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Turnover of ^{3}H-5-Hydroxytryptamine to ^{3}H-5-Hydroxyindoleacetic Acid and the ^{3}H-5-Methoxyindoles in Nondeprived and 24 Hr Food Deprived Rats[1]

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KANTAK, K. M., M. J. WAYNER, H. A. TILSON AND A. SVED. *Turnover of ^{3}H-5-hydroxytryptamine to ^{3}H-5-hydroxyindoleacetic acid and the ^{3}H-5-methoxyindoles in nondeprived and 24 hr food deprived rats.* PHARMAC. BIOCHEM. BEHAV. 6(2) 221–225, 1977. – Serotonin turnover in the lateral hypothalamus (LH) was determined in nondeprived and 24 hr food deprived rats. The LH was infused with 0.5 μCi of ^{3}H-5-hydroxytryptamine 1 hr prior to push-pull perfusion. The percentage of nCi/μCi of radioactivity was analyzed by thin layer chromatography and liquid scintillation spectrometry. There was significantly more 5-hydroxyindoleacetic acid and 5-methoxytryptamine formed in the 24 hr food deprived rats. These results indicate a faster 5-hydroxytryptamine turnover rate in the LH of 24 hr food deprived rats than in nondeprived rats.

Push-pull perfusion Serotonin Food deprivation Lateral hypothalamus 5-Hydroxyindoleacetic acid 5-Methoxytryptamine 5-Methoxyindoleacetic acid 5-Methoxytryptophol

WHOLE brain 5-hydroxyindoleacetic acid (5-HIAA) increases following 22–24 hr food deprivation [2,12]; whereas, brain serotonin (5-HT) either increases slightly following food deprivation [2] or is unchanged [12]. Regional determinations following 24 hr food deprivation indicate increases in 5-HT and/or 5-HIAA in the cortex, striatum, cerebellum, and midbrain plus hippocampus. No changes in 5-HT or 5-HIAA are found in the hypothalamus following 24 hr hr food deprivation [6]. Since the lateral hypothalamus (LH) contains 5-HT nerve terminals [4] and 5-HT metabolism within the hypothalamus has not been studied in food deprived animals, the present experiment was conducted to determine lateral hypothalamic changes in 5-HT, 5-HIAA, 5-MT (5-methoxytrptamine), 5-MIAA (5-methoxyindoleacetic acid) and 5-MTPhol (5-methoxytryptophol) in 24 hr food deprived and nondeprived rats. The methoxyindoles were also determined because 5-MT has been found in the rat hypothalamus using gas chromatographic-mass spectrometric techniques [5, 7, 8]. In addition, serotonin can serve as a substrate for indole-N-methyl transferase with 5-methyltetrahydrofolic acid as the methyl donor [1,9]. Under these conditions, mainly 5-MT is formed as the methylated product of 5-HT.

METHOD

Animals

Ten male hooded rats (356–430 g) from our colony were used in this experiment. Animals were housed in individual living cages. They had free access to Purina Lab Chow blocks and water unless otherwise stated. Animals were kept on a constant light-dark cycle. The 12 hr light phase began at 0600 hr and was followed by a 12 hr dark phase. The room temperature was maintained at 70° ± 2° F.

Surgery and Histology

Surgery was performed under Equi-Thesin anesthesia (Jensen-Salsbery Laboratories) at a dose of 3 cc/kg. Each animal was implanted with a concentric push-pull cannula in the right LH according to predetermined DeGroot [3] coordinates: AP −5.4, L − 1.8, V −3.0 mm from the interaural line. The tip of the implanted outer cannula was situated 0.5 mm above the LH. The inner cannula extended 0.25 to 0.50 mm beyond the end of the outer cannula. Four stainless steel screws were used to attach the cannula to the skull and the implant was secured with acrylic dental

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cement. There were 2 weeks of postoperative care prior to the start of the experiment. At the end of the experiment all animals were perfused intracardially, first with 0.9% NaCl and then with neuralized 10% Formalin plus 0.9% NaCl. The brains were removed, frozen, and sectioned at 60 μ. Tissue was stained with cresyl violet and examined to determine location of the cannula tip.

