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RAPID COMMUNICATION

Patterns of Extracellular 5-Hydroxyindoleacetic Acid (5-HIAA) in the Paraventricular Hypothalamus (PVN): Relation to Circadian Rhythm and Deprivation-Induced Eating Behavior

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STANLEY, B. G., D. H. SCHWARTZ, L. HERNANDEZ, S. F. LEIBOWITZ AND B. G. HOEBEL. *Patterns of extracellular 5-hydroxyindoleacetic acid (5-HIAA) in the paraventricular hypothalamus (PVN): Relation to circadian rhythm and deprivation-induced eating behavior.* PHARMACOL BIOCHEM BEHAV **33**(1) 257–260, 1989.—Daily rhythms in extracellular levels of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were examined in the region of the paraventricular nucleus (PVN), using intracerebral microdialysis combined with high performance liquid chromatography and electrochemical detection. Samples of PVN dialysate, from 11 rats on a 12/12 hr light/dark cycle, were collected and assayed for 5-HIAA every 2 hr for 3 days. During the first 2 days the rats were given free access to food. During the 3rd day they were deprived of food for a 24-hr period and then given food for 4 hr. The results showed that in freely-feeding rats, there was a 24-hr rhythm in the levels of 5-HIAA, with a marked transient peak just after the beginning of the dark portion of the light/dark cycle and stable levels at all other times. When the animals were food-deprived, PVN levels of this metabolite remained stable, and the early dark peak was abolished, suggesting that it might have been consequent to the eating behavior which normally occurred at this time. In the 4-hr refeeding period, there were no changes in 5-HIAA levels, despite the intense eating behavior which occurred during this time. These patterns of 5-HIAA in the PVN region, taken together with previous evidence, suggest that PVN serotonin metabolism may increase in association with feeding specifically in the early portion of the nocturnal eating period, when it may play a role in controlling food intake and macronutrient selection.

Circadian rhythm Eating behavior Microdialysis Serotonin 5-Hydroxyindoleacetic acid Paraventricular nucleus

CONVERGING evidence suggests that serotonergic neurons innervating the paraventricular nucleus (PVN) have a suppressive role in the control of eating behavior. Central administrations of serotonin (5-HT), or of drugs which release endogenous 5-HT, are most effective in the PVN (20,26), and conversely, electrolytic lesions of this nucleus are highly effective in attenuating the anorexia produced by peripheral injections of these drugs (25). A more specific function of PVN 5-HT may be to suppress consumption of a particular macronutrient, namely, carbohydrate. PVN injections of 5-HT or its agonists reduce ingestion of carbohydrate while having little or no effect on fat or protein intake (18, 20, 24). Most recently, this effect was found to vary in relation to the natural circadian cycle, with 5-HT exhibiting its greatest effectiveness at the start of the active (nocturnal) cycle (13, 20, 21, 24), a time when rats normally exhibit a preference for carbohydrate (22). This finding has been interpreted as reflecting a natural daily rhythm of serotonergic activity in the PVN.

In a recent microdialysis study, primarily focused on daily rhythms of PVN NE (20), we also measured the serotonin metabolite 5-HIAA and observed results which may support this

interpretation. In the present paper we report these observations that in freely-feeding animals the levels of extracellular 5-HIAA peak in the area of the PVN near the onset of the nocturnal eating period.

METHOD

Subjects and Surgery

A total of 11 adult male Sprague-Dawley rats, with indwelling 21-gauge stainless steel cannulas terminating above the PVN, were adapted to a 12:12 light/dark cycle, with lights on at 0900 hr and off at 2100 hr.

Microdialysis Procedures

Dialysis probes were constructed as described previously (6,19), such that when inserted into the guide cannula the 2.0 mm long, 0.25 mm diameter hollow semipermeable cellulose fiber tip penetrated the PVN. The dialysis membrane had a molecular weight cutoff of 6000, resulting in approximately 5% relative recovery (concentration in dialysate/concentration in test solution) of 5-HIAA in vitro (6). Ringer's solution (189 mM NaCl, 3.9 mM KCl, and 3.37 mM $CaCl_2$), pumped through the probe at a rate of 1 μl/min, was collected in 400 μl vials (containing 5 μl of 0.1 N HCl/100 μM EDTA) clipped to the rat's headpiece. The probe was connected to the syringe pump via a counterbalanced swivel arm at the top of the test chamber, thus allowing the subjects unrestricted movement.

