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Effects of Various Periods of Food Deprivation on Serotonin Synthesis 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 synthesis in the lateral hypothalamus.* PHARMAC. BIOCHEM. BEHAV. 9(4) 535–541, 1978.—One hr following an infusion of ^{3}H-L-tryptophan, the lateral hypothalamus was perfused with physiological bacteriostatic saline for 40 min. Samples of perfusate, which corresponded to 75–90 min post-infusion, were analyzed by thin layer chromatography for estimation of ^{3}H-labelled L-tryptophan, 5-hydroxytryptophan and 5-hydroxytryptamine. The results indicate that tryptophan uptake and serotonin synthesis are enhanced as a function of hours of food deprivation.

Serotonin synthesis 5-Hydroxytryptamine L-tryptophan Food deprivation Lateral hypothalamus Push-pull perfusion

FOOD deprivation results in an increase in brain tryptophan. Blood tryptophan is bound to albumin and is also in the free form which crosses the blood-brain barrier [22]. During food deprivation plasma albumin bound tryptophan decreases due to its displacement from albumin binding sites by nonesterified free fatty acids which increase as a result of lipolytic processes associated with food deprivation [23]. As plasma albumin bound tryptophan decreases, plasma free form tryptophan increases with food deprivation and more tryptophan becomes available in the brain [27].

Serotonin (5-HT) turnover increases in the whole brain [3,26], in several brain regions [21], and in the lateral hypothalamus [19,20] following 24 hr of food deprivation. In addition whole brain and regional tryptophan concentrations increase. Following longer periods of food deprivation 5-HT turnover in the lateral hypothalamus remains elevated [19]. The purpose of the present study was to investigate the effects of food deprivation on 5-HT synthesis in the lateral hypothalamus. Food deprivation periods of 0, 24, 48 and 72 hr were used. Serotonin synthesis was determined using push-pull cannulae perfusions with radiolabelled L-tryptophan to measure the efflux of tritium labelled 5-hydroxytryptophan (5-HTP) and 5-HT.

METHOD

Animals

Twenty male hooded rats, 314–486 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 cannula. Four stainless steel screws were used to attach the cannula to the skull and the implant was secured with acrylic dental cement. There was 1 week of post-operative 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

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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 Tracerlab Corumatic 100 A scintillation counter.

Procedure

Following the 1 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 (13.0 ng) of ^{3}H-L-tryptophan (specific activity = 7.9 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 one 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 μg PPO/1 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 correspond to 75–90 min post-infusion of ^{3}H-L-tryptophan, 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 serotonin creatinine sulfate (Calbiochem) and 0.50 μl (5.0 μg dissolved in 1.0 N formic acid) of L-tryptophan and L-5-hydroxytryptophan ethylester HCL (Calbiochem) were spotted on each plate as cold carrier standards. A ^{3}H-L-tryptophan standard plate was prepared in the above manner for each perfusion. However, 20 μl of freshly prepared solution of ^{3}H-L-tryptophan, 0.9% NaCl and 1.0 N formic acid were spotted on the plate. There were approximately 2000–4000 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 3 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 were 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, 5.8–4.2. There was no evidence that the unilateral lateral hypothalamic destruction due to the cannula had any effect on daily food and water intakes and body weight.

Aliquot Analysis

Data collected from the eight 5 min samples were analyzed by a 4×8 analysis of variance with repeated measures. The factors were the 4 groups and the 8 time periods post-infusion. The group factor was not significant. As would be expected, there was a significant main effect in the μCi/5 min for the time factor, $F(7,112)=5.85$, $p<0.01$. Tukey A tests of the differences among the 75–80, 80–85, and 85–90 min samples were not significant. However, the efflux of total radioactivity was not similar for each group because there was a significant Group × Time interaction effect, $F(21,112)=2.02$, $p<0.05$. Analysis by simple main effects of Groups at each level of Time revealed significant group differences at time 60–65, 65–70, 70–75, 75–80, and 80–85 min post-infusion, $p<0.05$. Further testing by Tukey A tests demonstrated that there was significantly more total radioactivity per 5 min in the 0 hr food deprivation control group than in the 24, 48 or 72 hr deprivation groups for each time sample analyzed, $p<0.05$. In addition, there were no significant differences among the 24, 48 and 72 hr groups in the total radioactivity per 5 min at any of the time samples analyzed. Because radioactivity in the perfusate is picked up from extraneuronal spaces [33] and food deprivation has been shown to increase the uptake of tryptophan intraneuronally [7], these data clearly demonstrate that 24, 48 and 72 hr of food deprivation increase the uptake of ^{3}H-L-tryptophan into serotonergic neurons in the lateral hypothalamus. The ^{3}H-washout curves are presented in Fig. 1 for all groups of rats.

