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Effects of Rapid Depletion of Phenylalanine and Tyrosine on Sleep and Behavior[1]

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BARRATT, E. S., P. M. ADAMS, P. L. POFFENBARGER, R. R. FRITZ AND C. W. ABELL. *Effects of rapid depletion of phenylalanine and tyrosine on sleep and behavior.* PHARMAC. BIOCHEM. BEHAV. 5(1) 47–53, 1976. – The effects of fluctuations of free amino acid concentrations in plasma on sleep patterns and operant behavior in the squirrel monkey were studied. Plasma phenylalanine (PHE) and tyrosine (TYR) were rapidly lowered to trace levels within 4 hr by intraperitoneal administration of phenylalanine ammonia-lyase (PAL), an enzyme which specifically deaminates both PHE and TYR to inactive products. Significant alterations in sleep patterns and in performance on a chained operant task involving hold and reaction time components were found, but no significant effect on the performance of a simple operant task was observed. Administration of saline or trans-p-cinnamic acid and trans-p-coumaric acid, the products of PHE and TYR deamination, produced no changes in behavior or sleep patterns. The reduction of plasma PHE and TYR resulted in a significant decrease in PHE and TYR levels in whole rat brain. Brain serotonin levels were increased within 4 hr after PAL administration, whereas, dopamine and norepinephrine levels were decreased subsequently (within 8 hr). These studies suggest that circulating levels of PHE and TYR are involved directly or indirectly in the modulation of certain parameters of brain function.

Sleep patterns Operant Behavior Phenylalanine Tyrosine Squirrel monkey Amino acids

ALTHOUGH previous investigations indicate that neural transmission and, thus, brain functioning and behavior are related to particular amino acid metabolites, the relationship of these biogenic amines to the concentration of precursors has not been defined. One of the difficulties in relating amino acid metabolism to brain functioning and behavior has been the lack of techniques to selectively and reversibly manipulate levels of amino acids. Techniques used to date, such as dietary manipulation [30] or loading procedures [18] have been nonspecific and difficult to control. The study reported here involved the administration of an enzyme, phenylalanine ammonia-lyase (PAL), to achieve specific and rapid reduction of plasma and brain levels of two amino acids, phenylalanine and tyrosine. Thus, brain activity can be studied in relationship to changes in these amino acids.

PAL is widely distributed in plants [8, 17, 31], in some fungi, and in yeast [16, 23, 25, 26]. It has been purified from the yeast *Rhodotorula glutinis* and evaluated as a therapeutic agent against leukemias [1, 2, 28]. Because of its selective action on phenylalanine and tyrosine, its potential for studying the role of these two amino acids, especially tyrosine, in brain functioning and behavior was feasible. Tyrosine is a precursor of dopamine and norepinephrine, both of which have been suggested as important neurochemical components in affective behaviors and in the production of mental aberrations including schizophrenic symptoms [18].

The experiments reported here were designed to determine the effects of PAL on: plasma and brain levels of phenylalanine and tyrosine; brain levels of dopamine, norepinephrine, and serotonin; sleep patterns; performance of a simple operant task; and performance of a more complex chained operant task. Sleep was chosen as a dependent variable because changes in sleep patterns have been related to the levels of phenylalanine and tyrosine derivatives and to psychotic disorders. Both a simple and a more complex operant task were chosen because performance of simple operant tasks is often resistent to drugs that can alter performance of more complex ones.

METHOD

PAL Preparation and Measurement

The yeast *Rhodotorula glutinis* (IFO 0559, IFO denotes the Institute of Fermentation, Osaka, Japan) was either purchased from P-L Biochemicals, Inc. or grown in a 14 L fermentor and PAL (E.C. 4.3.1.3) was purified according to the method of Fritz, *et al.* [13]. PAL was assayed by a modification of the method of Hodgins [16]. The reaction

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mixture contained 0.833 mM L-phenylalanine or 0.833 mM L-tyrosine in Tris-HCl buffer (0.1 M, pH 8.5). The formation of trans-p-cinnamic acid was monitored at 290 mm using a Gilford Model 240 Recording Spectrophotometer. Under these conditions, PAL catalyzes the deamination of L-tyrosine to about 25% of phenylalanine. One unit of enzyme is defined as that amount of protein that catalyzed the appearance of one μmole of cinnamic acid or coumaric acid per minute at 30°C.

The enzyme preparation was sterilized using a positive pressure Millipore filter (0.22 μ) and stored in potassium phosphate buffer (0.2 M, pH 7.2) at −60°C. The protein concentration of the sterilized preparation was in the range of 20 to 30 mg/ml with a specific activity of 2.5 to 3.0 units/mg.