HPLC Procedures

As previously described (6,19), the dialysate was injected directly into the HPLC and analyzed by reverse phase liquid chromatography with electrochemical detection. The mobile phase contained 116.8 mM NaOH, 144.7 mM monochloroacetic acid, 100 μM EDTA, 1.38 mM l-octanesulfonic acid and 2% v/v acetonitrile at pH 3.1, pumped at a rate of 1 ml/min. The oxidation potential was 0.71 V and 5-HIAA had a retention time of 5.5 min. The minimum detectable quantity of 5-HIAA, at a 4:1 signal to noise ratio, was approximately 1 pg or 5 fmoles.

Free Feeding, Deprivation and Refeeding

Microdialysis probes were inserted into the guide cannulas approximately 24 hr prior to the experimental session, to allow time for recovery of physiological function in brain tissue around the probe tip (2, 8, 27). Collection of 20-min samples of dialysate took place every 2 hr for a period of 3 days, starting at 1200 hr. Purina rat chow pellets were available during the first 2 days. After this period, the food was removed at 1200 hr, and collection of samples continued every 2 hr for a third 24-hr period. Food was then returned at 1200 hr, and samples of dialysate were collected every 20 min for 1 hr and then every hr for an additional 3 hr. Food intake was measured every 20 min for 1 hr and at the end of the 4-hr test.

Statistics

Main effects were determined using one-way or two-way repeated measures ANOVA, followed by a Duncan's New Multiple range test ($p<0.05$) to determine the contribution of specific subgroups. A gradual decline in 5-HIAA was observed during the 48-hr free-feeding period (slope = −6.8 pg/measurement; $p<0.001$ by Linear Regression). To eliminate the possibility that this decline might contribute to the statistical effects, analysis was also conducted on data that was transformed by adding increasing increments of 6.8 pg to successive values from each subject. Correlations were calculated using Pearson's correlation coefficient.

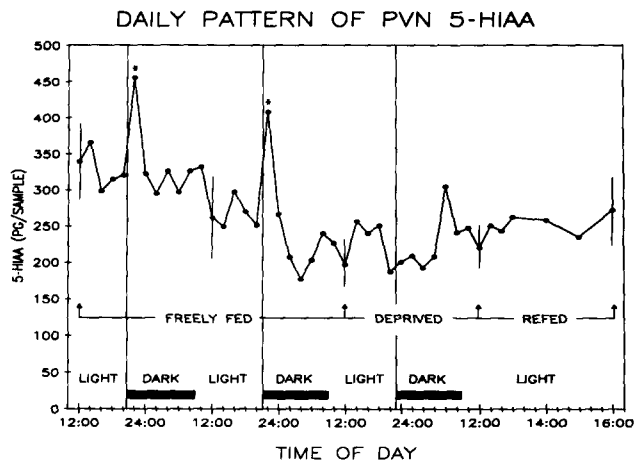

FIG. 1. Pattern of 5-HIAA (mean pg ± SEM at each 1200 hr) as a function of the time of day in samples of dialysate from the PVN area. The 11 subjects had free access to food for the first 2 days and were deprived the last day. In freely-feeding rats, 5-HIAA levels were significantly ($p<0.05$) greater at 2200 hr than at any other time. This was 1 hr after the onset of the dark phases, which are indicated by the horizontal bars above the x-axis. The 5-HIAA peak was abolished by food deprivation. Subsequently, food was returned and the animals ate vigorously, but 5-HIAA did not change. Note that the time scale was increased for better resolution during this period of refeeding.

Histology

After the experiments, the rats were anesthetized with pentobarbital and intracardially perfused with 10% buffered formalin. The brains were then removed, sectioned, and stained with cresyl violet.