TLC Analysis

Figure 2 represents the mean nCi/5 min of ^{3}H-L-tryptophan 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, $F(3,16)=6.24$, $p<0.01$. A Dunnett's test revealed that the ^{3}H-L-tryptophan detected after 24 ($p<0.025$), 48 ($p<0.025$), and 72 ($p<0.01$) hr of food deprivation was significantly less from the 0 hr deprivation control group. These differences must be related to changes in serotonergic neuronal activity in the lateral hypothalamus following food deprivation and again indicate that 24, 48 and 72 hr of food deprivation increase the uptake of tryptophan intraneuronally.

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

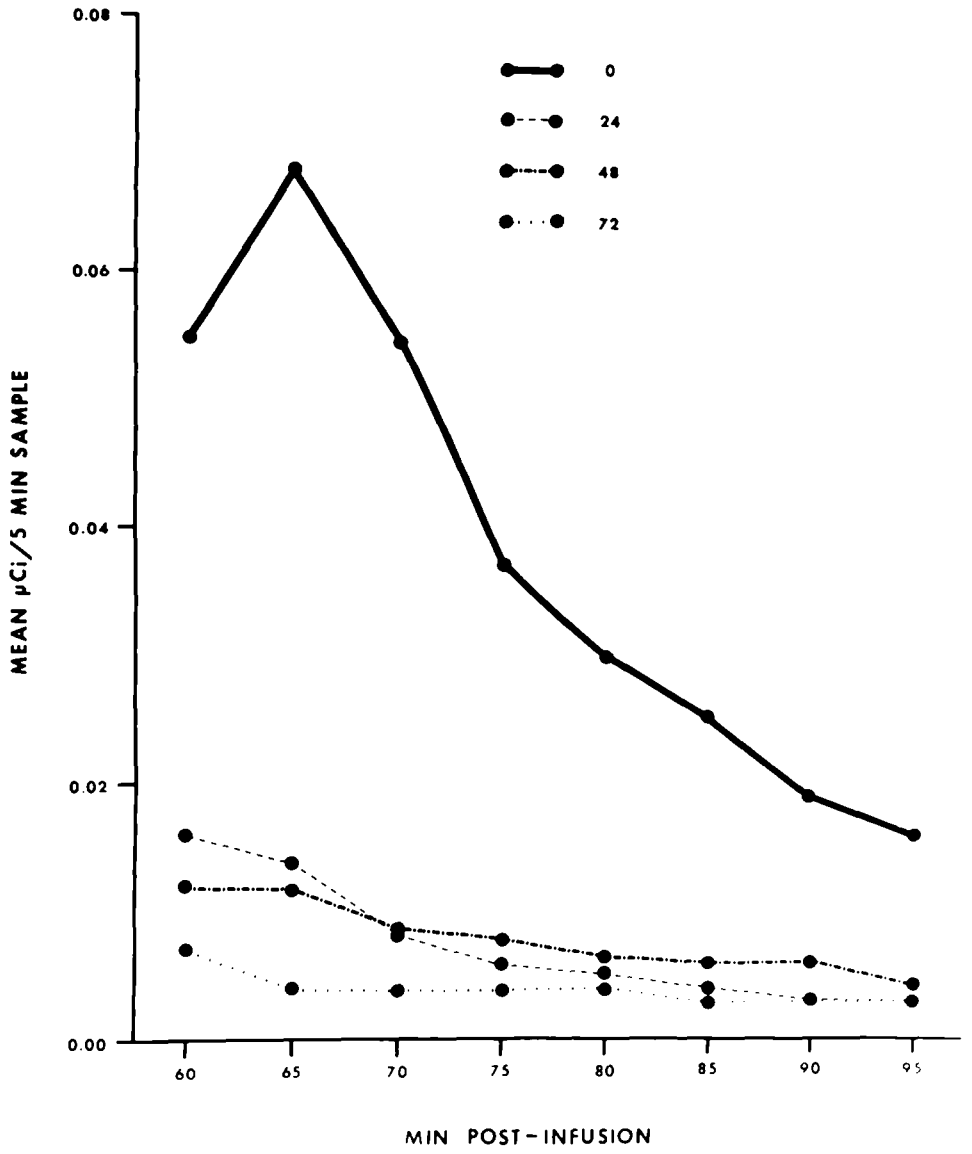

FIG. 1. Mean μCi/5 min sample of total radioactivity. Time is in min post-infusion of ^{3}H-L-tryptophan. 0 hr ●——●; 24 hr ●– – –●; 48 hr ●–·–·–●; and 72 hr ●·····● food deprived groups.

does not necessarily imply a lack of metabolism. Because most of the radioactivity from a push-pull perfusion remains 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-HTP formed from ^{3}H-L-tryptophan, 75–90 min post-infusion. There were no significant differences in the nCi/5 min when each deprivation group was compared to the 0 hr food deprivation control group.