Highly purified PAL, demonstrating a single major band on polyacrylamide gels, was injected intraperitoneally into squirrel monkeys at a concentration of 50 or 100 units/kg body weight. Blood samples were collected in heparinized capillary tubes, which were centrifuged in an Adams Micro Hematocrit Centrifuge. Circulating enzyme activity was then measured in diluted plasma using the assay indicated above.

Sleep Patterns Study

Sleep recordings of three adult male squirrel monkeys weighing 500–650 g were made following recovery from surgery. Chronic indwelling bipolar electrodes of stainless steel were implanted under nembutal anesthesia in the midbrain reticular area (A 4.0, H 2.5, D-V 2.0); stainless steel screw electrodes were placed bilaterally over the medial parietal-temporal cortex (A 5.0, H 12.0) and superior temporal cortex (A 0.0, H 10.0). Coordinates were determined according to the atlas of Gergen and MacLean [15]. Silverball electrodes were implanted bilaterally at the outer canthus of each eye for electro-oculogram (EOG) recordings. A reference electrode was placed over the frontal cortex using a stainless steel screw.

Recordings of spontaneous EEG and EOG were made from the cortical and subcortical sites beginning at 6 p.m. and ending at 6 a.m. Throughout the 12 hr, continuous recordings were made on a Grass Model IV electroencephalograph for 5 min periods with 10 min intervals of no recording. The recordings were scored for the different stages of sleep-wakefulness according to criteria established for the squirrel monkey [3]. Baseline recordings were made biweekly (3 to 4 days apart) for thirty days to obtain stable sleep-wakefulness profiles.

On the day that PAL was to be administered, assays of plasma amino acids were performed on a 3:30 pm femoral vein blood sample (1.5 ml). PAL (50 units/kg) was then adminstered intraperitoneally at 3:55 pm and the monkey immediately placed into the recording cubicle. Behavioral observations and EEG recordings were made continuously for 2 hr. A second blood sample was drawn at 5:55 pm (+2 hr) to confirm the expected reduction in PHE and TYR and the animal replaced in the cubicle.

Sleep-wakefulness patterns were then recorded for the 6 p.m. - 6 a.m. period and scored for the different sleep stages. A third blood sample was drawn at 24 hr following PAL administration. At 72 hr after PAL treatment, a follow-up recording session began and sleep-wakefulness patterns were recorded.

Simple Operant Behavior

Five adult male squirrel monkeys weighing 500–650 g and housed individually were maintained on a daily diet of 30 g each of fresh apple and orange and a high protein supplement (Smith Kline French) adjusted according to the number of reward pellets received during experimental sessions.

The monkeys were placed on the above diet for several weeks prior to the beginning of the experiments and were trained to press a lever for sucrose pellet rewards (94 mg Noyes pellets) on a schedule requiring a variable number of responses for each reward. This schedule [12] produced a stable level of responding throughout each daily session. The length of the daily session was held constant at 30 min.

Once a stable level of performance was established, as measured by the number of lever presses per session, the experiments began. Initially, a protocol was done to determine the effects of venipuncture alone (1.5 ml/time) on the performance of simple operant behavior. A period of 30 days elapsed to provide sufficient time for recovery from loss of blood before the effects of PAL were studied.

A single injection of PAL (100 units/kg) was administered intraperitoneally immediately after obtaining an 8 am blood sample. Additional blood samples were taken at 4, 8, and 24 hr after the injection of enzyme. The effects of PAL treatment on the simple operant performance were studied 30 min prior to obtaining the 4 hr blood sample. The above protocol was repeated with PAL in another group of five monkeys at a dosage of 50 units/kg.

Complex Operant Behavior

The complex operant task performed by four monkeys involved sequential response contingencies in a discrete trial format [22]. Each trial consisted of a ratio component requiring a number of lever presses, a hold period requiring a sustained lever depression for several seconds, and a reaction time (RT) component following the hold period; the RT was elicited by a stimulus cue to release the lever in order to receive a sucrose pellet reward. A 3 sec maximum release time contingency was in effect in order to maintain stimulus control. Between trials, a 10 sec period of nonreward for any lever response was signalled by a flashing yellow light. A lever response during the last 5 sec of this period resulted in resetting a 5 sec timer and prevented the initiation of the next trial. Baseline measurements of the number of correct trials, latencies to commence the ratio component, reaction times, and the number of non-rewarded responses made between trials were carried out for daily sessions of 90 trials.

