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Effects of Various Periods of Food Deprivation on Serotonin Turnover in the Lateral Hypothalamus[1]

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KANTAK, K. M., M. J. WAYNER AND J. M. STEIN. *Effects of various periods of food deprivation on serotonin turnover in the lateral hypothalamus.* PHARMAC. BIOCHEM. BEHAV. 9(4) 529–534, 1978.—Preliminary results indicated enhanced serotonin turnover in the lateral hypothalamus of 24 hr food deprived rats as compared to non-deprived rats. In the present study, the periods of food deprivation were extended in order that the effects of 0, 24, 48 and 72 hr of food deprivation on serotonin turnover could be measured. One hr following an infusion of ^{3}H-5-hydroxytryptamine the lateral hypothalamus was perfused with physiological bacteriostatic saline for 40 min. Samples of perfusate, which corresponds to 75–90 min post-infusion, were analyzed by thin layer chromatography for estimation of ^{3}H-labelled precursor and metabolites. The results indicate that serotonin turnover is enhanced as a function of hours of food deprivation.

Serotonin turnover 5-Hydroxytryptamine Food deprivation Lateral hypothalamus Push-pull perfusion

FOLLOWING 22 to 24 hr of food deprivation, whole brain 5-HIAA increases [3,14], whereas brain 5-HT has been shown to either increase [3] or remain unchanged [14]. Regional determination following 24 hr of food deprivation indicates increases in 5-HT and/or 5-HIAA in the cortex, striatum, cerebellum and midbrain plus hippocampus [8]. No changes in 5-HT or 5-HIAA were found in the whole hypothalamus following 24 hr food deprivation even though tryptophan concentration in this brain region was greatly enhanced. Preliminary results demonstrated that 5-HT turnover in the lateral hypothalamus increases with 24 hr food deprivation [7]. Increases in 5-HIAA and 5-methoxytryptamine (5-MT) were detected following 24 hr of food deprivation. These data do not contradict earlier results in which whole hypothalamic 5-HT metabolism did not change during 24 hr of food deprivation. Since the hypothalamus is comprised of several distinct nuclei, assaying the whole hypothalamus could easily obscure discrete variation in neurochemical activity [10]. Electrophysiological evidence indicates that various hypothalamic neurons are differentially sensitive to L-tryptophan [16,17] and to 5-HT [11,12]. In addition, the serotonin content of various hypothalamic nuclei are affected differently by stress [13]. These data indicate significant differences in serotonin metabolism within the hypothalamus.

The purpose of the present study was to investigate the effects of food deprivation on serotonin turnover in the lateral hypothalamus. Food deprivation periods of 0, 24, 48 and 72 hr were used. The effects of long periods of food deprivation on serotonin turnover in the hypothalamus have not been investigated. Serotonin turnover was determined using push-pull cannulae perfusions with ^{3}H-5-HT and the efflux of ^{3}H-labelled metabolites was measured by thin layer chromatography and a scintillation counter.

METHOD

Animals

Twenty male hooded rats, 319–430 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 lateral hypothalamus according to predetermined DeGroot [4] coordinates: AP 5.4, L 1.8 and V 3.0 mm from the interaural line. The tip of the implanted outer cannula was situated 0.5 mm above the lateral hypothalamus. The inner cannula extended 0.5 mm beyond the end of the outer

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cannula. Four stainless steel screws were used to attach the cannula to the skull and the implant was secured with acrylic dental 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 neutralized 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 perfusion chamber consisted of a 20×20×50 cm Plexiglas box with a standard stainless steel rod grid floor enclosed in an illuminated sound attenuating cubicle fitted with an exhaust fan. Push-pull perfusions were performed using a Sage Instruments Model 375 A tubing pump. All radioactive determinations were made with a Beckman Model LS 100-C scintillation counter or a Packard Tri Carb Model scintillation counter.