Figure 4 represents the ^{3}H-5-HT formed from ^{3}H-L-tryptophan, 75–90 min post-infusion. The nCi/5 min were significantly higher following 24 hr of food deprivation ($U=4$, $n_1=4$, $n_2=8$, $p<0.024$) when compared to the 0 hr control group. The ^{3}H-5-HT measured following 48 hr and 72 hr of food deprivation were not significantly different from the 0 hr control group.

The ^{3}H-L-tryptophan standard plates showed good specificity for the TLC separations (Table 1). The majority of the radioactivity on the standard plates was detected at the spot for tryptophan and the origin. A small percentage of radioactivity was non-specific with respect to the tryptophan spot and appeared at the spots of all metabolites co-chromatographed with the ^{3}H-L-tryptophan. In a percentage comparison of radioactivity from lateral hypothalamic perfusate and the standard plates (Table 2), the majority of the

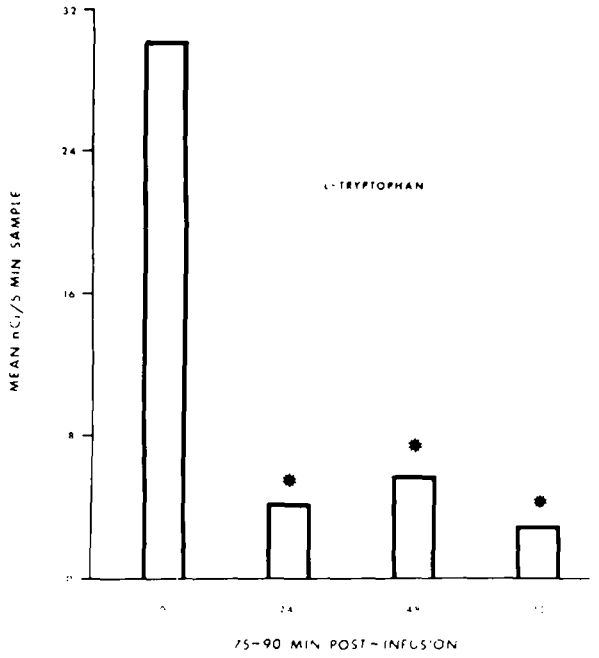

FIG. 2. Mean nCi/5 min of ^{3}H-L-tryptophan, 75–90 min post-infusion in 0, 24, 48 and 72 hr food deprived groups. *Significantly different from the 0 hr group.

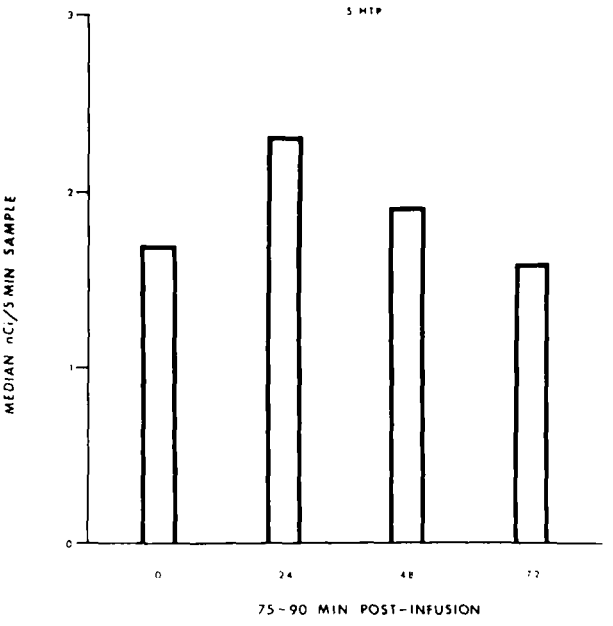

FIG. 3. Median nCi/5 min of ^{3}H-5-HTP, 75–90 min post-infusion in 0, 24, 48 and 72 hr food deprived groups.