Training to perform this task took approximately 90 days and stable daily performance occurred after another 30 days. The effects on the daily performance of saline and drawing blood were then measured as were the effects of intraperitoneal administration of trans-p-cinnamic acid and trans-p-coumaric acid at a concentration equivalent to twice the total circulating value of their respective precursors, PHE and TYR. Blood was drawn at 8 a.m., 12 noon, and 4 p.m. as described above. Thirty days after the control experiment was performed, the effects on performance of 100 units/kg of PAL were studied. The behavioral effects were assessed beginning 2 hr after the injection. The above protocol was repeated with 50 units/kg of PAL in another group of four monkeys.

Plasma Amino Acid Profile

Heparinized whole blood samples were centrifuged and the plasma protein was precipitated with an equal amount of cold 10% sulfosalicylic acid. The supernatant was stored frozen and analyzed for free amino acids on a Beckman Model 119 Amino Acid Analyzer using a two column system. The results of the analysis were reported in μmoles of amino acid/ml of plasma.

Brain Amino Acid Profile

The effects of 100 units/kg of PAL administered intraperitoneally on free amino acid levels in whole brain were studied in a group of 90–100 g male rats. Intraperitoneal injections of sterile 10 mM potassium phosphate buffer, pH 7.2, served as the control. Both groups (3 rats each) were sacrificed by decapitation 4 hr following injection. The brains were removed within 1 min and homogenized in 10 ml of cold 20% sulfosalicylic acid and centrifuged at 2,000 x g for 20 min. The supernatants were removed and frozen for amino acid assays.

Brain Biogenic Amines

Twenty-four male rats (190–220 g) were given either PAL at 100 units/kg body weight or potassium phosphate buffer (10 mM, pH 7.2) intraperitoneally. The rats were maintained ad lib until 4 hr prior to sacrifice. The animals were decapitated at 4, 8, or 24 hr postinjection, and the brains were removed (2–3 min) and immediately placed in a -60° C freezer. The brains were homogenized in perchloric acid and prepared for assay using the method of Shellenberger and Gordon [27]. The determination of dopamine was based on the method of Anton and Sayre [4] using periodate oxidation following aluminum oxide extraction. Norepinephrine was determined by the method of Laverty and Taylor [20] and serotonin by the procedure of Curzon and Greene [9].

RESULTS

Sleep-Wakefulness Effects

The effects of 50 units/kg of PAL on the amount of time spent in each of the stages of sleep-wakefulness are shown in Table 1. The baseline data represent the sleep-wakefulness profile for 12 hr sessions after 4 weeks of bi-weekly recording. The baseline values are in line with sleep profiles reported earlier [3] for adult squirrel monkeys. PAL produced significant changes in the amount of time spent in all of the stages of sleep except for Stages 1 and 2.

TABLE 1

EFFECTS OF A SINGLE INJECTION OF PAL (50 UNITS/KG) ON THE PERCENT TIME SPENT IN THE STAGES OF SLEEP-WAKEFULNESS IN SQUIRREL MONKEYS*

	Baseline Session	Session Beginning 2 Hr After PAL	Session Beginning 72 Hr After PAL
Awake	14.5 ± 0.11	38.2 ± 14.7†	19.3 ± 5.8
Stage 1	31.6 ± 1.21	34.1 ± 6.9	32.3 ± 5.7
Stage 2	36.3 ± 2.26	23.4 ± 12.9	33.5 ± 4.6
Stage 3-4	6.3 ± 0.89	1.7 ± 1.5†	5.4 ± 4.3
Rapid Eye Movement	11.4 ± 1.11	2.6 ± 2.2†	9.5 ± 6.8

*Values are expressed as means and standard errors based from 3 squirrel monkeys for the period 6 pm-6 am.

†These values are significantly different from baseline values at the 0.05 level.

Statistical analysis of the differences in the amount of time spent in each stage of sleep for the last baseline, the PAL treatment, and the 72 hr recovery night was performed using the Kruskal-Wallis analysis of variance technique [19]. There was a significant increase ($p<0.05$) in awake time accompanied by a significant decrease ($p<0.05$) in States 3–4 (slow wave sleep) and in rapid eye movement (REM) sleep ($p< 0.05$).

At 72 hr following PAL administration, the amount of time spent in each of the sleep stages had returned to normal levels (no differences between recovery and baseline recordings of the amount of time spent in each stage were statistically significant).

Simple Operant Performances

Baseline levels of lever pressing were found to average 1140 responses during a 30 min period. The effects of venipuncture and of PAL alone are shown in Table 2. Venipuncture was found to decrease the mean number of lever presses to 78% of baseline while administration of 50 units/kg of PAL also affected this parameter to approximately the same extent. A dosage of 100 units/kg increased the level of responding by 15% on the day of PAL administration with a decrease comparable to that observed in control and treated (50 units/kg) monkeys on the following day. None of these observed changes were statistically significant.

