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Long-Lasting Changes in Regional Brain Amino Acids and Monoamines in Recovered Pyrithiamine Treated Rats

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Rats were subjected to a severe bout of thiamine deficiency induced by daily pyrithiamine + a thiamine deficient diet, reversed by thiamine administration and allowed to recover. Pyrithiamine treated animals demonstrated impaired retention of a 24 h recall of passive avoidance. Regional brain concentration of norepinephrine, dopamine, serotonin, 3,4-dihydroxyphenylacetic acid, 5-hydroxyindoleacetic acid, GABA, glutamate, aspartate, glutamine, and glycine were determined after 2 and 9 weeks of nutritional recovery. A significant increase in NE content of cerebellum from the pyrithiamine treated animals was observed at both 2 and 9 week recovery periods. The concentrations of serotonin and its metabolite were significantly elevated in midbrain-thalamus and striatum. Significant reductions of GABA and glutamate were also observed in midbrain-thalamus. Amino acid levels in all other brain areas were unchanged from pair-fed controls. These results suggest regionally specific, chronic alterations in GABA, glutamate, serotonin, and norepinephrine activity following recovery from an acute bout of pyrithiamine-induced thiamine deficiency. The absence of a permanent reduction of cortical norepinephrine similar to that observed in an earlier study is discussed.

KEY WORDS: Amino acids; monoamines; thiamine deficiency; recovery; midbrain; thalamus; striatum.

INTRODUCTION

Wernicke-Korsakoff's disease is characterized by neurologic symptoms, cognitive-memory deficits and neuropathologic lesions of select structures within the diencephalon and brainstem. Although this disorder occurs most frequently among chronic alcoholics, thiamine deficiency is generally recognized as the primary etiologic factor (1, 2). In rats, an acute bout of thiamine deficiency induced by administration of pyrithiamine, a selective inhibitor of brain thiamine pyrophosphokinase (3) (EC 2.7.6.2) in combination with a thiamine deficient diet (PTD) produces acute neurologic disturbances, pathologic lesions (4) and chronic, long-lasting learning and memory deficits (5, 6) similar to those observed in human Wernicke-Korsakoff's disease.

The neurobiological disturbances underlying the permanent behavioral abnormalities are unknown but disruptions of monoamine and amino acid neurotransmitter activity have been proposed (7 for a review). In our reports of recovered PTD rats, norepinephrine (NE) concentration in cortex-hippocampus region was significantly reduced (by 35%) in the first study (5) but was only slightly re-

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duced in a subsequent study (8). Differences in the length of recovery and severity of treatment employed in these two studies may have contributed to these discrepancies in cortical NE change. The importance of establishing the long term effects of PTD treatment on cortical NE stems from reports that behavioral deficits similar to those observed in PTD rats can be produced by depletion of catecholamines in cortex (9–11) and reversed by treatment with catecholamine agonists (11, 12).

An additional neurotransmitter disturbance underlying PTD induced behavioral deficits has been suggested by the recent study of Thompson and McGeer (13). In this study of eight brain regions, glutamate decarboxylase (GAD) activity was significantly reduced in thalamus but not other brain areas of PTD treated rats after two weeks of thiamine therapy and nutritional restitution (13). This latter finding suggested that synthesis of GABA, an important inhibitory amino acid transmitter, may be permanently reduced within thalamus. This brain region is consistently damaged by acute thiamine deficiency and is considered a major neuroanatomic substrate for the learning and memory deficits characteristic of Korsakoff's disease.

The current study examined the hypothesis that a severe bout of PTD treatment produces a large depletion of cortical NE which undergoes a gradual but incomplete recovery following restitution of a normal diet. Rats were subjected to a severe bout of PTD treatment similar to that used in our original study (5). This particular PTD treatment produces consistent pathologic lesions within thalamus (4, 6, 14) and permanent learning and memory deficits (5, 6). Regional brain concentrations of norepinephrine (NE), dopamine (DA), serotonin (5-HT), and the metabolites 5-hydroxyindoleacetic acid (5-HIAA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were measured following 2 and 9 weeks of nutritional recovery. In order to provide a behavioral comparison of the current to previous groups of PTD rats, control and PTD animals in the 9 week recovery groups were tested on Passive Avoidance. Significant retention deficits of passive avoidance learning were observed in the original group of severely treated animals (5) but not in the second study involving more mildly treated animals (6). The concentrations of GABA, glutamate, aspartate, glycine, and glutamine were also measured in the same regions to explore the recent suggestion of a long-term loss of neurotransmitter amino acids, particularly GABA, in recovered PTD rats (13).