RESULTS

As shown on the left half of Fig. 1, when the animals were freely fed, 5-HIAA peaked 1 hr after the beginning of the dark period. This peak was evident on both days and contrasts with the stable levels at all other times of the cycle. The average level of 5-HIAA was 294 ± 37 pg/sample during this period, and the values on days 1 and 2 were significantly correlated (r = .77). Statistical analysis revealed a significant main effect of Time of Day [$F(12,240)=4.27$, $p<0.0001$; or $p<0.001$ on the transformed data], with no significant difference between Day 1 and Day 2, $F(1,20)=1.62$, n.s., and no significant interaction between Days and Time of Day, $F(12,240)=0.5$, n.s. Post hoc comparisons revealed that the only statistically significant effect ($p<0.05$) was at 2200 hr, when the levels of 5-HIAA increased by an average of 147 pg/sample.

This pattern was apparent in 9 of the 11 animals, each of which had their greatest 5-HIAA levels at 2200 hr. This is illustrated for a representative rat in Fig. 2. Over the 24-hr period shown, 5-HIAA levels in this subject were relatively stable, averaging 410 ± 43 pg. However, at 2200 hr, one hr into the dark phase, 5-HIAA was markedly elevated to a level of 805 pg, after which it returned toward previous levels.

As shown in the central portion of Fig. 1, the peak of 5-HIAA at dark onset was abolished under food deprivation conditions. Moreover, food deprivation produced no significant changes in the

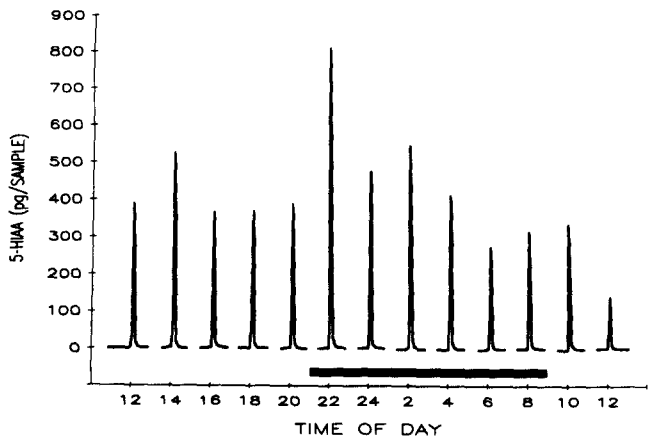

FIG. 2. Pattern of 5-HIAA (pg/sample) as a function of time of day in a representative subject. These peaks were traced from a series of this subject's chromatograms. The highest peak was one hr after the onset of the dark phase, as indicated by the horizontal bar above the x-axis.

baseline levels of 5-HIAA, $F(12,85)=0.88$, n.s., although there was a small nonsignificant peak at 0600 hr.

The right side of Fig. 1 shows the levels of 5-HIAA during the 4 hr after food was returned. There were no significant changes in 5-HIAA during this period, $F(6,53)=0.21$, n.s., even though the subjects initially exhibited intense eating behavior. Measures of food intake revealed that all subjects began eating within 60 sec, and they continued to eat throughout the first 20 minutes. In successive 20-min periods, they ate an average of 3.9 ± 0.4 g, 1.5 ± 0.2 g, and 1.0 ± 0.2 g for a total of 6.4 g in the first hr. During the subsequent 3 hr, they ate an additional 3.3 g.

Analysis of histology showed that the tip of the probe passed through the PVN, or immediately adjacent to its borders, in 8 of the 10 subjects examined. In the remaining 2 animals, the probe was located either anterior or lateral to the PVN. The average level of 5-HIAA (197 pg) in these "off target" subjects was lower than the overall mean (294 pg) but there was no clear difference in their pattern of 5-HIAA.

DISCUSSION

This study demonstrates that in the area of the PVN of freely-feeding rats, there is a 24-hr rhythm of extracellular 5-HIAA, characterized by a marked transient peak near the beginning of the dark period and stable levels at other times, under these conditions. This early dark peak of 5-HIAA was abolished during food deprivation. There were no significant alterations in 5-HIAA levels during or after the intense eating behavior consequent to food deprivation. Others have shown that extracellular 5-HIAA may increase during feeding in rats on a food restriction schedule (7a). Our findings suggest that, in freely feeding rats, the metabolism of 5-HT to 5-HIAA by neurons innervating the PVN area peaks at the beginning of the dark phase, and that this peak may be specifically related to the feeding behavior which normally occurs at this time.