TABLE 2

EFFECTS OF PAL ON SIMPLE OPERANT BEHAVIOR IN MONKEYS*

	24 Hr	4 Hr	24 Hr
Treatment	Before enzyme	After enzyme	After enzyme
PAL (50 units/kg)	1286 ± 440	965 ± 239	954 ± 317
PAL (100 units/kg)	1243 ± 228	1429 ± 389	953 ± 379
	Before blood sample	After blood sample	After blood sample
None	1140 ± 470	888 ± 249	711 ± 362

*Values reported are mean number of lever presses for a 30 minute period and standard errors for 5 squirrel monkeys. No significant differences were observed.

Complex Operant Performance

The effects of PAL on the ability of squirrel monkeys to perform the complex operant task are shown in Table 3. There were no significant changes in any of the dependent measures of performance after saline, trans-p-cinnamic and trans-p-coumaric acid administration. Two hours following 50 units/kg of PAL, a significant disruption ($p<0.05$) in performance was observed. The performance was characterized by a slowing of latency and reaction time responses, a marked decrease in the number of correct trials and an

TABLE 3

EFFECT OF PAL ON THE PERFORMANCE OF A COMPLEX OPERANT TASK

Dependent Measures	Saline	P-Cinnamic Acid + P-Coumaric Acid	PAL (50 units/kg) 2 Hr	24 Hr
Correct trials	87	88	2	78
Length of session (min)	27	27	115	32
Latency to make first lever press response at Beginning of each trial (sec)	0.53	0.55	15.0	3.06
Reaction time (sec)	0.30	0.42	13.4	0.79

increase in the time required to complete the 90 trials. At 24 hr, performance on the complex operant task had recovered considerably; however, some impairment in latency and reaction time was still evident.

When 100 units/kg of PAL was administered 2 hr prior to the behavioral session, a marked increase in errors occurred characterized by premature releases of the response lever before the cue was presented for the reaction time component. At approximately 150 min postinjection, the monkey stopped responding and remained inactive but awake for the remainder of the behavioral session. Performance was improved 24 hr after the injection, but latency and reaction time were still significantly longer than for the controls ($p<0.05$).

Circulating Levels of PAL and Amino Acids

The activity of PAL in the peripheral blood was determined 4, 8, and 24 hr after a single injection of the enzyme (Fig. 1a). After intraperitoneal injection, the enzyme reached a maximum in the circulation within 8 hr. Circulating enzyme activity was dependent upon the dose administered as were the plasma levels of PHE and TYR (Fig. 1b). As shown in Fig. 2, an inverse relationship was found between the levels of circulating enzyme and PHE or TYR in plasma.

Analysis of the levels of amino acids in blood at 0, 4, 8, and 24 hr after PAL injection are shown in Fig. 3. Although the most pronounced effects were a reduction of plasma PHE and TYR levels, the levels of some other amino acids such as threonine, serine, isoleucine, and leucine were also decreased. Still other free amino acids did not undergo significant changes. Tryptophan was not determined. The observed difference in the basal values of the amino acids is due to the variability between animals used for the 50 and 100 unit/kg study. The same animals were used for the control and 100 unit/kg PAL while different animals were used for the 50 unit/kg PAL study.

Brain Amino Acid Levels

The analyses of amino acids in the brains of rats after PAL administration are shown in Table 4. These studies were performed 4 hr after PAL treatment to approximate the time of the observed behavioral disruption on the complex operant task for the squirrel monkey. The levels of several amino acids were significantly altered following PAL injection; however, statistically TYR decreased most markedly.

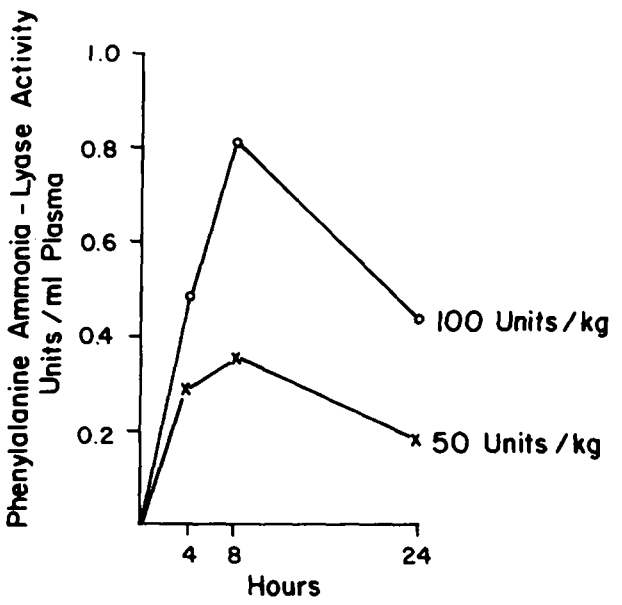

FIG. 1a. PAL levels in plasma at various time intervals and doses (0–0, 100 units/kg body weight; x–x, 50 units/kg body weight). Half-life of circulating enzyme was calculated to be 17 ± 1 hr. Each curve represents duplicate samples from an average of 5 squirrel monkeys.