This material has been published in abstract form (15).

EXPERIMENTAL PROCEDURE

Animals. Male Long-Evans rats, 50 days old (200–250 g) were treated with either daily pyrithiamine (0.5 mg/kg, i.p.) and a thiamine deficient chow (Teklad Mills) (PTD group, $n = 17$) or matched amounts of the test diet plus daily thiamine injection (0.4 mg/kg, i.p.) (Control group, $n = 17$) as previously described (5, 15). Within one to two hours following the onset of the acute neurologic stage of opisthotonos, thiamine deficient animals were given a large dose of thiamine (100 mg/kg, i.p.) and received repeated thiamine injections every 6 h thereafter until the acute neurologic signs disappeared. The PTD and control animals were then placed on the same nutritionally balanced diet (Purina Lab Blox) and sacrificed after 2 ($n = 10$, each group) or 9 ($n = 7$, each group) weeks of recovery. Since many of the experimental animals were anorectic for varying lengths of time following reversal, control intake was restricted during the recovery period to maintain body weights equivalent to the paired PTD animals.

Behavioral Measurement. One week prior to sacrifice, all seven control and seven PTD animals in the 9 week recovery groups were tested on a 1 trial acquisition and 24 h retention of a passive avoidance task. Passive avoidance was tested in the same apparatus and with procedures identical to those described previously (5). Briefly, animals were placed in a brightly lit startbox, facing the startbox gate, and the time was recorded by photocells for them to move their heads (T1) and bodies (T2) into a darkened alleyway. After the startbox gate was closed, a brief scrambled shock (1.2 mA, 2 s in duration) was delivered to the floor of the alleyway, and the animal was removed and placed back in its homecage. Twenty-four h later, each animal was placed back in the brightly lit startbox and the latencies to T1 and T2 were again measured. Retention trial was terminated after 10 min if an animal had not yet broken both photocells.

Neurochemical Measurements. Following decapitation, the areas of cerebellum, pons-medulla, midbrain-thalamus, hypothalamus, striatum, cortex-hippocampus and olfactory bulb were removed and frozen at −70C until extracted in 0.1 M perchloric acid (PCA) as previously described (5). Aliquots of the 0.1 M PCA tissue extracts were analyzed for monoamine and metabolite concentrations using a single high-performance liquid chromatographic (HPLC), four cell coulometric electrochemical detector (EC) assay (8). Brain tissue amino acids were measured by precolumn treatment of the 0.1 M PCA extract with orthopthaldialdehyde (OPA), separation of amino acid-OPA derivatives using isocratic, reversed phase HPLC, and quantitation by electrochemical detection. An aliquot of PCA tisue extract (20–80 μl) in a final volume of 570 μl methanol/water (1:1) was reacted at room temperature with 130 μl of OPA-mercaptoethanol derivitizing agent (16). Amino acid derivatives were separated by injection of 20–50 μl volumes of the reaction mixture onto a uBondapak C18 reverse phase column (30 cm × 4.6 mm) protected by a guard column packed with C18 corasil (all from Waters Corp.). The mobile phase consisted of 0.1 M sodium phosphate buffer, 34% methanol, 1.5% acetonitrile, pH 5.40 delivered at a 1.5 ml/min and passed through a silica saturation precolumn (Whatman Corp.). Detection was achieved with a coulometric electrochemical detector apparatus (Coulchem Model

5100A, ESA Inc.) equipped with a conditioning cell and a dual electrode analytical cell (Model 5010). The column eluate was conditioned at +40 mV and the separated amino acid derivatives detected at the series electrodes (T1 & T2) operated at +350 mV and +800 mV, respectively. The identification and purity of the separated chromatographic peaks was determined by comparison of their retention time and T1/T2 signal ratio to similar measures obtained from the analysis of authentic standards. Quantitation of regional brain amino acid content was performed by comparison of tissue extract peak heights to those of standard peak heights obtained at T1. Concentration of amino acids, monoamines and metabolites are based on tissue protein (17).

Biochemical data were examined by analysis of variance using a two way (Treatment vs Recovery Period) classification. When justified, subsequent comparisons between means were made using Newman-Keuls tests. Passive avoidance data were analyzed with Mann-Whitney U-test (one-tailed).