radioactivity attributable to the perfusate metabolites was specific except for the 0 hr deprivation group. In this group, the percentage spread of radioactivity was similar in the perfusate and standard plates. This probably represents little if any functional synthesis and might be related to the ex-

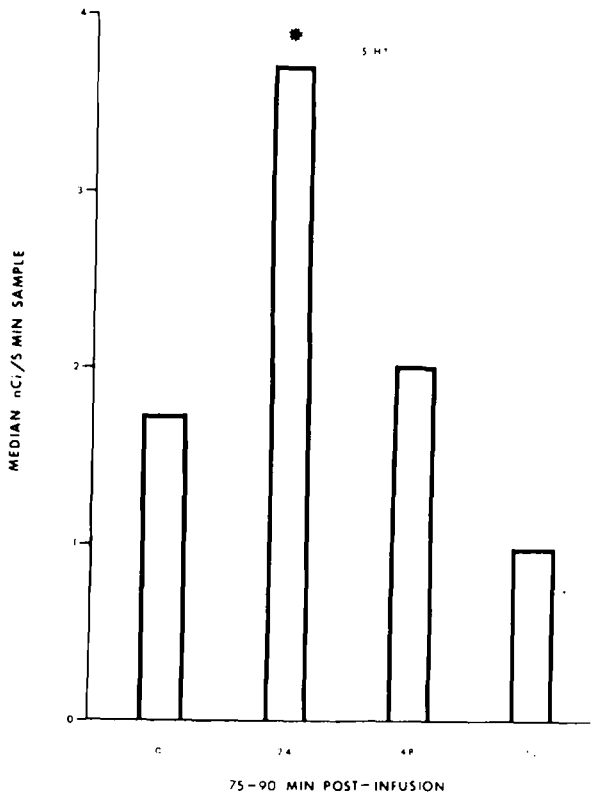

FIG. 4. Median nCi/5 min of ^{3}H-5-HT, 75–90 min post-infusion in 0, 24, 48 and 72 hr food deprived groups. *Significantly different from the 0 hr group.

tremely slow 5-HT synthesis rate found in the hypothalamus compared to other brain regions [2,21].

The Rf values are presented in Table 3. 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.

DISCUSSION

The results indicate an enhanced synthesis of serotonin in the lateral hypothalamus as a function of hours of food deprivation. Following 24 hr of food deprivation there was an increased formation of ^{3}H-5-HT. There were no differences in ^{3}H-5-HT formation following 48 and 72 hr of food deprivation. However, statements concerning synthesis can not be made without consideration of turnover [13]. As demonstrated in a previous study [19], there was significantly more 5-HIAA formed following 48 and 72 hr of food deprivation than at 0 and 24 hr of deprivation. The data indicate that more of the ^{3}H-5-HT formed is being metabolized at a faster rate following 48 and 72 hr of food deprivation. Under these conditions, increased synthesis would be masked by faster turnover. Increases synthesis could be detected following 24 hr of food deprivation because the turnover to ^{3}H-5-HIAA is not as great as that following 48 and 72 hr food deprivation. The interpretation that synthesis is increased following 24, 48 and 72 hr of food deprivation is further supported by the data showing that tryptophan uptake is enhanced as a result of 24, 48 and 72 hr deprivation. If the rate of synthesis of

TABLE 1

^{3}H-L-TRYPTOPHAN STANDARD PLATES

	0	24	48	72
Tryptophan + Origin	99.27% ± 0.51%	97.27% ± 1.59%	97.75% ± 0.48%	97.03% ± 0.68%
5-HTP	0.25% ± 0.17%	1.27% ± 0.49%	0.76% ± 0.27%	1.51% ± 0.36%
5-HT	0.48% ± 0.21%	1.45% ± 0.86%	1.49% ± 1.04%	1.46% ± 0.41%

