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Alterations in Catecholamine Levels and Turnover in Discrete Brain Areas After Food Deprivation

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JHANWAR-UNIYAL, M., M. DARWISH, B. E. LEVIN AND S. F. LEIBOWITZ. *Alterations in catecholamine levels and turnover in discrete brain areas after food deprivation.* PHARMACOL BIOCHEM BEHAV **26**(2) 271–275, 1987.—The present study determined the levels and turnover of norepinephrine (NE), epinephrine (EPI) and dopamine (DA) in discrete brain areas of rats after 48 hr food deprivation. The steady-state levels of NE, EPI and DA in saline-treated food-deprived rats, relative to satiated rats, remained basically unchanged. However, 48 hr deprivation caused a site-selective potentiation, specifically in the hypothalamic paraventricular nucleus, in the depletion of NE after α-methyl-p-tyrosine injection (IP, 200 mg/kg), indicating an increase in NE turnover. While changes in EPI turnover could not be demonstrated, an apparent increase in DA turnover was detected in the perifornical lateral hypothalamus and anterior hypothalamic nucleus after deprivation, while decreased DA turnover was seen in the hypothalamic dorsomedial nucleus and caudate nucleus. These results may reflect specific functions of hypothalamic catecholamines in control of food intake.

Food deprivation Norepinephrine Epinephrine Dopamine Levels Turnover Hypothalamus

A great deal of evidence has shown that levels and turnover of hypothalamic catecholamines (CA) are responsive to a variety of pharmacological manipulations, as well as to changes in the animal's endocrinological and nutritional state. In particular, biochemical studies have implicated norepinephrine (NE) and dopamine (DA) in the regulation of feeding behavior [10, 22, 23, 34], an idea strongly supported by various pharmacological and behavioral studies involving the injection of neurotransmitters directly into discrete hypothalamic sites of rats [12, 13, 15]. These pharmacological studies indicate the possible existence of an α_2-noradrenergic system in the medial paraventricular hypothalamus for stimulation of food intake and more specifically carbohydrate intake [5, 11, 13, 29]. On the other hand, there may also exist CA receptor systems (β-adrenergic and dopaminergic) in the peripheral lateral hypothalamus which reduce feeding, particularly of protein [13, 14, 17].

With regard to biochemical studies supportive of hypothalamic CA function in feeding behavior, it has been documented that 48 hr food deprivation, which enhances feeding, increases NE turnover in the whole hypothalamus as well as medial hypothalamus [3, 4, 33, 34], while decreasing turnover in the basal hypothalamus, medial preoptic area, and cortex [26]. Moreover, food deprivation has been shown to increase DA metabolism and turnover in parts of the hypothalamus and amygdala [3, 7, 30].

While these earlier studies investigating turnover have provided regional analyses of the effects of food devrivation on CA metabolism, they leave unchartered more discrete sites which may respond differentially to changes in nutritional state. The present biochemical study, which used the CA synthesis inhibitor α-methyl-p-tyrosine (α-MpT) to deplete endogenous CA and thereby estimate tunrnover, examines CA metabolism in microdissected tissue of animals exposed to 48 hr food deprivation.

METHOD

Adult male albino Sprague-Dawley rats (n=32), weighing 350–400 g, were group-housed in a temperature controlled (24°C) colony room and maintained ad lib on Purina lab chow pellets and water, with a 12/12 hr light-dark cycle and lights on at 06.00 hr.

Four groups of animals were examined. Two groups, one satiated (n=8) and one 48 hr food deprived (n=8), received intraperitoneal saline injection (0.5 ml) 3 hr prior to decapitation, to measure changes in brain CA levels. To estimate CA turnover, two additional groups of satiated (n=8) and food deprived (n=8) rats were injected intraperitoneally with α-MpT (200 mg/kg) 3 hr prior to decapitation. All rats were sacrificed by decapitation between 07.00 and 09.00 hr. The brains were rapidly removed, frozen on dry ice, and serial sections of 300 μm were cut in a cryostat and placed under a

