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HPLC Determination of Biogenic Amines in Discrete Brain Areas in Food Deprived Rats[1]

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LOULLIS, C. C., D. L. FELTEN AND P. A. SHEA. *HPLC determination of biogenic amines in discrete brain areas in food deprived rats.* PHARMAC. BIOCHEM. BEHAV. 11(1) 89–93, 1979.—Norepinephrine (NE), dopamine (DA), 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), and 5-hydroxyindole acetic acid (5-HIAA) levels in the lateral hypothalamus (LH), ventromedial hypothalamus (VMH), median raphe (MR) and dorsal raphe (DR) were determined in nondeprived and 48 hr food deprived rats. Simultaneous determination of these compounds was accomplished by means of high performance liquid chromatography (HPLC) with electrochemical detection. When compared with controls, food deprived animals showed significant increases in 5-HT and 5-HIAA levels in the raphe nuclei, significant increases in 5-HIAA in the LH, but no changes in either 5-HT or 5-HIAA levels in the VMH. No changes in catecholamine levels were found in any of the brain areas studied. These results show that indoles in the raphe nuclei, as well as in the LH, are affected by food deprivation. The lack of change in indole levels in the VMH indicates that specific nuclei within the hypothalamus are differentially affected by food deprivation.

Catecholamines Indoles Food deprivation High performance liquid chromatography
Lateral hypothalamus Ventromedial hypothalamus Raphe nuclei

FOOD deprivation has been shown to influence the levels of indoles in the brain. Following food deprivation, whole brain 5-HIAA increases [2,10]. Serotonin, on the other hand, has been shown to either increase [2] or remain unchanged [10]. Increases in 5-HT and/or 5-HIAA have also been shown in the cortex, striatum, cerebellum, and midbrain plus hippocampus [7]. No changes in whole hypothalamic 5-HT and 5-HIAA were found [7]. Recently, however, increases in serotonin synthesis and turnover were demonstrated in the LH using push-pull cannula perfusions [4, 5, 6]. These studies appear contradictory. However, since the hypothalamus is comprised of several distinct nuclei, it has been suggested that assay of the entire hypothalamus might obscure discrete variation in neurochemical parameters in smaller regions [5].

Food deprivation has also been shown to increase whole hypothalamic DA levels and NE and DA synthesis [3]. Release of NE and DA from the medial hypothalamus of food deprived animals was shown to decrease when animals were allowed free access to food [14]. Furthermore, whole brain levels of homovanillic acid and dihydroxyphenylacetic acid, two DA metabolites, increased when food deprived rats were allowed free access to food [1].

The purpose of the present study was to determine the effects of food deprivation on biogenic amines in the LH, VMH, MR and DR. Determination of the biogenic amine levels in these brain areas was accomplished by a new, rapid HPLC electrochemical method.

METHOD

Animals

Twelve 3 month old male Wistar rats, with an average body weight of 296 ± 16 (SD) g, were allowed to adapt in individual home cages for 13 days. Animals were kept on a constant light-dark cycle (light 0600–1800 hr) and had free access to Purina Lab chow blocks and tap water. The room temperature was maintained at 70° ± 2° F.

Procedure

On Day 14 animals were randomly divided into two groups. The control group, N=6, continued to have free access to food and water whereas the experimental group, N=6, was deprived of food for 48 hr. On Day 16 all animals were sacrificed, between 0900 and 1100 hr, using the near freezing method of Takahashi and Aprison as modified by Shea and Aprison [11,13]. Individual brain parts were dissected in a cold box at −10°C. Tissue samples were stored frozen, at −70°C.

Dissection

Transverse cuts were made perpendicular to the axis of the brain stem with the ventral surface of the brain facing upward. In the hypothalamus, a perpendicular cut was made through the caudal portion of the infundibulum, corresponding approximately to A3990 μm in the König and Klippel

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atlas [8], as shown in Fig. 1. A second cut was made approximately 1 mm rostral to the first cut, caudal to the optic chiasm, producing a 1 mm thick section of diencephalon. The section was placed with the caudal surface facing upward. Two angled cuts at the base of the hypothalamus removed the arcuate nucleus and the median eminence bilaterally. Additional angled cuts dissected the ventromedial nucleus of the hypothalamus as a rectangular piece of tissue, oriented obliquely in the direction of the long axis of the nucleus. The lateral hypothalamus was dissected with two vertical cuts, one just lateral to the fornix, the other just medial to the internal capsule. Two additional oblique cuts removed the lateral hypothalamus as a trapezoidal piece of tissue. These hypothalamic nuclei were removed bilaterally.