Brain Biogenic Amines

Analysis of several biogenic amines (Table 5) demonstrated that PAL (100 units/kg) increased the level of serotonin approximately 3-fold within 4 hr, but the values subsequently decreased to normal within 24 hr. Conversely, levels of dopamine and norepinephrine remained unchanged within 4 hr after PAL administration but were reduced approximately 18% and 50%, respectively, within 8 hr and remained suppressed for 24 hr.

DISCUSSION

These studies demonstrate that the reduction of plasma and brain levels of PHE and TYR is accompanied by marked alterations in brain function and behavior. The importance of a relationship between these amino acids and sleep-wakefulness patterns was substantiated by dramatic changes in the sleep profile after PAL administration and its recovery following clearance of the enzyme. The reduction

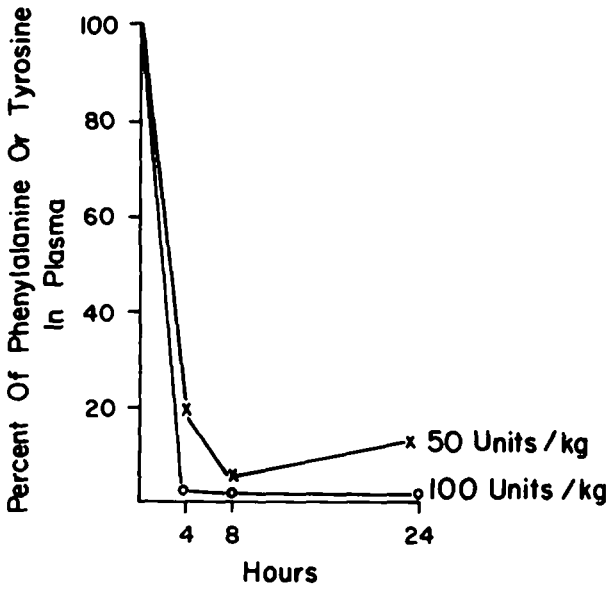

FIG. 1b. Percent of normal circulating phenylalanine or tyrosine levels in plasma at various time intervals after the administration of PAL at 50 (x–x) and 100 (0–0) units/kg body weight.

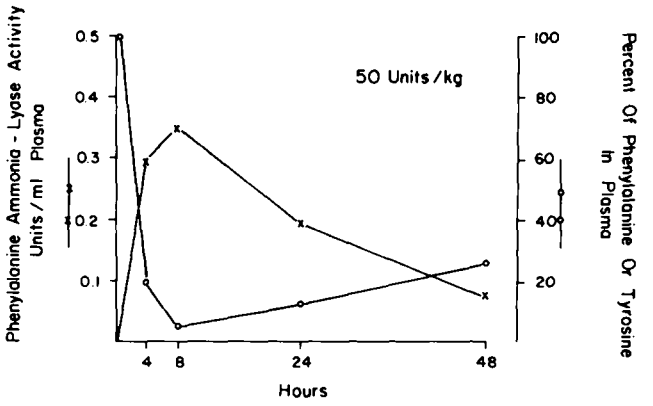

FIG. 2. Relationship between circulating PAL following intraperitoneal injection at 50 units/kg body weight and the percent of normal circulating phenylalanine or tyrosine as determined by amino acid analysis. Duplicate samples for both assays are from 5 squirrel monkeys and values shown above are the average mean.

of REM and SWS sleep following PHE and TYR reduction is consistent with the results of earlier work [30] on the effects of diets low in phenylalanine and tyrosine on REM sleep. In addition, previous studies on the blockade of norepinephrine synthesis by alpha-methyl-tyrosine revealed a decrease in REM sleep in primates [29].

The diminished amounts of SWS and REM sleep may reflect a change in brain catecholamine levels or of an imbalance in the brain amines. Because of the specificity of PAL and the rapid recovery within 72 hours of the altered amino acid levels to normal, this enzyme may provide a useful method to relate brain amines to the sleep-wakefulness mechanism.

The observed behavioral changes on the complex task also coincided with low levels of PHE and TYR. It is important to note that PAL had no effect on simple behavioral tasks which required considerable overtraining but considerably altered the performance of more complex well learned behaviors which required close attention to and/or memory of stimulus-response contingencies.