TABLE 1

LEVELS OF CATECHOLAMINES (pg/μg PROTEIN) AFTER 48 HR FOOD DEPRIVATION

	PVN	DMN	VMH	POM	AH	PFH	CAUD	FC	HiCA1
Norepinephrine									
Satitated	59.7±5.7 (6)	28.4±2.9 (8)	30.0±1.0 (7)	28.5±3.4 (8)	21.4±1.9 (8)	37.0±3.1 (8)	ND	5.3±0.3 (8)	7.7±2.1 (7)
Food-Deprived	58.8±6.5 (6)	28.6±5.6 (8)	31.9±4.3 (8)	21.9±2.6 (8)	19.7±1.3 (8)	29.0±2.7 (8)	ND	5.0±0.4 (4)	5.1±0.7 (6)
Epinephrine									
Satiated	3.9±0.5 (7)	2.0±0.4 (6)	3.5±0.9 (7)	1.7±0.4 (6)	2.3±0.3 (5)	1.7±0.5 (4)	ND	ND	ND
Food-Deprived	3.5±0.8 (5)	2.1±0.6 (5)	3.6±1.3 (7)	1.4±0.2 (2)	2.8±0.4 (6)	1.9±0.8 (6)	ND	ND	ND
Dopamine									
Satiated	7.3±2.2 (8)	6.8±1.2 (7)	8.7±1.3 (6)	5.7±1.6 (6)	5.0±1.4 (6)	6.1±1.1 (6)	80.2± 7.5 (7)	ND	ND
Food-Deprived	8.4±1.7 (8)	4.9±0.4 (6)	10.1±2.1 (7)	5.7±1.0 (7)	6.4±0.9 (4)	4.7±0.8 (8)	38.6±10** (4)	ND	ND

The table indicates the levels of norepinephrine, epinephrine and dopamine measured in satiated and 48 hr food-deprived rats. Given are mean±SEM, with the number of animals used in parentheses. ND=not detected. PVN, paraventricular nucleus; DMN, dorsomedial nucleus; VMH, ventromedial hypothalamus; POM, medial preoptic area; AH, anterior hypothalamus; PFH, perifornical hypothalamus; CAUD, caudate; FC, frontal cortex; HiCA1, hippocampus (dorsal). **Significantly different from satiated rats ($p<0.01$).

dissecting microscope. The tissue samples were microdissected according to the method of Palkovits [25]. Six hypothalamic nuclei, namely, the paraventricular nucleus (PVN), dorsomedial nucleus (DMN), ventromedial hypothalamus (VMH), lateral perifornical hypothalamus (PFH), medial preoptic area (POM) and anterior hypothalamus (AH), and also three extrahypothalamic areas, namely, the caudate nucleus (CAUD), frontal cortex (FC) and the dorsal cell-containing region of the hippocampus (HiCA1), were examined for the levels and turnover of NE, epinephrine (EPI) and DA.

For measurement of CA levels, bilateral tissue samples were placed in 150 μl of cold 0.2 N perchloric acid in microfuge tubes (1000 μl capacity) and homogenized by sonification at 4°C for 5 sec. The samples were acidified and stored at −70°C until assayed within 1–14 days. The tissue CA levels were measured by high performance liquid chromatography, as described by Levin [19]. For the protein analysis, aliquots of 25 μl of the tissue homogenate were immediately taken prior to acidification and estimated according to the method of Lowry *et al.* [20]. The measures of CA levels are expressed in pg/μg protein, and CA turnover is estimated by the percent differences of α-MpT-treated satiated or food-deprived rats from their saline baseline rats. The mean CA levels in food-deprived and satiated rats were statistically analyzed by single factorial analysis of variance and by appropriate post hoc tests for direct comparisons between individual means.

RESULTS

The impact of 48 hr food deprivation on steady-state CA levels is illustrated in Table 1. Relative to the baseline of satiated rats, food derpivation failed to produce any significant changes in NE content in any of the discrete brain sites analyzed. This includes the PVN, which had by far the highest level of endogenous NE. The only brain area which showed a significant difference in DA content after food deprivation was the CAUD, which decreased its DA level by 52% ($p<0.01$). None of the six hypothalamic areas showed a significant change in EPI levels, in the food-deprived rats as compared to satiated rats.