In the brain stem, a transverse cut was made at the rostralmost extent of the pontine fibers on the ventral surface of the brain stem. This cut passed through the rostral zone of the trigeminal nerve at approximately A350 μm in the König and Klippel atlas [8] as shown in Fig. 2. A second transverse cut was made approximately 1 mm caudal to the first cut, through the ventral mid-pontine fibers. The resultant 1 mm thick section was placed with the rostral surface facing upward. The central gray and medial longitudinal fasciculi served as landmarks for the dissection of the raphe nuclei. The dorsal raphe nucleus was dissected from the ventral midline of the central gray, while the median raphe nucleus was dissected from the midline of the ventral tegmentum, above the pontine nuclei and fibers. Each nucleus was dissected as a single piece of tissue.

Assay of Biogenic Amines

Tissue samples were homogenized in 300 μl of ice cold IN formic acid/acetone 15:85 (v/v) and centrifuged for 10 min at 900 × g. The pellets were resuspended in 200 μl of the formic acid/acetone, recentrifuged, and the supernatants pooled. The extracts were then washed with 0.5 ml of heptane/chloroform 8:1. The aqueous portion containing the compounds of interest was then dried in a vacuum centrifuge, resuspended in 100 μl of the HPLC buffer and stored until analysis at −70°C. Protein was determined by the method of Lowry *et al.* [9] on the pellets from the formic acid/acetone extract after overnight digestion in 2N NaOH. Determinations of NE, DA, 5-HTP, 5-HT, and 5-HIAA were performed by HPLC with electrochemical detection, using a modification of the procedure of Shea and Jackson [12]. A standard curve was established by taking varying amounts of the compounds of interest (range 10–100 pmoles) and adding these to equal amounts of control brain extracts following the second formic acid/acetone centrifugation step. One of the extracts contained no addition of standard and was used as a blank. Two internal standards, dihydroxybenzylamine (150 pmoles) and N-methyl-5-HT (50 pmoles) were added to all samples in order to correct for recoveries of the catechols and indoles, respectively.

The HPLC system (Bioanalytical Systems, Inc., West Lafayette, IN) utilized a C-18 reverse phase column (25 cm × 3.2 mm, Waters Company) coupled with a glassy carbon detector set at a potential of 0.8 volts versus the reference electrode. The electronic controller was set at 5 nA/v and the recorder at 0.1 volt full scale. The HPLC buffer was 0.1 M citrate-disodium phosphate, pH 3.5, containing 0.004% sodium octyl sulphate and 12.5% methanol by volume. The buffer was filtered and degassed under vacuum before the addition of methanol. The flow rate of the HPLC

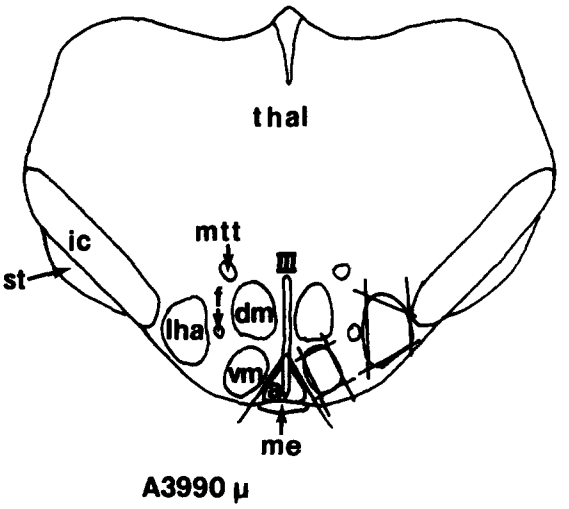

FIG. 1. Cross-section through the diencephalon at A3990μ in the König and Klippel atlas [8], caudal surface up, from which the ventromedial nucleus and lateral hypothalamic area were dissected. Abbreviations: thal—thalamus: st—stria terminalis: ic—internal capsule: mtt—mammillothalamic tract: f—fornix: lha—lateral hypothalamic area: dmn—dorsomedial nucleus: vmn—ventromedial nucleus: a—arcuate nucleus: me—median eminence: III—third ventricle. The lines represent scalpel cuts for the dissection.