Apparatus

The test chamber consisted of a 20 x 20 x 50 cm Plexiglas box with a standard stainless steel rod grid floor, enclosed in an illuminated sound attenuated cubicle fitted with an exhaust fan. Push-pull perfusions were performed using a Sage Instrument Model 375A tubing pump. All radioactive determinations were made with a Beckman Model LS 100-C scintillation counter.

Procedure

Following the 2 week postoperative period, all animals were placed into individual living cages for 10 days prior to push-pull perfusion. Daily home cage food and water intakes and body weights were recorded at 1600 hr. On Day 10 the animals were divided into two groups of 5 animals each. One group of animals continued feeding on an ad lib basis on Day 10. The other group of animals was deprived of food at 1000 hr on Day 10. On Day 11, 0.5 μl (23.0 ng) of ^{3}H-5-hydroxytryptamine binoxalate (specific activity = 5.7 Ci/mmole, New England Nuclear) was infused via the push-pull cannula into the LH of each animal at 1000 hr. This procedure utilized a Harvard infusion pump. Liquid was infused at a rate of 1.0 μl/min. Following the infusion, animals were placed into the testing chamber for 1 hr. At 1100 hr the animals were perfused for 40 min with 0.9% bacteriostatic NaCl (Eli Lilly and Co.) at an average rate of 19.57 ± 1.11 μl/min. Eight 5 min samples of perfusate were collected. Each collection vial contained 0.5 ml of 1.0 N formic acid. Each animal received only 1 perfusion. A 20 μl aliquot was taken for each 5 min sample and pipetted into a glass scintillation counting vial (Kimble Products). This vial contained 5 drops of Bio Solv (Beckman Instruments) and 10 ml of a liquid scintillation cocktail (6 g PPO/1 toluene). The 8 vials for each perfusion were then counted in the scintillation counter. The cpm were corrected for background (21.80 ± 1.08 cpm), efficiency (39–44%), and dilution with formic acid (49.1 ± 0.72%). Final dpm were converted to μCi/5 min sample.

Samples 4, 5, and 6, which corresponded to 75–90 min postinfusion of ^{3}H-5-hydroxytryptamine, were further analyzed by thin layer chromatography (TLC). Twenty μl of perfusate from samples 4, 5, and 6 were spotted on individual cellulose coated TLC plates (Brinkman). In addition, 0.25 μl (2.5 μg dissolved in 1.0 N formic acid) of the following cold carrier standards were spotted on each plate: serotonin creatinine sulfate (Calbiochem); 5-hydroxyindoleacetic acid cyclohexylammonium salt (Calbiochem); 5-methoxytryptamine (Calbiochem); 5-methoxytryptophol (Sigma); and 5-methoxyindole-3-acetic acid (Regis). A ^{3}H-5-hydroxytryptamine standard plate was prepared in the above manner for each perfusion. However, 20 μl of a freshly prepared solution of ^{3}H-5-hydroxytryptamine, 0.9% NaCl and 1.0 N formic acid were spotted on the plate. There were approximately 4000–6000 dpm/20 μl. A bidirectional solvent system was used to develop the TLC plates. Solvent I consisted of butanol, 1.0 N formic acid and methanol (3:1:1). Solvent II consisted of isopropanol, ammonia and triple distilled water (8:1:1). Upon removal from the second solvent the 5 spots on each plate were detected with Erlich's Reagent (7% v/v). Each of these spots and the origin were cut into two 1 x 2 cm strips. Each strip was placed into an individual counting vial containing 1.0 ml methanol. The strips in methanol were allowed to elude for 24 hr before the addition of the scintillation cocktail. The vials were then counted in the scintillation counter. The cpm were corrected for background (20.82 ± 1.70 cpm), efficiency (10–44%), dilution with formic acid (49.41 ± 0.72%), and recovery of counts from the corresponding aliquot vial (35.15 ± 9.90%). Final dpm for each compound were converted to the percentage of nCi/μCi of total radioactivity.

RESULTS

Histology

The anterior-posterior extent of the lesions were as follows: 6.6–4.4 mm in the ad lib control animals and 5.8–5.0 mm in the 24 hr food deprived rats. This is well within the anterior-posterior limits of the LH according to the DeGroot atlas [3]. There was no evidence that these unilateral LH lesions had any effects on daily food and water intakes and body weight.