RESULTS

Behavioral Observations-Body Weights and Feeding Habits. Following reversal of treatment by administration of thiamine, a large number of the PTD animals remained anorectic and required forced feeding for various lengths of time during the recovery period. Four of the ten PTD rats examined at two weeks and one of the seven PTD rats studied at 9 weeks of recovery required hand feeding of wet mash up to the time of sacrifice. The remaining 2 week PTD animals were anorectic for periods of 1–2 days (n = 3) and 7–8 days (n = 3). The remaining 9 week PTD animals were anorectic for periods of 2–8 days (n = 3) and 15–28 days (n = 3) following thiamine reversal before returning to self-feeding. At the time of sacrifice, no significant differences (Student's t tests, one-tailed) were observed between body weights of the control and PTD groups examined at the 2 week (Control = 203.4 ± 14.5 g vs PTD = 213.6 ± 38.4 g) or the 9 week (Control = 380.6 ± 23.6 g vs PTD = 353.6 ± 66.3 g) recovery periods.

Passive Avoidance. One week prior to sacrifice, all 9 week control and PTD animals were studied on passive avoidance. On the training day, control and PTD animals exhibited comparable latencies to move their heads and bodies into the darkened alleyway. On the 24 hr recall, however, the PTD animals were significantly (Mann-Whitney U-test) faster for moving their heads (T1; $Z = -2.24$, $p = .024$) but not their bodies (T2; $Z = -0.89$, p = n.s.) into the alleyway. Every control animal displayed a maximum 600 s latency to move both their heads and bodies into the darkened alleyway on the 24 h recall. Within the PTD group, however, only two animals reached the maximum 600 s duration for T1 latency. Four of the remaining five PTD had T1 latencies less than 200 s. Failure to detect significant differences between controls and PTD animals for T2 latencies may have been due to a ceiling effect since trials were terminated after 600 s if an animal had not broken both photocells. All seven control but only five PTD animals attained maximum T2 latency.

Other animals treated along with the present PTD groups were used in electrophysiological and histopathological studies. Consistent lesions within the intralaminar and mediodorsal nuclei of the thalamus but not the mammillary bodies, brainstem or locus coeruleus regions were observed along with alterations in cortical discharge patterns (Armstrong-James et al., unpublished observations).

Monoamines and Metabolites. Regional brain concentrations of NE, DA, 5-HT, DOPAC, and 5-HIAA are shown in Table I. Analyses of variance revealed a significant overall treatment effect on the concentration of NE in cerebellum; DA in striatum and cortex; 5-HT and 5-HIAA in midbrain-thalamus and cortex; and 5-HIAA in midbrain-thalamus, striatum and hypothalamus. Post hoc analyses demonstrated a significant (Newman-Keuls test) increase of NE in cerebellum of the PTD animals at both the 2 and 9 week recovery period. The concentration of 5-HT in the PTD animals was significantly elevated in midbrain-thalamus and cortex at 9 weeks and in the striatum at both recovery periods. The level of 5-HIAA in the PTD group was also significantly increased compared to controls in midbrain-thalamus and striatum at 9 weeks and in the hypothalamus at 2 weeks following recovery. Dopamine levels of the PTD groups were significantly elevated in the striatum at 2 weeks. There were no significant differences in DOPAC concentration between the PTD and control groups for any brain area. Length of nutritional recovery (2 weeks vs 9 weeks) had a significant (ANOVA) effect on monoamine and metabolite levels in certain brain regions of both control and PTD animals. A significant increase from the second to ninth week of recovery was observed in cortical NE of the PTD but not control group. The level of NE, 5-HT and 5-HIAA declined within cerebellum and increased within striatum and olfactory bulb of both controls and PTD animals from the 2 week to 9 week period. A significant elevation of cortical DOPAC (by 64%) was observed in the control but not PTD treated animals.

