhibited so as to provide the appropriate neurochemical signal for the animal to consume food. Thus the local imbalance in glucose detected in the PVN would subsequently be restored. Whether input from the dorsomedial hypothalamus also is involved in the release of NE in the PVN cannot be determined from the present study; however, radioenzymatic data show that endogenous NE can increase in this nucleus and in the perifornical region during feeding [29].

On the other hand, when the rat is already fasted, the presynaptic efflux of endogenous NE presumably is at its maximal level, because the neurochemical signal to feed is already in full operation. Therefore, no modification in the activity in NE would be required. Hence, the local application of 2-DG, which again would cause a state of localized glucoprivation within the PVN should not necessarily enhance further the already elevated output of the catecholamine from this nucleus. The results of the present experi-

ments have tended to corroborate these theoretical viewpoints and in fact, correspond precisely with the independent observations made earlier [14]. That is, in the satiated rat, 2-DG infused systemically evokes the release of NE from sites within the medial hypothalamic but exerts little or no effect on the catecholamine's activity in the LH [14].

In the fasted rat, insulin perfused within the PVN does not alter consistently the output of NE, and in the satiated animal insulin can suppress its release. Within the LH, insulin exerts little or no effect during the sated condition but induces an intense and sustained efflux of NE after the animal is food-deprived. Given systemically to the satiated rat, insulin generally attenuates the release of NE from sites of perfusion in the LH [14]. Therefore, it is apparent that the local action of the pancreatic hormone on brain tissue differs substantially from the effect produced when the hormone acts via the periphery on hypothalamic neurons [14]. The regional level of insulin is thought to reflect a feedback signal denoting the oncoming state of satiety [6,31] since the hormone in circulating plasma may rise in conjunction with the ingestion of food and the subsequent termination of the meal. As a consequence, noradrenergic neurons in the PVN could detect a rise in the local titre of insulin as reflecting the condition of satiety, and would thereby suppress the release of NE or fail to affect its output. When the animal is food-deprived, insulin should not necessarily alter the already elevated release of the catecholamine from the medial structures. In relation to this is the *in vitro* finding that the presence of insulin even in neurons in primary cell culture can markedly alter the activity of NE [4].

The state-dependent difference in insulin's action on the kinetics of NE in the LH and PVN corresponds well with recent experiments on the neuronal binding properties of this hormone in the hypothalamus [19]. In the food-deprived rat, the specific binding of insulin to medial hypothalamic receptors is reduced, but unchanged in the LH receptors; however, insulin binding is lower in the LH of the sated rat than in its medial hypothalamus [18]. Further, it is of interest that in the fasted animal [26] the concentrations of 3 methoxy-4-hydroxy-phenylglycol (MHPG) in the whole hypothalamus, which reflects NE metabolism, are suppressed in the insulin-deficient rat fed a carbohydrate meal but stimulated by a protein meal supplemented with tyrosine [7]. Therefore, the idea that insulin could serve in a feedback manner as a metabolic signal to both populations of neurons in the LH and medial hypothalamus is supported [6,31].

Finally, it should be noted that the influence of 2-DG or insulin on other putative neurotransmitters is not yet known. For example a recent study using HPLC-EC showed that a peptide acting directly on hypothalamic tissue can influence other monoaminergic transmitters including serotonin and dopamine [22]. Thus, future research utilizing a similar experimental strategy is required to delineate the interaction of 2-DG and insulin with these and other synaptic processes.

ACKNOWLEDGEMENTS

This research was supported in part by National Science Foundation Grant BNS-84-10663 and by the U.S.-Spain Joint Committee for Cultural and Educational Cooperation. The authors are indebted to D. M. Collins, T. Privette and S. Crovi for their excellent technical assistance.