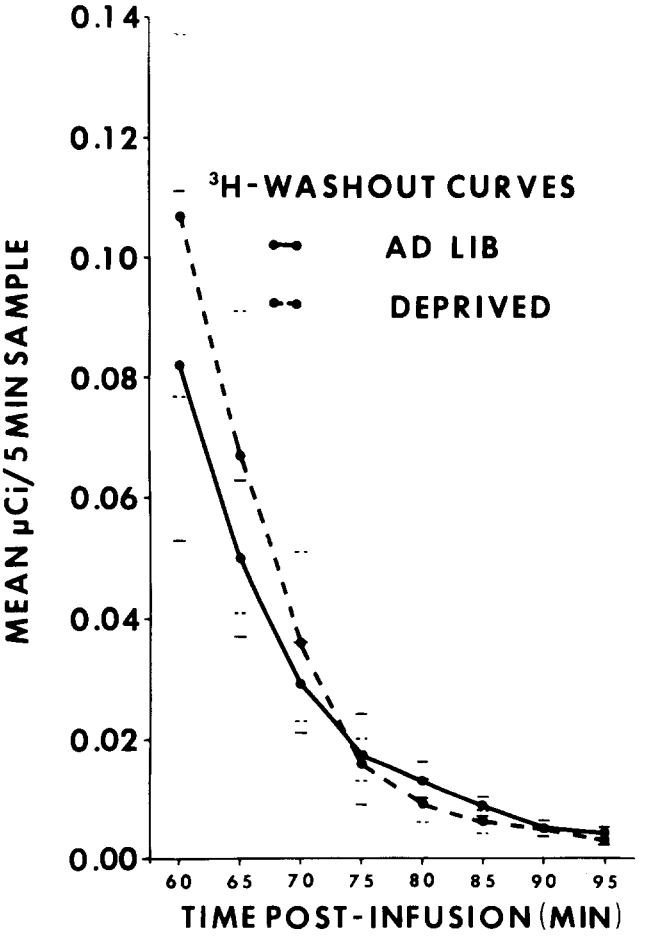

FIG. 1. Mean μCi ± SEM of total ^{3}H-washout per 5 min sample collected in the perfusate of ad lib and 24 hr food deprived rats. Each sample is expressed as time postinfusion of ^{3}H-5-HT. Ad lib rats (●——●): 24 hr food deprived rats (●– – –●).