The turnover of NE, EPI and DA was measured in satiated and food-deprived rats pretreated with α-MpT. The results of these tests can be found in Fig. 1. With regard to NE (top panel of Fig. 1), only the PVN showed a significant increase, $F(3,23)=9.02$, $p<0.05$, in turnover, as reflected by a 50% drop in NE levels in food deprived α-MpT-treated rats versus only a 30% drop in satiated α-MpT-treated rats. All other seven discrete brain areas, namely the DMN, VMH, POM, AH, PFH, FC and HiCA1, showed no difference in NE turnover between the deprived and satiated rats. Measurements of DA, presented in the bottom panel of Fig. 1, revealed that food deprivation caused a significant increase in DA turnover (i.e., a greater depletion) in two hypothalamic areas, namely, the AH, $F(3,17)=4.5$, $p<0.05$, and PFH, $F(3,20)=7.77$, $p<0.05$, in contrast to a decrease in DA turnover (a smaller depletion) in the DMN, $F(3,20)=15.7$, $p<0.001$, and CAUD, $F(3,26)=5.3$, $p<0.05$. The effect of food deprivation on EPI turnover (middle panel of Fig. 1) was minimal.

DISCUSSION

The results demonstrate that, while steady-state levels of the three amines in discrete hypothalamic nuclei are basically unaltered by 48 hr food deprivation, changes in NE turnover may occur specifically in the PVN, in contrast to alterations in DA turnover that may be detected in at least four brain sites (PFH, AH, DMN and CAUD). The failure to observe changes in steady-state CA levels following food

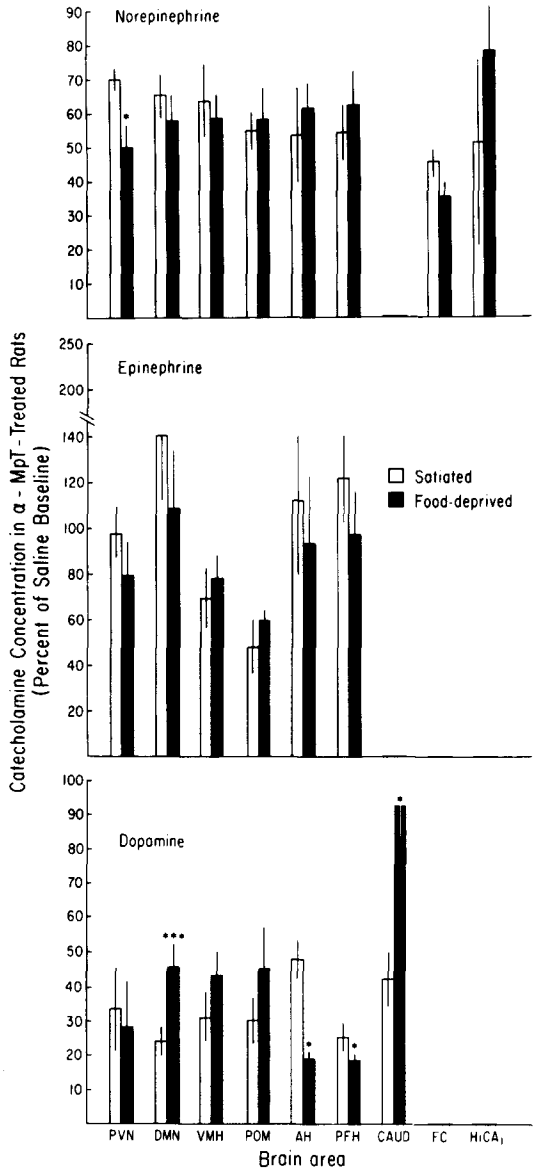

FIG. 1. Effect of food deprivation on brain catecholamine metabolism. Satiated (open bar) and 48 hr food-deprived (solid bar) rats were treated with saline or α-methyl-p-tyrosine (200 mg/kg, α-MpT, IP) and decapitated 3 hr later. Each bar represents the scores of the percent difference of α-MpT-treated satiated or food-deprived rats compared to their respective saline-injected groups (used as baseline). The results of statistical comparison by *t*-test for post-hoc comparisons after ANOVA revealed significant differences at $*p<0.05$ and $***p<0.001$. PVN, paraventricular nucleus; DMN, dorsomedial nucleus; VMH, ventromedial hypothalamus; POM, medial preoptic area; AH, anterior hypothalamus; PFH, perifornical hypothalamus; CAUD, caudate nucleus; FC, frontal cortex; HiCA1, hippocampus (dorsal).

deprivation corroborates earlier studies of whole hypothalamus [3] and lateral hypothalamus, DMN, and POM [33]. However, food deprivation decreases NE content in the whole, medio-basal and ventromedial hypothalamus [4, 28, 33], whereas a provision of palatable food for a restricted period produces an increase in NE content in the PVN, suprachiasmatic nucleus, and amygdala [27].