Procedure

Following the 2 week postoperative period, all animals were placed in individual living cages for 10 days prior to push-pull perfusion. Daily home cage food and water intakes and body weight were recorded at 1600 hr. On Day 8 the animals were divided into 4 groups of 5 animals each. One group of animals continued to feed on an ad lib basis. The second group of animals was food deprived on Day 10, 24 hr prior to push-pull perfusion. The third group of animals was food deprived on Day 9, 48 hr prior to push-pull perfusion. The fourth group of animals was food deprived on Day 8, 72 hr prior to push-pull perfusion. On Day 11, 0.5 μCi (23.0 ng) of ^{3}H-5-hydroxytryptamine binoxalate (specific activity – 5.7 Ci/m mole, New England Nuclear) was infused via the push-pull cannula into the lateral hypothalamus of each animal at 1000 hr. This procedure utilized a Harvard infusion pump and 0.5 μl was infused at a rate of 1.0 μl/min. Following the infusion, animals were placed into the perfusion chamber for 1 hr. At 1100 hr the animals were perfused for 40 min with 0.9% bacteriostatic NaCl (Eli Lilly and Co.) at a rate of approximately 20 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/l toluene). The 8 vials for each perfusion were then counted in the scintillation counter. The cpm were corrected for background, efficiency and dilution with formic acid. Final dpm were converted to μCi/5 min sample.

Samples 4, 5 and 6, which corresponds to 75–90 min post-infusion of ^{3}H-5-hydroxytryptamine, were further analysed 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 cyclohexyl-ammonium 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 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 1×2 cm strips. Each strip was placed into an individual counting vial containing 1.0 ml methanol. The strips in the methanol were allowed to elute 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, efficiency, dilution with formic acid and recovery of counts from the corresponding aliquot vial. Final dpm for each compound was converted to nCi/5 min sample.

RESULTS

Histology

The tips of all cannulae were in the lateral hypothalamus, lateral to the fornix, and medial to the cerebral peduncle. All placements were within the anterior-posterior limits of the lateral hypothalamus according to the DeGroot atlas, 6.2–4.2. There was no evidence that unilateral lateral hypothalamic destruction due to the cannula had any effect on daily food and water intakes and body weight.

Aliquot Analyses

Data collected from the eight 5 min samples were analyzed by a 4×8 analysis of variance with repeated measures [18]. The factors were the 4 groups and the 8 time periods post-infusion. There were no significant differences among the μCi/5 min of ^{3}H-washout for the group factor or the group×time interaction. As would be expected, there was a significant difference in the μCi/5 min for the time factor, $F(7,112)=32.52$, $p<0.01$. Tukey A tests of the differences among the 75–80, 80–85 and 85–90 min samples were not significant. Therefore, the efflux of total radioactivity was similar in all samples upon which the TLC analyses were performed. The ^{3}H-washout curves are presented in Fig. 1 for all groups of rats.

TLC Analyses

Figure 2 represents the mean nCi/5 min of ^{3}H-5-HT detected in the perfusate after bidirectional TLC separation from samples taken 75–90 min post-infusion. A one-way analysis of variance was performed and significant differences were found, $F(3,16)=58.46$, $p<0.01$. A Dunnett's test revealed that the ^{3}H-5-HT detected after 48 hr of food deprivation was significantly less than the 0 hr deprivation control group ($p<0.005$). The means after 24 and 72 hr of food deprivation were not significantly different from the 0 hr control.

For statistical analysis of the metabolite data nonparametric statistics were used because of the non-normal nature of these data. With the methods employed in these experiments, the minimum detectable amount or sensitivity is in the picogram range. Thus when no radioactivity is detected over the background, the lack of measurable counts does not necessarily imply a lack of metabolism. Because most of the radioactivity from a push-pull perfusion remains