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-L-tryptophan + Origin	99.47%	99.27%	83.79%	97.27%	94.47%	97.75%	90.56%	97.03%
^{3}H-Metabolite	0.53%	0.73%	16.21%	2.73%	5.53%	2.25%	9.44%	2.97%

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
Tryptophan	0.49 ± 0.01	0.35 ± 0.01	0.49 ± 0.01	0.36 ± 0.01	0.46 ± 0.01	0.39 ± 0.01	0.40 ± 0.01	0.34 ± 0.01
5-HTP	0.75 ± 0.01	0.93 ± 0.01	0.74 ± 0.01	0.93 ± 0.01	0.69 ± 0.01	0.94 ± 0.01	0.71 ± 0.01	0.93 ± 0.01
5-HT	0.49 ± 0.01	0.75 ± 0.01	52 ± 0.01	0.78 ± 0.01	0.46 ± 0.01	0.75 ± 0.01	0.48 ± 0.01	0.72 ± 0.01

Values are the Mean ± S.E.M.

5-HT were the same following 0, 48 and 72 hr of deprivation, then one would not expect differences in tryptophan uptake and 5-HT turnover to 5-HIAA. These 2 measures are indicative of 5-HT synthesis rate [8]. Therefore, an increased synthesis of 5-HT in the lateral hypothalamus is indicated following 24, 48 and 72 hr of food deprivation. In addition, if the data are expressed as a ratio of metabolite to substrate [19], then the formation of ^{3}H-5-HT from ^{3}H-L-tryptophan is significantly enhanced following 24, 48 and 72 hr of food deprivation.

The increased serotonin synthesis and turnover observed following food deprivation is important. Since turnover of extraneuronally released serotonin is the best index of functional serotonin utilization [9], more serotonin must be functionally active following food deprivation up to 72 hr in the lateral hypothalamus. The behavioral significance of the increased serotonin utilization might be involved in the increased motor activity which accompanies food deprivation and which is mediated by hypothalamic mechanisms [32].

There is considerable pharmacological evidence that substances such as L-tryptophan [8, 10, 17, 24], 5-hydroxytryptophan [24], fluoxetine [15], chlorimipramine [24], para-chloroamphetamine [29], fenfluramine [29], quipazine [12] and veratramine [16] produce a specific serotonin-mediated hyperactivity syndrome. All these substances can act to either increase functional serotonin availability or mimic serotonin. When functionally active serotonin is increased, so is motor activity [9]. Although the hyperactivity is serotonin-mediated, it is also dopamine dependent [10,17]. The activity of the serotonergic neurons which produce the hyperactivity depends upon adequate dopamine concentration. Dopamine depletion or blockade breaks the neuronal sequence necessary for the behavioral expression of 5-HT receptor site stimulation.

The hypothesis that increased serotonin utilization is involved in the production of motor activity is further supported by data showing that, within normal circadian variation, serotonin turnover and tryptophan concentration are highest during the dark phase of a light/dark cycle for rats [13, 14, 25]. This occurs not only in the whole brain but also regionally in the cortex, brain stem and hypothalamus. Rats are normally more active during the dark phase and food deprivation up to 96 or 120 hr has been shown to increase motor activity as well [1,28]. Since serotonin turnover has been shown to increase under both conditions, the increased activity might be serotonin mediated. The increased activity following food deprivation cannot be accounted for solely by the catecholamines. Following 22 hr of food deprivation, in-

creases in hypothalamic dopamine level and norepinephrine and dopamine synthesis are found [5]. Other investigators found that the increased hypothalamic norepinephrine release following 16 hr food deprivation is not correlated with motor activity [31]. However, the increased dopamine release following 16 hr of food deprivation is correlated with motor activity but at a time lag of 10 min. This might be related to the dopamine dependency of serotonin mediated hyperactivity.

Motor activity is not the only behavior which might involve serotonin utilization. There is evidence that serotonin might be involved in many different behaviors such as sleep [18], sexual behavior [6], and aggression [30]. The exact role of serotonin in any of these behaviors is unknown. If serotonin is directly involved in the mediation of these behaviors or interacts with other neurotransmitters or neuropeptides to produce these effects remains to be determined. An important point is that serotonin utilization might be involved with the facilitation of behavior as well as its inhibition.

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