The plasma amino acid profiles demonstrated the importance of circadian factors as well as individual differences across animals which contribute to the variability of the analyses [5]. However, the reduction to trace levels of PHE and TYR following PAL demonstrates the potential of the enzyme for amino acid manipulation.

Our present studies imply the existence of a close relationship between PHE-TYR and brain-behavior function. Although tryptophan was not measured, brain serotonin levels were found to be increased within 4 hours after PAL administration while dopamine and norepinephrine levels were not reduced until 8 hr. These results suggest that

TABLE 4

BRAIN AMINO ACID PROFILE IN RATS SACRIFICED 4 HOURS AFTER BUFFER OR PAL TREATMENT*

Amino Acid	Buffer	PAL (100 units/kg)	p
Taurine	111.06 ± 1.21	109.77 ± 6.61	NS
Aspartic Acid	29.16 ± 1.59	24.65 ± 7.71	NS
Threonine	3.84 ± 0.16	4.44 ± 0.56	NS
Serine	9.60 ± 0.22	11.08 ± 0.86	NS
Glutamine/Asparagine	23.39 ± 6.88	9.95 ± 5.35	<.05
Glutamic acid	104.22 ± 3.39	112.83 ± 5.51	NS
Glycine	8.77 ± 0.32	9.87 ± 1.40	NS
Alanine	4.68 ± 0.39	5.35 ± 0.50	NS
Methionine	0.21 ± 0.01	0.32 ± 0.06	<.05
Isoleucine	0.34 ± 0.02	0.35 ± 0.06	NS
Leucine	0.69 ± 0.04	0.75 ± 0.09	NS
Tyrosine	0.78 ± 0.08	0.25 ± 0.04	<.01
Phenylalanine	0.53 ± 0.06	0.36 ± 0.07	<.05

*Values are reported in μ moles/g of tissue ± S.E. Probability values were determined on the basis of a Kruskal-Wallis analysis of buffer and PAL treatment differences. Proline, valine, and cystine were undetected in this system.

TABLE 5

EFFECT OF PAL ON LEVELS OF DOPAMINE, NOREPINEPHRINE, AND SEROTONIN IN RAT BRAIN*

Hrs After Injection	Dopamine		Norepinephrine		Serotonin	
	Buffer	PAL	Buffer	PAL	Buffer	PAL
4	0.73 ± 0.08	0.65 ± 0.07	0.34 ± 0.02	0.28 ± 0.03	0.48 ± 0.06	1.46 ± 0.25†
8	0.70 ± 0.03	0.57 ± 0.03*	0.34 ± 0.02	0.15 ± 0.003‡	0.54 ± 0.04	0.77 ± 0.06†
24	0.67 ± 0.03	0.55 ± 0.03*	0.34 ± 0.02	0.15 ± 0.003‡	0.48 ± 0.02	0.37 ± 0.08

*PAL (100 units/kg body weight) was administered intraperitoneally. Values of amines are expressed as mean ± S.E. in g/g wet weight brain.
*$p<0.05$.
†$p<0.02$.
‡ $p<0.001$.

greater transport of plasma tryptophan into the brain may occur when plasma PHE and TYR are reduced [10,11]. Previous investigations show the importance of mutual competition for transport into the brain among the neutral amino acids [7,24]. Alternatively, synthesis of serotonin from pre-existing brain tryptophan could be increased by reduction of PHE and TYR, inhibitors of tryptophan hydrolylase [21]. Other studies indicate that increased tryptophan produces disturbance in behavioral [6] and sleep-wakefulness patterns [14]. Our observed alterations in sleep patterns and behavior as a function of PAL are compatible with these observations and further substantiate the importance of brain levels of these amino acids in neural functioning.