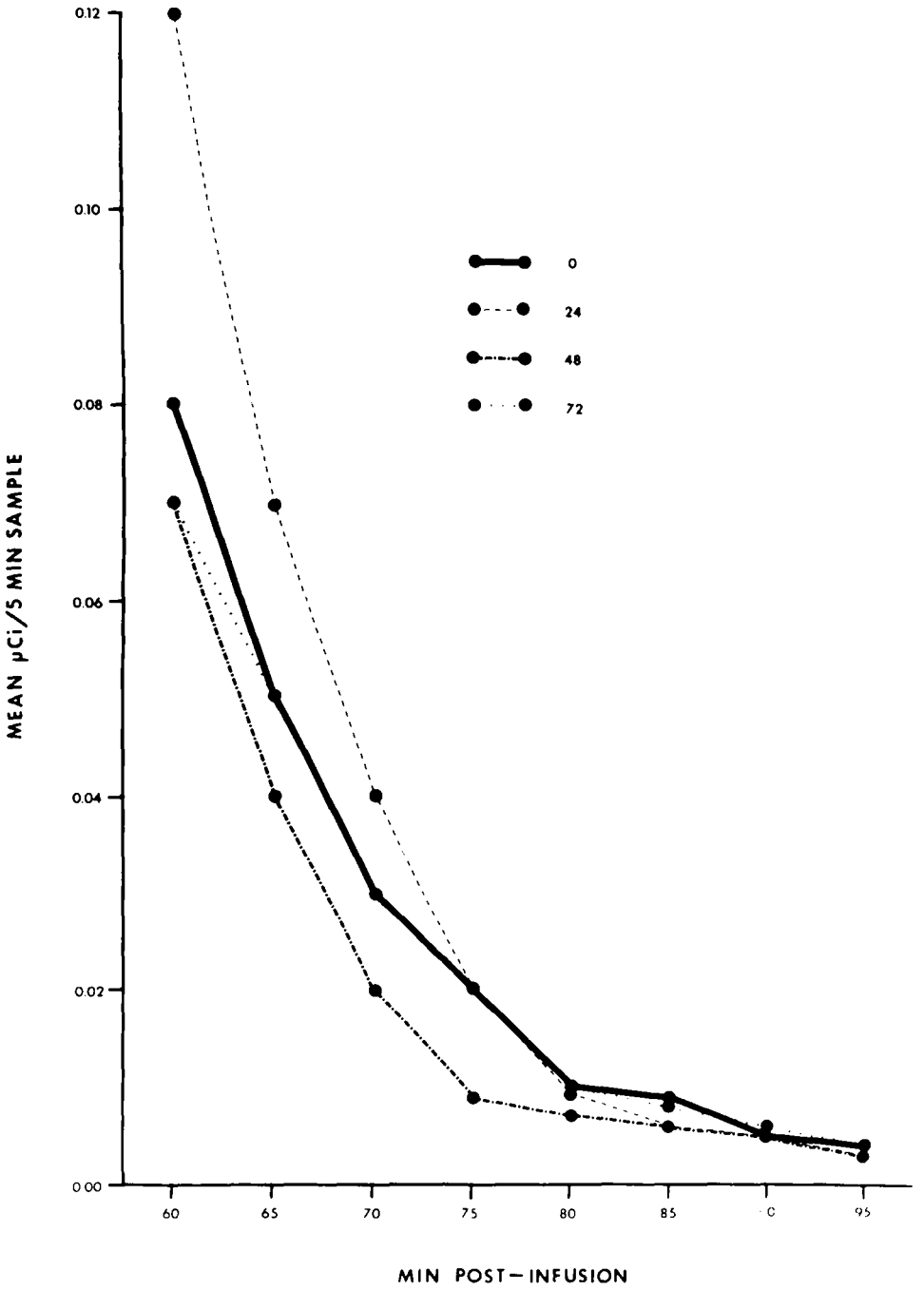

FIG. 1. Mean μCi/5 min sample of total radioactivity. Time is in min post-infusion of ^{3}H-5-HT. O hr ●———●; 24 hr ●– – – –●; 48 hr ●–·–·–●; and 72 hr ●·····●, food deprived groups.

as the labelled compound used, there are no problems in detection and the data conforms properly to parametric statistics. However, the metabolites from the labelled compound represent small amounts of radioactivity and it is common in some samples to fail to detect any counts over background. Because the validity of the zero values could not be determined, a non-parametric statistic, the Mann-Whitney U test, was used for statistical analysis of the metabolite data. Zero values were not used in the analysis.

Figure 3 represents the ^{3}H-5-HIAA formed from ^{3}H-5-HT, 75–90 min post-infusion. The nCi/5 min were significantly higher following 48 hr ($U=21$, $n_1=8$, $n_2=13$, $p<0.025$) and 72 hr ($U=21$, $n_1=8$, $n_2=11$, $p<0.05$) of food deprivation when compared to the 0 hr deprivation control group. Although the median nCi/5 min was 34% higher following 24 hr of food deprivation, there were no significant differences between this group and the 0 hr food deprivation control group.

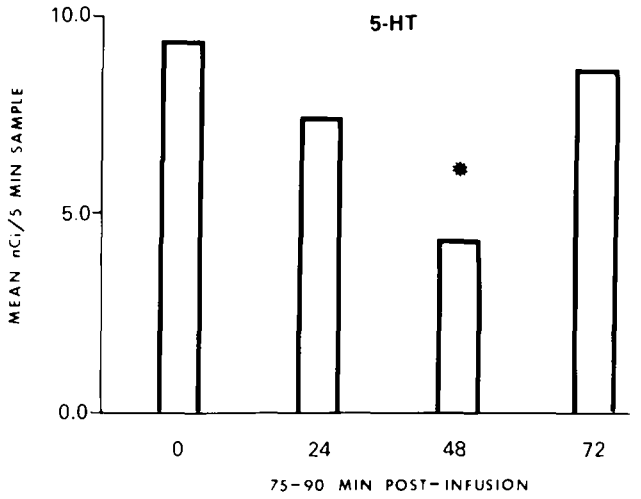

FIG. 2. Mean nCi/5 min of ^{3}H-5-HT, 75–90 min post-infusion in 0, 24, 48 and 72 hr food deprived groups. *Significantly different from the 0 hr group.