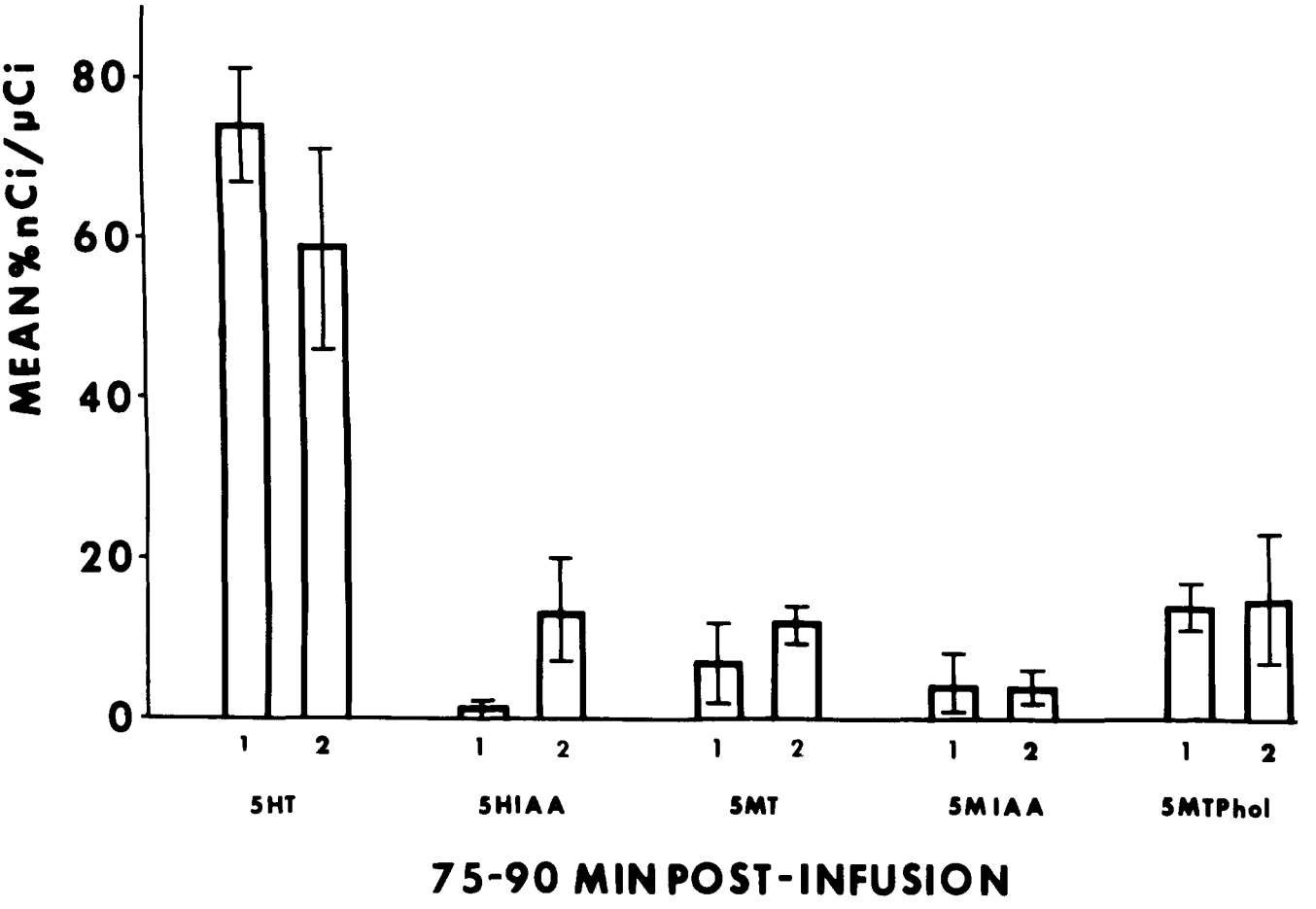

FIG. 2. Mean percent ± SEM of nCi of ^{3}H-5-HT and ^{3}H-metabolites per μCi of total radioactivity for 75–90 min postinfusion of ^{3}H-5-HT. 1, ad lib rats; 2, 24 hr food deprived rats.

Aliquot Analysis

Data collected from the eight 5 min samples were analyzed by a 2 × 8 analysis of variance. There were no significant differences between the μCi of ^{3}H-washout for the group main effect. As would be expected, there was a significant main effect in the μCi for the time postinfusion, $F(1,7) = 17.33$, $p<0.01$. Tukey A tests of the differences among the 75–80, 80–85 and 85–90 min samples were not significant. Therefore, the efflux of radioactivity was similar in the samples upon which the TLC analyses were performed. The ^{3}H-washout curves are presented in Fig. 1 for both groups of rats.

TLC Analysis

Turnover of 5-HT to all four metabolites was detected in both groups of rats during the 75–90 min postinfusion period. However, 5-HT turnover was different in nondeprived and 24 hr food deprived rats. For the total 15 min period, there was a significantly higher percentage of nCi/μCi for 5-HIAA and 5-MT in the 24 hr food deprived group (Mann-Whitney U, $p<0.025$). No differences were found in the percentage of nCi/μCi for 5-HT, 5-MIAA, and 5-MTPhol during this 15 min period. These data are presented in Fig. 2. Examination of the 5 min TLC data revealed that the 5-HIAA increases with food deprivation occurred during the 75–80 and 85–90 min periods. The increases in 5-MT with food deprivation occurred during the 85–90 min period. This was due to a significant increase in 5-MT over time in the 24 hr food deprived group, $F(2,30) = 5.05$, $p<0.05$. Analysis of variance of the time differences on these 5 min data were carried out after the percents were transformed to the arc-sin. There were no significant increases or decreases over time in all other compounds for both groups of rats. These data are presented in Figs. 3 and 4.