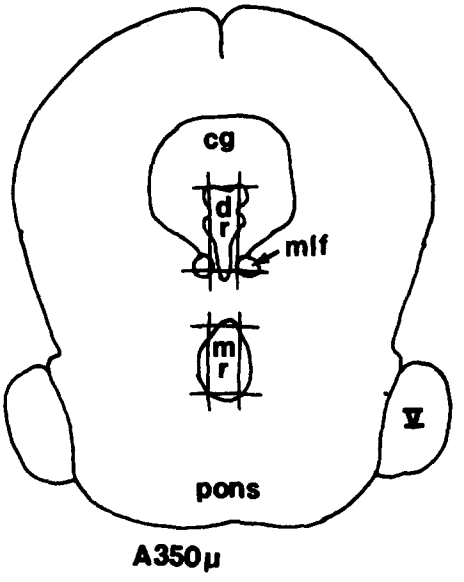

FIG. 2. Cross-section through the pons at A350μ in the König and Klippel atlas [8] rostral surface up from which dorsal and median raphe nuclei were dissected. Abbreviations: cg—central gray: dr—dorsal raphe nucleus: mlf—medial longitudinal fasciculus: mr—median raphe nucleus: V—trigeminal nerve. The lines represent scalpel cuts for the dissection.

was maintained at 1.0 ml per min. Twenty microliters of each sample were injected on the HPLC. The compounds of interest (identified by retention times of standards) were quantified by determining the area under the curves using an integrator (Supergrator II, Columbia Scientific Company) and their contents determined from standard curves. Chromatographs of standards without tissue (A) and a tissue extract of the VMH (B) are shown in Fig. 3.

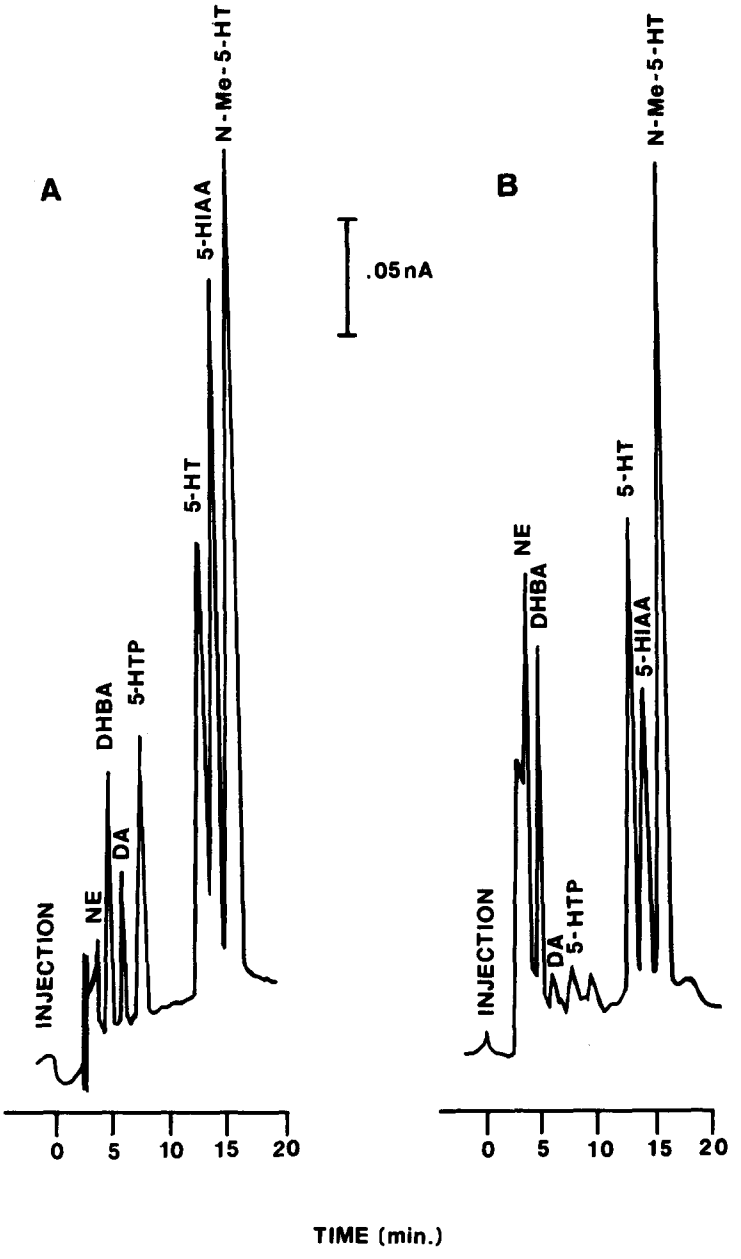

FIG. 3. Chromatographs of NE, DA, 5-HTP, 5-HT and 5-HIAA with internal standards dihydroxybenzylamine (DHBA) and N-methyl-5-HT (N-Me-5-HT). Part A is standards without tissue and Part B is a typical tissue extract (VMH, 8 mg).