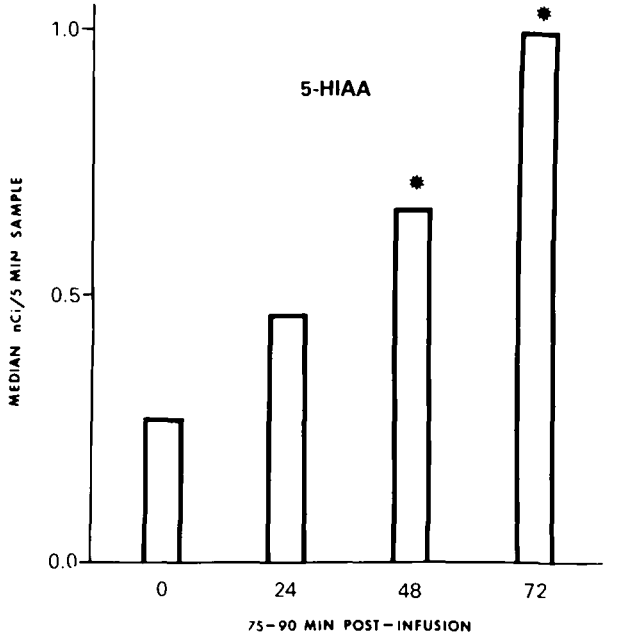

FIG. 3. Median nCi/5 min of ^{3}H-5-HIAA, 75–90 min post-infusion in 0, 24, 48 and 72 hr food deprived groups. *Significantly different from the 0 hr group.

Figure 4 represents the ^{3}H-5-MT formed from ^{3}H-5-HT 75–90 min post-infusion. No significant differences in the nCi/5 min were found when each food deprivation group was compared to the 0 hr food deprivation control group.

Figure 5 represents the ^{3}H-5-methoxytryptophol (5-MTPhol) formed from ^{3}H-5-HT, 75–90 min post-infusion. The nCi/5 min were significantly lower following 24 hr ($U=16$, $n_1=7$, $n_2=6$, $p<0.025$), 48 hr ($U=9$, $n_1=7$, $n_2=11$, $p<0.01$), and 72 hr ($U=2$, $n_1=7$, $n_2=6$, $p<0.002$) of food deprivation when compared to the 0 hr food deprivation control group.

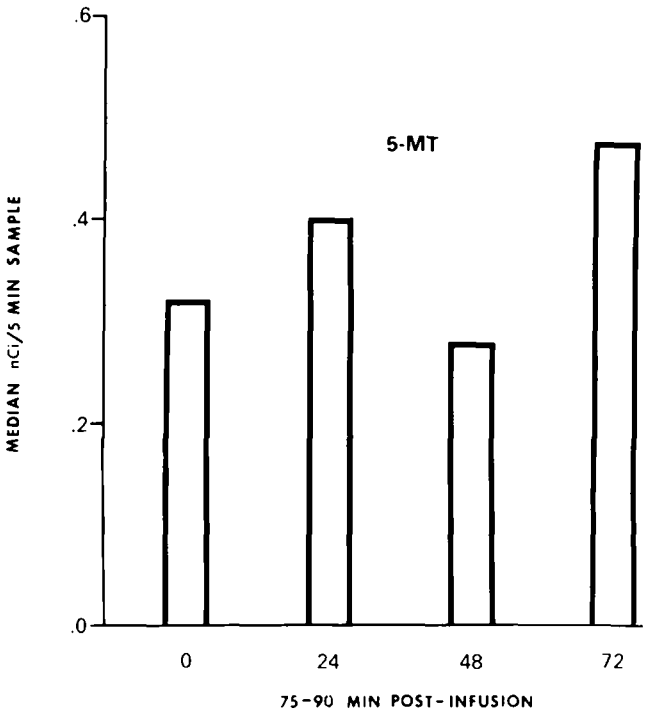

FIG. 4. Median nCi/5 min of ^{3}H-5-MT, 75–90 min post-infusion in 0, 24, 48 and 72 hr food deprived groups.