Table I. Regional Brain Monoamine and Metabolite Concentrations of Control and Recovered Pyrithiamine Treated Rats

ANALYTE	Treatment group	Pons-medulla	Midbrain-thalamus	Cerebellum	Striatum	Cortex	Olfactory bulb	Hypothalamus
NE	CT-2 wks	4.87(.20)	4.87(.22)	4.28(.18)	1.76(.31)	4.99(.28)	5.62(.30)	28.23(1.53)
	PTD-2 wks	5.06(.21)	5.21(.25)	5.29(.33)[b]	1.78(.20)	3.85(.20)	4.89(.28)	28.21(1.23)
	CT-9 wks	4.31(.24)	5.12(.30)	3.32(.26)	2.31(.27)	5.24(.31)	6.22(.34)	29.32(2.54)
	PTD-9 wks	4.66(.11)	5.80(.29)	4.03(.17)[a]	3.54(.48)	6.03(.36)	6.10(.29)	30.84(3.41)
DA	CT-2 wks	0.18(.01)	1.22(.18)	0.05(.00)	69.40(3.62)	3.40(.59)	0.73(.08)	4.66(.36)
	PTD-2 wks	0.18(.01)	1.69(.32)	0.05(.01)	88.65(2.15)[a]	4.04(.47)	0.67(.05)	4.52(.16)
	CT-9 wks	0.23(.05)	1.71(.15)	0.04(.00)	84.51(6.91)	3.30(.36)	0.52(.05)	3.50(.36)
	PTD-9 wks	0.19(.01)	1.87(.29)	0.04(.00)	89.06(4.94)	4.77(.51)	0.65(.05)	4.74(.31)
DOPAC	CT-2 wks	0.10(.01)	0.86(.12)	0.05(.00)	21.42(2.45)	2.84(.30)	0.88(.12)	2.11(.14)
	PTD-2 wks	0.10(.01)	1.06(.18)	0.06(.00)	19.43(1.05)	3.37(.44)	0.68(.06)	3.12(.59)
	CT-9 wks	0.14(.03)	1.29(.16)	0.05(.01)	22.67(2.15)	4.67(.60)	0.52(.07)	1.75(.27)
	PTD-9 wks	0.16(.02)	0.96(.11)	0.09(.04)	25.27(4.17)	3.91(.34)	0.88(.27)	2.13(.24)
5-HT	CT-2 wks	1.74(.09)	3.15(.32)	0.49(.03)	3.49(.27)	2.45(.22)	2.00(.14)	5.79(.44)
	PTD-2 wks	1.89(.13)	3.39(.17)	0.50(.04)	4.70(.28)[a]	2.60(.24)	2.12(.12)	5.91(.39)
	CT-9 wks	1.82(.12)	3.96(.35)	0.35(.05)	5.13(.32)	2.35(.15)	2.61(.18)	5.21(.18)
	PTD-9 wks	1.96(.08)	5.34(.49)[b]	0.42(.08)	6.99(.66)[b]	3.22(.21)[b]	3.00(.15)	6.46(.58)
5-HIAA	CT-2 wks	2.15(.09)	4.73(.38)	0.74(.04)	4.07(.37)	3.67(.21)	2.72(.27)	7.98(.41)
	PTD-2 wks	2.42(.17)	5.83(.40)	0.74(.05)	4.67(.29)	3.77(.21)	2.73(.20)	10.48(.86)[b]
	CT-9 wks	2.50(.19)	4.61(.37)	0.62(.04)	6.18(.40)	4.60(.45)	3.56(.20)	6.87(.70)
	PTD-9 wks	2.75(.12)	5.72(.36)[a]	0.69(.02)	8.19(.62)[b]	4.32(.51)	4.00(.42)	8.08(.50)

[a] $P < 0.05$; [b] $P < 0.01$; when compared with control group, Newman-Keuls test. Values are expressed as Mean (SEM) in ng/mg protein. CT: Pair-fed controls given thiamine deficient diet + thiamine (0.4 mg/kg/day, i.p.) and sacrificed after 2 or 9 weeks of nutritional recovery. PTD: Experimental animals given pyrithiamine (0.5 mg/kg/day, i.p.) + thiamine deficient diet for 14–17 days and sacrificed after 2 or 9 weeks of nutritional recovery. There were ten animals in each group sacrificed at 2 weeks and seven animals in each group sacrificed at 9 weeks.