Existing studies demonstrate an increase in NE turnover in whole and medial hypothalamus after food deprivation [3, 33, 34]. The present study, investigating discrete hypothalamic areas, indicates an increase in NE turnover exclusively in the PVN, apparently reflecting a deprivation-induced enhancement of noradrenergic activity. This is in contrast to the medio-basal hypothalamus where a decrease in NE turnover is found in 5-day deprived rats treated with α-MpT [26]. This difference in response observed in the basal and dorsal portions of the medial hypothalmus may reflect either an anatomical differentiation or possibly the differing periods of food deprivation studied, namely, 5 days in the basal hypothalamus and 2 days in the present study. Additional evidence, that feeding and increased hunger are associated with an increase in hypothalamic NE metabolism, is the enhanced NE release observed in the medial and medio-dorsal hypothalamus prior to or at the onset of feeding [10,35], followed by a gradual decline in NE release during and after feeding [35]. Further supporting the role of NE in feeding is the decreased NE release in the medial hypothalamus, in particular the PVN, subsequent to gastrointestinal loading [24], indicating a reversal of the deprivation-induced increase in NE turnover. It may be noted that these changes in the NE system may also be due, in part, to alterations in DA metabolism. Dopamine serves as a substrate for NE synthesis, and DA itself is known to play a role in feeding behavior [7, 23, 30].

As a consequence of this deprivation-induced change in presynaptic NE activity, α-adrenergic receptor sites within the hypothalamus may also be altered. This appears to occur with basal hypothalamic α_2-adrenergic receptors, which are up-regulated by 5 days of food deprivation [32] in association with a decrease in NE turnover [26]. In the dorso-medial portion of the hypothalamus, the α_2-adrenergic receptors are also significantly affected by deprivation; in this case, however, they are down-regulated by 48 hr food deprivation, as well as by 1–3 hr of deprivation specifically at the onset of the dark cycle [8,9]. This change in medial hypothalamic receptor binding appears to be attributed predominantly to alterations within the PVN itself [8,9]. In this nucleus, as opposed to other medial and lateral hypothalamic areas, we have observed a dramatic (65%) decrease in α_2-adrenergic receptor binding following deprivation and, in the present study, a site-specific increase in NE turnover. This biochemical evidence is consistent with pharmacological studies indicating that NE and α_2-adrenergic receptors, specifically in the PVN, act to stimulate food ingestion, possibly by inhibiting local satiety neurons [11–13].

In addition to these changes in NE metabolism, an increase in DA turnover, in whole and posterior hypothalamus, VMH and amygdala [3, 7, 34], has been detected after food deprivation or food intake, as well as in response to the presentation of stimuli anticipating the presence of food by hungry rats [30]. In other studies, no change in DA levels, after 4 hr limited access to food, was demonstrated in the medial hypothalamus [7, 30, 34]. As with the above studies, the findings of the present investigation indicate that food deprivation has a differential effect on DA turnover, which is apparently decreased in the DMN, increased in the PFH and AH, and not changed in other areas (Fig. 1, bottom panel).

This deprivation-induced change in DA turnover in the PFH is of particular interest in light of cannula mapping studies, distinguishing this site as most responsive to the feeding suppressive effect of direct CA stimulation (dopaminergic and β-adrenergic) [12,14]. Systemic in-

jections of drugs, such as amphetamine and L-DOPA which increase the release of brain CAs, are also most effective in the suppression of food intake at this site. Using histofluorescence techniques, it has been demonstrated that the PFH contains fairly dense clusters of CA varicosities [15], and the neurochemical projections mediating the anorexic affect of amphetamine-induced CA release appear to originate in the medulla (containing NE and EPI) and ventral midbrain (DA) and terminate in the lateral PFH [15,21]. Further supporting the role of PFH CA neurotransmitters in feeding behavior is the observation that gastric loading, which reduces food intake, increases NE release at this site [24]. The observation that food deprivation and intraperitoneal administration of amphetamine enhances DA metabolism within the PFH [16] indicates that DA at this site may play a role in normal feeding behavior. Food deprivation is also associated with a decrease in binding of ^{3}H-amphetamine to hypothalamic receptors, which is strongly correlated with the level of deprivation, as well as with circulating levels of blood glucose [6].