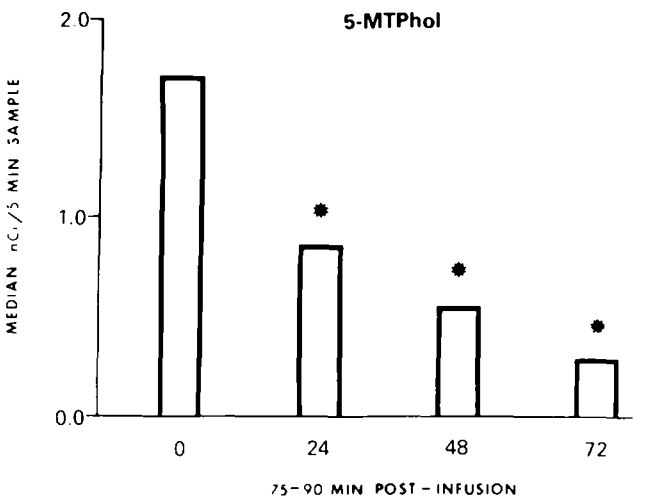

FIG. 5. Median nCi/5 min of ^{3}H-5-MTPhol, 75–90 min post-infusion in 0, 24, 48 and 72 hr food deprived groups. *Significantly different from the 0 hr group.

Figure 6 represents the ^{3}H-5-methoxyindoleacetic acid (5-MIAA) formed from ^{3}H-5-HT, 75–90 min post-infusion. The nCi/5 min were significantly lower following 24 hr ($U=1$, $n_1=3$, $n_2=9$, $p<0.01$) and 72 hr ($U=2$, $n_1=3$, $n_2=8$, $p<0.024$) of food deprivation when compared to the 0 hr food deprivation control group. The difference in the nCi/5 min between the 48 hr food deprived group and the 0 hr control group was not significant.

The ^{3}H-5-HT standard plates show good specificity for

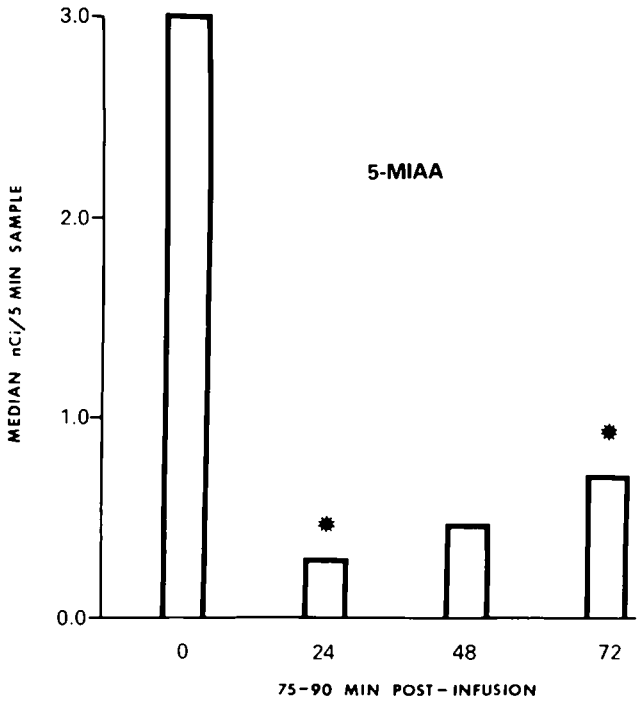

FIG. 6. Median nCi/5 min of ^{3}H-5-MIAA, 75–90 min post-infusion in 0, 24, 48 and 72 hr food deprived groups. *Significantly different from the 0 hr group.

the TLC separations (Table 1). The majority of the radioactivity on the standard plates was detected at the spot for 5-HT and the origin. A small percentage of radioactivity was non-specific with respect to the 5-HT spot and appeared at the spots of all metabolites cochromatographed with the ^{3}H-5-HT. In a percentage comparison of radioactivity from lateral hypothalamic perfusate and the standard plates (Table 2), the perfusate contained much more radioactivity attributable to the metabolites than the standard plates non-specific spread. Thus the radioactivity attributable to the metabolites in the perfusate represents functional metabolism and not just an artifact due to non-specific radioactive spread on the TLC plate.