Examination of the standard plates revealed good specificity for the TLC separations. The majority of the radioactivity on the standard plates was detected at the spot for 5-HT (93.42 ± 6.02%; $Rf_1 = 0.50 \pm 0.02$, $Rf_2 = 0.70 \pm 0.05$). The other 6.58% of radioactivity was nonspecific and appeared in the spots of the other compounds or remained at the origin. These values were: 5-HIAA, 1.50 ± 1.73% ($Rf_1 = 0.77 \pm 0.01$, $Rf_2 = 0.22 \pm 0.02$); 5-MT, 1.55 ± 2.99% ($Rf_1 = 0.65 \pm 0.02$, $Rf_2 = 0.85 \pm 0.04$); 5-MIAA, 0.60 ± 1.13% ($Rf_1 = 0.81 \pm 0.02$, $Rf_2 = 0.35 \pm 0.03$); 5-MTPhol, 1.26 ± 1.93% ($Rf_1 = 0.91 \pm 0.01$, $Rf_2 = 0.46 \pm 0.05$); and origin, 1.67 ± 3.07%.

DISCUSSION

Since larger amounts of 5-HIAA and 5-MT were formed

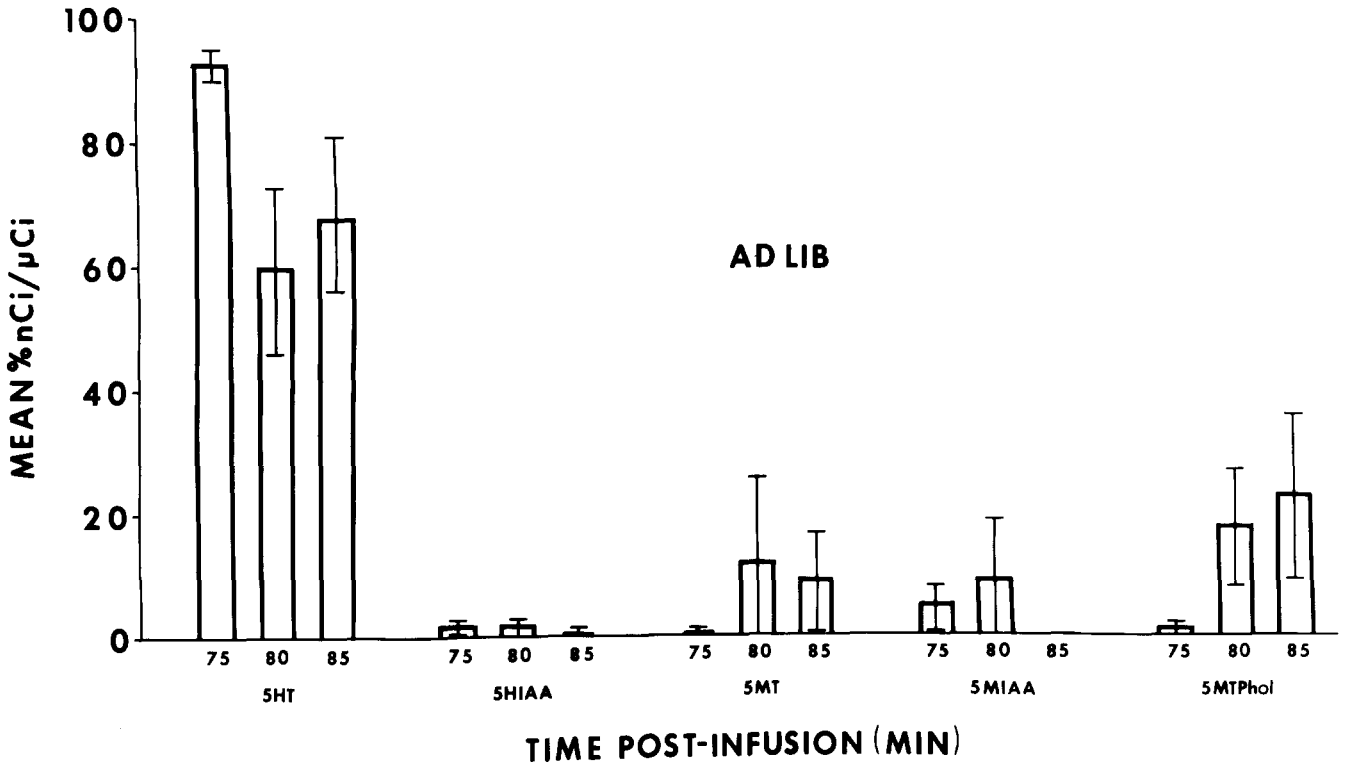

FIG. 3. Mean percent ± SEM of nCi of ^{3}H-5-HT and ^{3}H-metabolites per µCi of total radioactivity for 75–80, 80–85 and 85–90 min postinfusion samples of ad lib rats.