RESULTS

The effect of 48 hr food deprivation on catecholamines and indoles in the different brain areas is presented in Table 1. Comparisons of means between the control versus the deprived groups for each amine level in each brain area, were performed by means of two tailed *t*-tests.

In the raphe nuclei there were significant increases in 5-HT and 5-HIAA levels ($p<0.05$) for the food deprived group. No significant changes were found in any of the other compounds measured. In the lateral hypothalamus there was a significant increase in 5-HIAA levels ($p<0.05$) and no significant changes between the two groups in the other compounds measured. There were no significant differences between the two groups in the ventromedial hypothalamus. The apparent increase in DA in the VMH for the food deprived group can be attributed to a large value obtained from one animal in that group. Control and deprived group means, however, were not statistically different.

TABLE 1

EFFECT OF 48 HR FOOD DEPRIVATION ON CATECHOLAMINES AND INDOLES IN DIFFERENT BRAIN AREAS

Compounds		pmoles/mg protein			
		Median Raphe	Dorsal Raphe	Lateral Hypothalamus	Ventromedial Hypothalamus
Norepinephrine	Control	207.3 ± 61.2 (5)	116.6 ± 16.3 (6)	212.7 ± 41.4 (6)	31.9 ± 8.6 (6)
	Deprived	250.2 ± 72.1 (6)	161.3 ± 21.0 (6)	213.9 ± 67.2 (6)	35.4 ± 12.4 (5)
Dopamine	Control	24.3 ± 12.2 (4)	10.9 ± 2.3 (5)	35.9 ± 4.3 (6)	23.5 ± 8.0 (6)
	Deprived	38.9 ± 9.3 (4)	19.4 ± 5.9 (6)	25.1 ± 7.3 (6)	49.0 ± 19.4 (5)
5-Hydroxytryptophan	Control	————†	9.2 ± 2.1 (6)	27.9 ± 6.6 (6)	6.1 ± 1.8 (5)
	Deprived	————†	12.9 ± 4.7 (6)	22.4 ± 4.6 (6)	7.5 ± 2.2 (3)
5-Hydroxytryptamine	Control	65.8 ± 9.7 (4)	63.8 ± 10.0 (6)	37.7 ± 4.5 (6)	38.8 ± 7.1 (6)
	Deprived	154.0 ± 16.9 (5)*	95.4 ± 7.6 (6)*	43.5 ± 4.1 (6)	37.8 ± 3.8 (5)
5-Hydroxyindole Acetic Acid	Control	58.2 ± 10.6 (4)	65.0 ± 7.5 (6)	26.4 ± 1.4 (6)	14.1 ± 2.1 (6)
	Deprived	219.2 ± 20.1 (5)*	129.4 ± 7.8 (6)*	37.5 ± 4.3 (6)*	18.1 ± 1.3 (5)

Values are the mean ± S.E.M. Number of determinations are in parenthesis.
*Significantly different from control mean, $p<0.05$.
†Reliable group means could not be calculated because of overall recovery losses in some samples.

DISCUSSION

The HPLC assay procedure described here represents a simple, sensitive, and rapid method for the simultaneous determination of NE, DA, 5-HTP, 5-HT and 5-HIAA content from the same small brain sample. The major advantages over existing methods are: (1) the absence of any prepurification procedure before detection: (2) the separation and detection are simultaneous and under the same assay conditions (e.g., no splitting of sample for different reaction conditions): (3) the sensitivity is greater than fluorometric methods and equal to enzymatic procedures: and (4) the recoveries are essentially 100% (only loss is due to tissue extraction, <5%).

The increases in 5-HT and 5-HIAA levels found in the raphe nuclei, an area not previously investigated under these conditions, suggest an increase in 5-HT synthesis associated with food deprivation. With regard to the lateral hypothalamus, the results reported in our study are in agreement with previous reports of increases in 5-HIAA levels following 48 hr food deprivation [4,5]. The fact that increases in 5-HT did not occur in the LH is probably due to an increase in 5-HT turnover [5]. There were no changes in 5-HT or 5-HIAA levels in the VMH. This suggests that specific nuclei within the hypothalamus are differentially affected by food deprivation.

No changes in the levels of NE and DA were found in either of the hypothalamic nuclei or raphe nuclei. These results do not necessarily mean that changes in the metabolism of these compounds do not take place following food deprivation. In fact, similar studies in whole hypothalamus indicate that changes in the metabolites of the catechols are occurring [1, 3, 14]. Further investigation, under the conditions used in the present study, is obviously needed in order to compare changes in the parent catechols and their metabolites.

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