The Rf values (the ratio of the distance of each spot from the origin to the distance of the solvent front from the origin) are presented in Table 3. This value represents the location of each spot in each solvent direction. As can be seen from the table, each spot is discretely separated from all other spots and the location is very consistent from plate to plate. Because of these discrete separations, the radioactivity from each compound is never cross-contaminated with radioactivity from another compound.

DISCUSSION

The results from this experiment indicate an enhanced turnover of 5-HT to 5-HIAA in the lateral hypothalamus following 48 and 72 hr of food deprivation. Turnover of 5-MT appears to be reduced following, 24, 48 and 72 hr of deprivation. These results do not agree with previously reported data which show that 5-HIAA and 5-MT are enhanced as a result of 24 hr of food deprivation [7]. The discrepancy is related to the ratio transformation of the data in the previous study. The data were expressed as a ratio of metabolite to substrate. A ratio transformation can produce a spurious correlation between 2 variables unless the variables are linearly related [1]. A positive correlation between 5-HT availability and 5-HIAA formation has been demonstrated for the whole brain [3, 6, 9] and several brain regions, including the hypothalamus [2, 5, 8, 15], following various physiological and pharmacological treatments. However these linear relations have not yet been demonstrated for the lateral hypothalamus. In the present study, if one assumes linearity and a ratio transformation is used, then the turnover

TABLE 1

^{3}H-5-HT STANDARD PLATES

	0	24	48	72
5-HT + Origin	98.28% ± 0.81%	91.90% ± 2.72%	98.73% ± 0.44%	95.87% ± 2.58%
5-HIAA	0.28% ± 0.28%	2.72% ± 0.82%	0.29% ± 0.21%	2.50% ± 1.25%
5-MT	0.72% ± 1.33%	2.38% ± 2.03%	0.41% ± 0.13%	0.36% ± 0.33%
5-MTPhol	0.02% ± 0.02%	2.50% ± 1.07%	0.82% ± 0.41%	1.53% ± 1.19%
5-MIAA	0.70% ± 0.78%	0.50% ± 0.32%	0.11% ± 0.13%	0.74% ± 0.72%

Values are the Mean ± S.E.M. % of total radioactivity.

TABLE 2

COMPARISON OF RADIOACTIVITY FROM THE PERFUSATE AND SPECIFIC AND NON-SPECIFIC SPREAD ON THE STANDARD PLATES

	0		24		48		72	
	Perf	Std	Perf	Std	Perf	Std	Perf	Std
^{3}H-5-HT + Origin	75.65%	98.28%	63.23%	91.90%	75.09%	98.73%	71.84%	95.87%
^{3}H-Metabolites	24.35%	1.72%	36.77%	8.10%	24.91%	1.27%	28.16%	4.13%

Values are the Mean % of total radioactivity found on the standard plates (Std) and in the perfusate (Perf) 75–90 min post-infusion.

TABLE 3
RF VALUES

	0		24		48		72	
	I	II	I	II	I	II	I	II
5-HT	0.48 ± 0.01	0.68 ± 0.03	0.52 ± 0.01	0.71 ± 0.01	0.47 ± 0.01	0.70 ± 0.01	0.45 ± 0.01	0.69 ± 0.02
5-HIAA	0.78 ± 0.01	0.24 ± 0.01	0.77 ± 0.01	0.20 ± 0.01	0.76 ± 0.01	0.27 ± 0.01	0.77 ± 0.01	0.21 ± 0.01
5-MT	0.63 ± 0.01	0.84 ± 0.03	0.66 ± 0.01	0.88 ± 0.01	0.62 ± 0.01	0.83 ± 0.01	0.60 ± 0.01	0.85 ± 0.01
5-MTPhol	0.92 ± 0.01	0.52 ± 0.02	0.90 ± 0.01	0.42 ± 0.01	0.91 ± 0.01	0.45 ± 0.01	0.93 ± 0.01	0.58 ± 0.02
5-MIAA	0.82 ± 0.01	0.36 ± 0.02	0.80 ± 0.01	0.35 ± 0.01	0.81 ± 0.01	0.41 ± 0.01	0.83 ± 0.01	0.36 ± 0.01

Values are the Mean ± S.E.M.

of 5-HT to 5-HIAA is significantly enhanced following 24, 48 and 72 hr of food deprivation while the turnover of 5-MT is not affected. These results are in agreement and extend our previously reported data on the effects of food deprivation on serotonin turnover in the lateral hypothalamus.

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