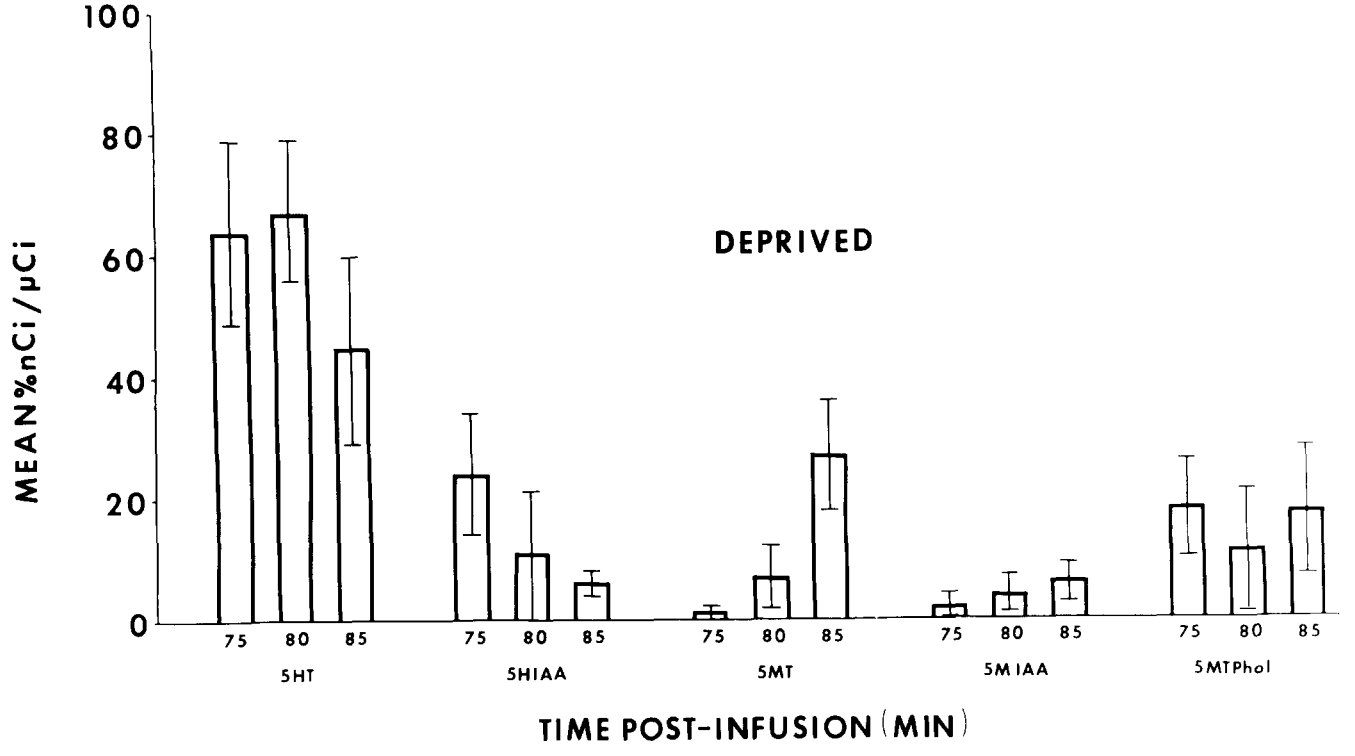

FIG. 4. Mean percent ± SEM of nCi of ^{3}H-5-HT and ^{3}H-metabolites per µCi of total radioactivity for 75–80, 80–85 and 85–90 min postinfusion samples of 24 hr food deprived rats.

in the LH of 24 hr food deprived rats, a higher 5-HT turnover rate is indicated under these conditions. This regional difference in 5-HT turnover could easily be obscured in the whole hypothalamic determination which was reported earlier and showed no difference in 5-HT metabolism during 24 hr food deprivation [6]. The assaying of a whole brain or fraction of a brain can obscure discrete variation in neurochemical activity [10]. The data reported here do support the differential effects of 5-HT on LH neurons when tested individually by means of microiontophoresis [11] and the histofluorescence of 5-HT terminals in this part of the hypothalamus [4].

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