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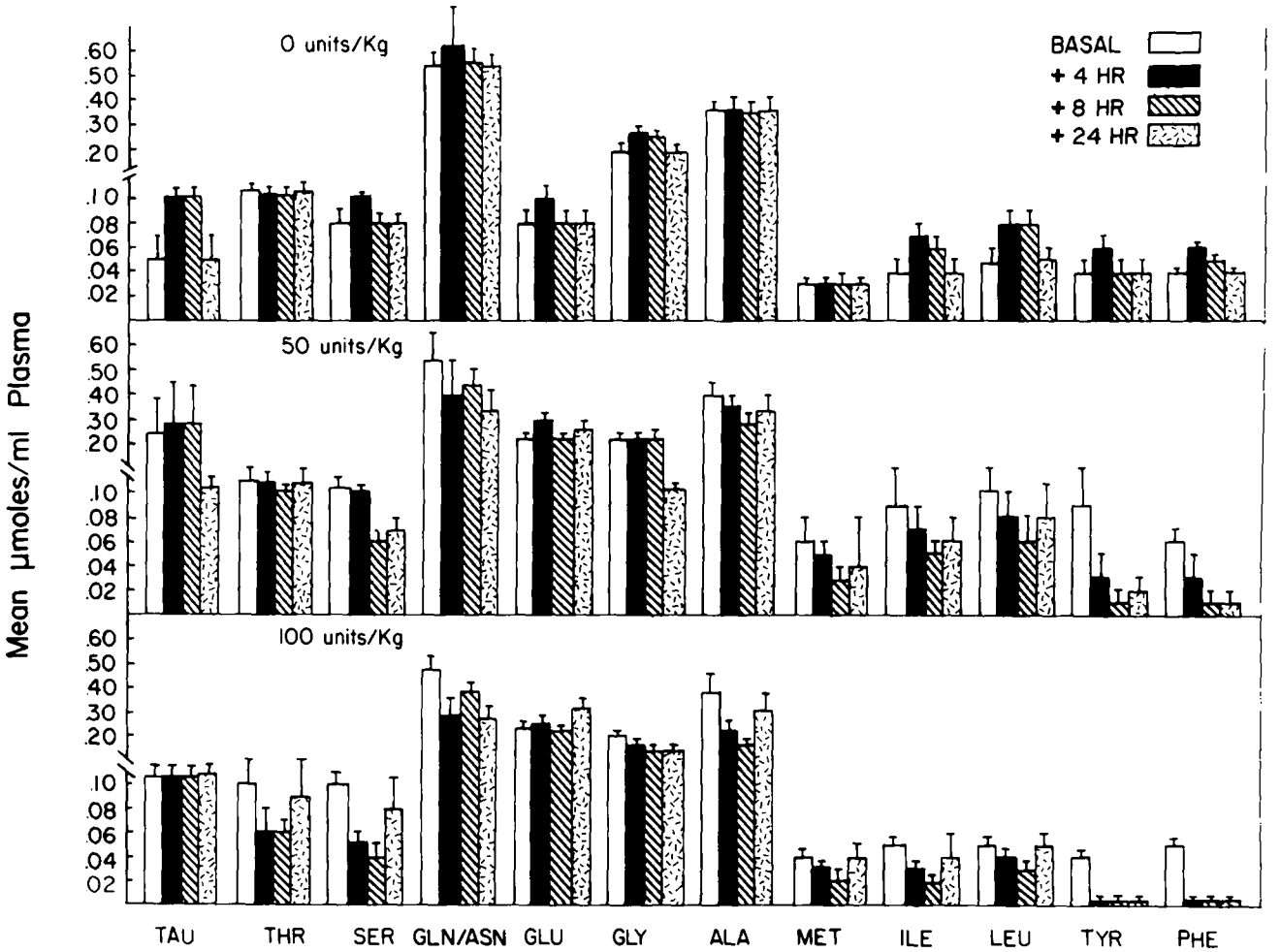

FIG. 3. Mean plasma levels of amino acids at 0, 4, 8, and 24 hr following PAL administration of 50 and 100 units/kg body weight. Control injection of isotonic saline is given as a comparison for profile differences at the 4 sample times.