Variations in blood glucose levels have been linked to the eating process. Specifically, decreased glucose levels are found to be correlated with the magnitude of the eating response that occurs consequent to a brief period of deprivation [1,18]. A strong positive correlation, between blood glucose levels and α_2-adrenergic receptor binding, has been detected in the medial hypothalamus [2], and in particular the PVN [8], after three or six hr food deprivation or after treatment with tolbutamide. Additional studies demonstrate that glucose levels are reciprocal to hypothalamic, possibly PVN, NE activity [31], that a glucose satiety signal causes a decrease in medial hypothalamic (PVN) NE release [24], and that injections of NE into the PVN increase circulating levels of glucose [2]. In light of this relationship between blood glucose levels, NE metabolism and α_2-adrenergic receptor binding in the PVN, we propose that food deprivation may possibly exert its effect on NE turnover in the PVN, in part, through its known impact on circulating glucose.

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REFERENCES

1. Campfield, L. A., P. Brandon and F. J. Smith. On-line continuous measurement of blood glucose and meal pattern in free-feeding rats: The role of glucose in meal initiation. *Brain Res Bull* **14:** 605–616, 1985.
2. Chafetz, M. D., K. Paiko, S. Diaz and S. F. Leibowitz. Relationships between medial hypothalamic α_2-receptor binding, norepinephrine, and circulating glucose. *Brain Res* **384:** 404–408, 1986.
3. Friedman, E., N. Starr and S. Gershon. Catecholamine synthesis and the regulation of food intake in the rat. *Life Sci* **12:** 317–326, 1973.
4. Glick, S. D., D. H. Waters and S. Milloy. Depletion of hypothalamic norepinephrine by food deprivation and interaction with D-amphetamine. *Res Commun Chem Pathol Pharmacol* **6:** 775–778, 1973.
5. Goldman, C. K., L. Marino and S. F. Leibowitz. Postsynaptic α_2-noradrenergic receptors in the paraventricular nucleus mediate feeding induced by norepinephrine and clonidine. *Eur J Pharmacol* **115:** 11–19, 1985.
6. Hauger, R., B. Hulihan-Giblin, I. Angel, M. D. Luu, A. Janowsky, P. Skolnick and S. M. Paul. Glucose regulates [^{3}H]-amphetamine binding and NA^+K^+ ATPase activity in the hypothalamus: A proposed mechanism for the glucostatic control of feeding and satiety. *Brain Res Bull* **16:** 281–288, 1986.
7. Heffner, T. G., J. A. Hartman and L. S. Seiden. Feeding increases dopamine metabolism in the rat brain. *Science* **208:** 1168–1170, 1980.
8. Jhanwar-Uniyal, M., M. P. Papamichael and S. F. Leibowitz. Rapid effects of food deprivation on α_2-receptor binding in the paraventricular nucleus: relation to circulating glucose. *Soc Neurosci Abstr* **12:** 415, 1986.
9. Jhanwar-Uniyal, M. and S. F. Leibowitz. Impact of food deprivation on α_1- and α_2-noradrenergic receptors in the paraventricular nucleus and other hypothalamic areas. *Brain Res Bull* **17:** 889–896, 1986.
10. Kruissink, N., J. Van der Gugten and J. L. Slangen. Short-term feeding-related changes in mediodorsal hypothalamic catecholamine release. *Pharmacol Biochem Behav* **24:** 575–579, 1986.
11. Leibowitz, S. F. Paraventricular nucleus: A primary site mediating adrenergic stimulation of feeding and drinking. *Pharmacol Biochem Behav* **8:** 163–175, 1978.
12. Leibowitz, S. F. Neurochemical systems of the hypothalamus. Control of feeding and drinking behavior and water-electrolyte excretion. In: *Handbook of the Hypothalamus*, vol 3A, edited by P. J. Morgane and J. Panksepp. New York: Dekker, 1980, pp. 299–437.
13. Leibowitz, S. F. Brain monoamines and peptides: role in the control of eating behavior. *Fed Proc* **45:** 1396–1403, 1986.
14. Leibowitz, S. F. and C. Rossakis. Mapping study of brain dopamine- and epinephrine-sensitive sites which cause feeding suppression in the rat. *Brain Res* **172:** 101–113, 1979.
15. Leibowitz, S. F. and L. Brown. Histochemical and pharmacological analysis of catecholaminergic projections to the perifornical hypothalamus in relation to feeding inhibition. *Brain Res* **201:** 315–345, 1980.
16. Leibowitz, S. F., M. Jhanwar-Uniyal and B. E. Levin. Effects of amphetamine on catecholamine levels and turnover in discrete hypothalamic areas. *Brain Res* **266:** 348–354, 1983.
17. Leibowitz, S. F., G. Shor-Posner, C. Maclow and J. A. Grinker. Amphetamine: Effect on meal patterns and macronutrient selection. *Brain Res Bull* **17:** 681–689, 1986.
18. Le Magnen, J. The metabolic basis of dual periodicity of feeding in rats. *Behav Brain Sci* **4:** 561–607, 1981.
19. Levin, B. E. Retrograde axonal transport of β-adrenoceptors in rat brain: Effect of reserpine. *Brain Res* **300:** 103–112, 1984.
20. Lowry, O., N. Rosebough, A. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
21. McCabe, J. T. and S. F. Leibowitz. Determination of the course of brainstem catecholamine fibers mediating amphetamine anorexia. *Brain Res* **311:** 211–224, 1984.
22. 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.
23. Martin, G. E. and R. D. Myers. Dopamine efflux from the brain stem of the rat during feeding, drinking and lever-pressing for food. *Pharmacol Biochem Behav* **4:** 551–560, 1976.
24. Myers, R. D. and M. L. McCaleb. Feeding: satiety signal from intestine triggers brain's noradrenergic mechanism. *Science* **209:** 1035–1037, 1980.