## REFERENCES

1. Ashley, D. V. M. Nutritional control of brain neurotransmitter synthesis and its implications. *Bib Nutr Dieta* **38:** 39–53, 1986.
2. Baskin, D. G., S. C. Woods, D. B. West, M. Van Houten, B. I. Posner, D. M. Dorsa and D. Porte. Immunocytochemical detection of insulin in rat hypothalamus and its possible uptake from cerebrospinal fluid. *Endocrinology* **113:** 1818, 1983.
3. Bernardis, L. L. Ventromedial and dorsomedial hypothalamic syndromes in the weanling rat: Is the "center" concept really outmoded? *Brain Res Bull* **14:** 537–549, 1985.
4. Boyd, F. T., Jr., D. W. Clarke, T. F. Muther and M. K. Raizada. Insulin receptors and insulin modulation of norepinephrine uptake in neuronal cultures from rat brain. *J Biol Chem* **260:** 15880–15884, 1985.
5. Clarke, D. W., F. T. Boyd, Jr., M. S. Kappy and M. K. Raizada. Insulin binds to specific receptors and stimulates 2-deoxy-D-glucose uptake in cultured glial cells from rat brain. *J Biol Chem* **259:** 11672–11675, 1984.
6. Figlewicz, D. P., D. M. Dorsa, L. J. Stein, D. G. Baskin, T. Paquette, M. R. C. Greenwood, S. C. Woods and D. Porte, Jr. Brain and liver insulin binding is decreased in Zucker rats carrying the 'fa' gene. *Endocrinology* **117:** 1537–1543, 1985.
7. Glanville, N. T. and G. H. Anderson. Hypothalamic catecholamine metabolism in diabetic rats: the effect of insulin deficiency and meal ingestion. *J Neurochem* **46:** 753–759, 1986.
8. Hall, G. H. and D. M. Turner. Effects of nicotine on the release of $^{3}$H-noradrenaline from the hypothalamus. *Biochem Pharmacol* **21:** 1829–1838, 1972.
9. Kasser, T. R., A. Deutsch and R. J. Martin. Uptake and utilization of metabolites in specific brain sites relative to feeding status. *Physiol Behav* **36:** 1161–1165, 1986.
10. Lee, T. F., A. H. Rezvani, J. R. Hepler and R. D. Myers. Neurotensin releases norepinephrine differentially from the perfused hypothalamus of the sated and fasted rat. *Am J Physiol* **252:** E102–E109, 1987.
11. Leibowitz, S. F. Neurochemical systems of the hypothalamus: control of feeding and drinking behavior and water-electrolyte excretion. In: *Handbook of the Hypothalamus*, Vol 3, edited by P. J. Morgane and J. Panksepp. New York: Marcel Dekker, 1980, pp. 299–437.
12. Leibowitz, S. F., G. R. Weiss, F. Yee and J. B. Tretter. Noradrenergic innervation of the paraventricular nucleus: Specific role in control of carbohydrate ingestion. *Brain Res Bull* **14:** 561–567, 1985.
13. Lowe, W. L., Jr., F. T. Boyd, D. W. Clarke, M. K. Raizada, C. Hart and D. LeRoith. Development of brain insulin receptors: structural and functional studies of insulin receptors from whole brain and primary cell cultures. *Endocrinology* **119:** 25–35, 1986.
14. McCaleb, M. L. and R. D. Myers. 2-Deoxy-D-glucose and insulin modify release of norepinephrine from rat hypothalamus. *Am J Physiol* **242:** R596–R601, 1982.
15. McCaleb, M. L., R. D. Myers, G. Singer and G. Willis. Hypothalamic norepinephrine in the rat during feeding and push-pull perfusion with glucose, 2-DG, or insulin. *Am J Physiol* **236:** R312–R321, 1979.
16. 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. *Pharmacol Biochem Behav* **5:** 491–494, 1976.
17. Martin, G. E. and R. D. Myers. Evoked release of ($^{14}$C) norepinephrine from the rat hypothalamus during feeding. *Am J Physiol* **229:** 1547–1555, 1975.
18. Melnyk, R. B. and J. M. Martin. Starvation-induced changes in insulin binding to hypothalamic receptors in the rat. *Acta Endocrinol* **107:** 78–85, 1984.
19. Melnyk, R. B. and J. M. Martin. Failure of chronic experimental hyperinsulinism to alter insulin binding to hypothalamic receptors in the rat. *Acta Endocrinol* **107:** 86–90, 1984.

20. Myers, R. D. Methods for perfusing different structures of the brain. In: *Methods in Psychobiology*, Vol II, edited by R. D. Myers. London: Academic Press, 1972, pp. 169–211.
21. Myers, R. D. Brain mechanisms in the control of feeding: A new neurochemical profile theory. *Pharmacol Biochem Behav* **3:** Suppl 1, 75–83, 1975.
22. Myers, R. D. and M. L. McCaleb. Feeding: satiety signal from intestine triggers brain's noradrenergic mechanism. *Science* **209:** 1035–1037, 1980.
23. Myers, R. D., H. S. Swartzwelder, J. M. Peinado, T. F. Lee, J. R. Hepler, D. M. Denbow and J. M. R. Ferrer. CCK and other peptides modulate hypothalamic norepinephrine release in the rat: Dependence on hunger or satiety. *Brain Res Bull* **17:** 583–597, 1986.
24. Rowland, N. E., L. L. Bellush and J. Carlton. Metabolic and neurochemical correlates of glucoprivic feeding. *Brain Res Bull* **14:** 617–624, 1985.
25. Savard, P., Y. Merand, J. Leblanc and A. Dupont. Limitation of access to highly palatable foods increases the norepinephrine content of many discrete hypothalamic and amygdaloidal nuclei of rat brain. *Life Sci* **33:** 2513–2519, 1983.
26. Schweiger, U., M. Warnhoff and K.-M. Pirke. Norepinephrine turnover in the hypothalamus of adult male rats: alteration of circadian patterns by semistarvation. *J Neurochem* **45:** 706–709, 1985.
27. Smythe, G. A., J. E. Bradshaw, M. V. Nicholson, H. S. Grunstein and L. H. Storlien. Rapid bidirectional effects of insulin on hypothalamic noradrenergic and serotonergic neuronal activity in the rat: role in glucose homeostasis. *Endocrinology* **117:** 1590–1597, 1985.
28. Storlien, L. H. The ventromedial hypothalamic area and the vagus are neural substrates for anticipatory insulin release. *J Auton Nerv Syst* **13:** 303–310, 1985.
29. Van der Gugten, J. and J. L., Slangen. Release of endogenous catecholamines from rat hypothalamus in vivo related to feeding and other behaviors. *Pharmacol Biochem Behav* **7:** 211–219, 1977.
30. Wolf, G. Elementary histology for neuropsychologists. In: *Methods in Psychobiology*, Vol 1, edited by R. D. Myers. New York: Academic Press, 1971, pp. 281–300.
31. Woods, S. C., D. Porte, Jr., E. Bobbioni, E. Ionescu, J.-F. Sauter, F. Rohner-Jeanrenaud and B. Jeanrenaud. Insulin: its relationship to the central nervous system and to the control of food intake and body weight. *Am J Clin Nutr* **42:** 1063–1071, 1985.

## NOTE ADDED IN PROOF

Recently it was reported that the release of epinephrine as well as NE from medial hypothalamic sites, corresponding to those in the present study, was enhanced during periods when the rat feeds and immediately thereafter (Kruissink, N., J. Van Der Gugten and J. L. Slangen. Short-term feeding-related changes in mediodorsal hypothalamic catecholamine release. *Pharmacol Biochem Behav* **14:** 575–579, 1986).