REFERENCES

1. Abell, C. W., W. J. Stith and D. S. Hodgins. The effects of phenylalanine ammonia-lyase on leukemic lymphocytes *in vitro*. *Cancer Res.* **32**: 285–290, 1972.
2. Abell, C. W., D. S. Hodgins and W. J. Stith. An *in vivo* evaluation of the chemotherapeutic potency of phenylalanine ammonia-lyase. *Cancer Res.* **33**: 2529–2532, 1973.
3. Adams, P. M. and E. S. Barratt. Nocturnal sleep in squirrel monkeys. *Electroenceph. clin. Neurophysiol.* **36**: 201–204, 1974.
4. Anton, A. H. and D. F. Sayre. The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. *J. Pharmac. exp. Ther.* **145**: 326–336, 1964.
5. Armstrong, M. and U. Stave. A study of plasma free amino acid levels. I. A study of factors affecting validity of amino acid analyses. *Metabolism* **22**: 549–560, 1973.
6. Birkmayer, W., W. Danielczk, E. Neumayer and P. Riedrer. The balance of biogenic amines as condition for normal behavior. *J. Neurol. Trans.* **33**: 163–178, 1972.
7. Blasberg, R. and A. Lajtha. Heterogeneity of the mediated transport systems of amino acid uptake in brain. *Brain Res.* **1**: 86–104, 1966.
8. Camm, E. and G. H. N. Towers. Phenylalanine ammonia-lyase. *Phytochemistry* **12**: 961–973, 1973.
9. Curzon, G. and A. R. Green. Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindole acetic acid in small regions of rat brain. *Br. J. Pharmac.* **39**: 653–655, 1970.
10. Fernstrom, J. D. and R. J. Wurtman. Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science* **178**: 414–416, 1972.
11. Fernstrom, J. D. and R. J. Wurtman. Control of brain serotonin levels by the diet. *Adv. Biochem. Psychopharmac.* **11**: 133–142, 1974.
12. Ferster, C. B. and B. F. Skinner. *Schedules of Reinforcement.* New York: Appleton-Century Crofts, 1957.
13. Fritz, R. R., D. S. Hodgins and C. W. Abell. Phenylalanine ammonia-lyase: induction and purification from yeast and clearance in mammals. *J. Biol. Chem.*, in press.
14. Frohman, C., K. Warren, C. Barry and R. Arthur. Neuroticism, physiological state and mood: an explanatory study of Friday/Monday changes. *Biol. Psychiat.* **1**: 201–211, 1969.
15. Gergen, J. A. and P. A. McLean. *A Stereotoxic Atlas of the Squirrel Monkey's Brain.* U.S. Public Health Service, Bethesda, Maryland, 1962.
16. Hodgins, D. S. Yeast phenylalanine ammonia-lyase. Purification, properties and the identification of catalytically essential dehydroalanine. *J. Biol. Chem.* **246**: 2977–2985, 1971.
17. Huault, C. Phytochrome regulation of phenylalanine ammonia-lyase activity in radish catyledons: a threshold mechanism. *Plant Sci. Letters* **3**: 149–155, 1974.
18. Kety, S. S. The Precursor-load Strategy in Psychochemical Research. In: *Psychochemical Research in Man,* edited by A. J. Mandell and M. P. Mandell. New York: Academic Press, 1969.
19. Kruskal, W. H. and W. A. Wallis. Use of ranks in one-criterion variance analysis. *J. Am. statist. Ass.* **47**: 583–621, 1952.
20. Laverty, R. and K. M. Taylor. The fluorometric assays of catecholamines and related compounds: improvements and extensions to the hydroxyindole technique. *Anal. Biochem.* **22**: 269–279, 1968.
21. Lovenberg, W., E. Jequier and A. Sjoerdsma. Tryptophan-hydroxylation in mammalian systems. *Adv. Pharmac.* **6A**: 21–36, 1968.
22. McDiarmid, C. G. and E. S. Barratt. Techniques for psychophysiological research with squirrel monkeys. *Proc. 2nd Int. Congr. Primat.* **1**: 246–253, 1969.
23. Ogata, K., K. Ucheyama, H. Yamada and T. Tochikura. Metabolism of aromatic amino acids in microorganisms. Part II. Properties of phenylalanine ammonia-lyase of *Rhodotorula. Agr. Biol. Chem.* **31**: 600–606, 1967.
24. Oldendorf, W. H. Brain uptake of radiolabelled amino acids, amines and hexoses after arterial injection. *Am. J. Physiol.* **221**: 1629–1639, 1971.
25. Parkhurst, J. R. and D. S. Hodgins. Phenylalanine and tyrosine ammonia-lyase activity in *Sporobolomyces pararoseus. Phytochemistry* **10**: 2997–3000, 1971.
26. Parkhurst, J. R. and D. S. Hodgins. Yeast phenylalanine ammonia-lyase properties of the enzyme from *Sporobolomyces pararoseus* and its catalytic site. *Arch. Biochem. Biophys.* **152**: 597–605, 1972.
27. Shellenberger, M. K. and J. H. Gordon. A rapid, simplified procedure for simultaneous assay of norepinephrine, dopamine, and 5-hydroxytryptamine from discrete brain areas. *Anal. Biochem.* **39**: 356–372, 1971.
28. Stith, W. J., D. S. Hodgins and C. W. Abell. Effects of phenylalanine ammonia-lyase and phenylalanine deprivation on murine leukemic lymphoblasts *in vitro*. *Cancer Res.* **33**: 966–971, 1973.
29. Weitzman, E. D., P. McGregor, C. Moore and J. Jacoby. The effect of alpha-methyl-para-tyrosine on sleep patterns of the monkey. *Life Sci.* **8**: 751–757, 1960.
30. Williams, H. L., B. K. Lester and J. D. Coulter. Monoamines and the EEG stages of sleep. *Activitas nervosa sup.* **11**: 188–192, 1969.
31. Wong, P. P., M. Zucker and L. L. Creasy. Induction of phenylalanine ammonia-lyase in strawberry leaf disks. *Plant Physiol.* **54**: 659–665, 1974.