In light of recent evidence dissociating 5-HIAA from 5-HT levels in the brain (10), the present data, showing changes in 5-HIAA alone, need to be interpreted cautiously. In some cases, changes in 5-HIAA may mimic changes in 5-HT (15); however, other biochemical studies demonstrate that the levels of these two chemicals can be dissociated by pharmacological and behavioral manipulations (8,17). Therefore, the present evidence does not permit the conclusion that there is a circadian rhythm in the synaptic release of 5-HT; it does, however, suggest that a daily cycle of physiological 5-HT metabolism may exist in the area of the PVN.

Consistent with this proposal are several other studies which do indicate that 5-HT activity peaks during the dark or active period. For example, electrophysiological measures of 5-HT unit activity in the dorsal raphe nucleus have revealed an increase in firing rate during the active period of the light/dark cycle in cats (23). This is consistent with biochemical studies of whole brain (7) or of PVN tissue (9), which suggest daily rhythms of 5-HT function with peak activity during the dark or active phase of the light/dark cycle. Moreover, in a voltammetry study of the SCN (4) and microdialysis studies of the ventromedial (15) or lateral hypothalamus (16), measures of extracellular 5-HIAA and/or 5-HT each demonstrated peak levels in the nocturnal period. Like the PVN, the SCN showed its peak specifically at dark onset (4), in contrast to the ventromedial hypothalamus which demonstrated a peak in the middle of the dark (15).

In the present study, food intake was necessary but not sufficient for the early dark peak of extracellular 5-HIAA to occur. This suggests that the peak was not due solely to the widespread increase in the levels of behavioral activity normally seen at this time. Nor did the peak seem to be due to an endogenous circadian pacemaker or a change in light intensity, as might have been suggested by neural inputs to the PVN from retinal ganglion cells and from the SCN (3,28). However, the finding that the early dark peak was dependent on the presence of food and thus presumably on feeding behavior, suggests that food consumption and/or the postingestive effects of the nutrients themselves are involved in the release of 5-HIAA into the extracellular fluid. This is consistent with the proposal that feeding-induced alterations in circulating nutrients can impact on brain neurotransmitter activity (5). The additional finding that this peak in 5-HIAA did not occur during feeding at other times, suggests that certain times of the circadian cycle might be more favorable than others for nutrient effects to be translated into neurotransmitter metabolism. Specifically, it appears that food intake is most effective as a stimulus for 5-HIAA rise at dark onset. This is consistent with the findings of Ashley *et al.* (1) demonstrating in humans that changes in circulating amino acid ratios occur after carbohydrate intake early in the active period (morning), as opposed to later in the active period (evening).

What do these patterns of 5-HT metabolism suggest as a function for 5-HT innervation of the PVN? It has previously been proposed that PVN 5-HT normally functions to suppress consumption of carbohydrate during the early dark period of the feeding cycle (12). This proposal was based on the findings that PVN injection of 5-HT, or of drugs that enhance or mimic its effects, are most effective in suppressing eating of carbohydrate, as compared to protein and fat, and that these effects are most apparent immediately after the onset of the dark period (13, 20, 21, 24). In fact, low doses of 5-HT are effective in reducing carbohydrate intake only during the first few hrs of the dark period (13), at a time when freely-feeding rats normally select carbohydrate diets in preference to protein or fat diets (22). The present results suggest that the documented rhythm in the effectiveness of PVN 5-HT in suppressing feeding may be related to the observed rhythm in the metabolism of 5-HT to 5-HIAA by neurons innervating the PVN.

We have recently provided evidence that NE , which is known to elicit eating behavior and a specific carbohydrate preference (11,14), is released in the PVN region during the early portion of the dark period (19). We have suggested that this early dark NE peak might function to initiate and/or maintain food intake and more specifically carbohydrate intake at this time (19). Consistent with published reports (12), the findings of the present study

suggest that PVN NE and 5-HT might act in opposition to control meal size and carbohydrate intake during the early portion of the dark phase, with NE acting to initiate carbohydrate meals and 5-HT acting to terminate these meals. Measures of changes in the extracellular levels of 5-HT in the PVN in relation to the initiation and termination of meal taking will be needed to clarify its actual role in control of eating behavior.

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