25. Palkovits, M. Isolated removal of hypothalamic or other brain nuclei of the rat. *Brain Res* **59:** 449–450, 1973.
26. Pirke, K. M. and B. Spyra. Catecholamine turnover in the brain and the regulation of luteinizing hormone and corticosterone in starved male rats. *Acta Endocrinol* **100:** 168–176, 1982.
27. Savard, P., Y. Merand, J. LeBlanc and A. Dupont. Limitation of access to highly palatable food increases the norepinephrine content of many discrete hypothalamic and amygdaloid nuclei of rat brain. *Life Sci* **33:** 2513–2519, 1983.
28. Schweiger, U., M. Warnoff 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.
29. Shor-Posner, G., A. P. Azar, S. Insinga and S. F. Leibowitz. Deficits in the control of food intake after hypothalamic paraventricular nucleus lesions. *Physiol Behav* **35:** 883–890, 1985.
30. Simansky, K. J., K. A. Bourbonais and G. P. Smith. Food-related stimuli increase the ratio of 3,4-dihydroxyphenylacetic acid to dopamine ratio. *Pharmacol Biochem Behav* **23:** 253–258, 1985.
31. Smythe, G. A., M. S. Gruntstein, J. E. Bradshaw, M. V. Nicholson and P. J. Crompton. Relationship between brain noradrenergic activity and blood glucose. *Nature* **308:** 65–67, 1984.
32. Spyra, B. and K. M. Pirke. Binding of [^{3}H] clonidine and [^{3}H] WB-4101 to the pre- and postsynaptic α-adrenergic receptors of the basal hypothalamus of the starved male rat. *Brain Res* **245:** 179–182, 1982.
33. Stachowiak, M., J. Bialowas and M. Jurkowski. Catecholamines in some hypothalamic and telencephalic nuclei of food deprived rats. *Acta Neurobiol Exp (Warsz)* **38:** 157–165, 1978.
34. Van der Gugten, J., E. R. DeKloet, D. H. G. Versteeg and J. L. Slangen. Regional hypothalamic catecholamine metabolism and food intake regulation in the rat. *Brain Res* **135:** 325–336, 1977.
35. 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.