Table II. Regional Brain Amino Acid Concentrations of Control and Recovered Pyrithiamine Treated Rats

ANALYTE	Treatment group	Pons-medulla	Midbrain-thalamus	Cerebellum	Striatum	Cortex	Olfactory bulb	Hypothalamus
GABA	CT-2 wks	14.2(0.5)	27.7(1.3)	19.6(1.9)	25.1(1.2)	21.1(1.1)	51.1(2.1)	54.2(1.5)
	PTD-2 wks	14.1(0.6)	25.9(2.0)	19.2(1.5)	25.0(1.1)	19.9(0.6)	50.0(2.4)	56.5(2.2)
	CT-9 wks	11.3(0.5)	28.2(1.3)	30.5(1.3)	27.9(1.1)	24.4(1.9)	42.9(2.0)	53.4(2.7)
	PTD-9 wks	11.3(0.2)	22.6(1.7)[a]	28.9(1.3)	31.8(0.9)	23.5(2.4)	41.9(1.3)	57.6(3.6)
GLUTAMATE	CT-2 wks	54.3(1.6)	66.6(2.2)	125.1(12.9)	97.6(2.8)	121.8(5.7)	118.1(2.4)	48.3(1.5)
	PTD-2 wks	56.7(1.6)	57.6(2.5)[a]	125.2(10.4)	93.2(5.3)	120.0(4.5)	112.2(2.9)	50.6(1.8)
	CT-9 wks	46.7(2.9)	64.3(3.1)	184.9(5.8)	106.6(5.5)	127.5(5.1)	104.2(4.6)	53.7(1.3)
	PTD-9 wks	47.8(2.2)	50.6(2.9)[b]	183.1(6.7)	101.4(3.0)	131.9(9.5)	100.4(4.2)	59.0(2.5)
GLUTAMINE	CT-2 wks	25.2(0.7)	31.3(1.5)	55.4(6.1)	42.6(2.0)	41.7(2.3)	66.1(2.0)	88.2(1.6)
	PTD-2 wks	27.8(1.0)[a]	33.7(1.7)	59.3(5.3)	47.0(2.4)	44.3(1.6)	63.5(2.2)	87.6(2.8)
	CT-9 wks	24.2(1.3)	35.0(1.9)	89.0(3.2)	52.5(3.0)	52.4(1.8)	58.3(1.3)	89.3(2.3)
	PTD-9 wks	27.2(1.1)	32.6(1.9)	90.8(3.8)	56.7(2.0)	55.7(4.1)	58.8(1.2)	89.3(3.9)
ASPARTATE	CT-2 wks	21.4(0.8)	17.7(1.1)	23.1(2.1)	17.7(0.8)	27.4(1.9)	31.3(1.2)	27.4(0.5)
	PTD-2 wks	21.1(0.9)	14.5(1.1)[a]	21.6(1.9)	15.3(1.0)	25.8(2.1)	29.3(1.2)	26.9(0.7)
	CT-9 wks	18.2(1.2)	18.5(1.0)	33.7(2.0)	19.4(1.4)	28.1(1.5)	27.6(0.6)	27.2(1.0)
	PTD-9 wks	19.9(1.1)	16.0(1.1)	33.5(1.7)	18.7(1.0)	28.0(2.2)	27.4(0.6)	29.9(1.3)
GLYCINE	CT-2 wks	37.5(1.2)	14.7(0.8)	13.7(1.4)	9.9(0.4)	10.6(0.4)	17.7(0.7)	17.1(0.5)
	PTD-2 wks	38.1(1.4)	14.7(0.7)	15.5(1.3)	9.3(0.3)	10.4(0.4)	16.8(0.6)	18.9(0.7)
	CT-9 wks	32.1(1.2)	15.0(0.9)	20.6(0.9)	10.9(0.8)	13.6(0.8)	14.0(0.4)	16.8(0.6)
	PTD-9 wks	34.6(0.9)	13.4(1.1)	20.4(0.9)	10.9(0.4)	12.7(0.9)	13.6(0.5)	17.9(1.1)

[a] $P < 0.05$; [b] $P < 0.01$, when compared with control group, Newman-Keuls test. Values are expressed as Mean (SEM) in μmol/g protein. CT: Pair-fed controls given thiamine deficient diet + thiamine (0.4 mg/kg/day, i.p.) and sacrificed after 2 or 9 weeks of nutritional recovery. PTD: Experimental animals given pyrithiamine (0.5 mg/kg/day, i.p.) + thiamine deficient diet for 14–17 days and sacrificed after 2 or 9 weeks of nutritional recovery. There were ten animals in each group sacrificed at 2 weeks and seven animals in each group sacrificed at 9 weeks.

Amino Acids. Regional brain concentration of the amino acids at both recovery periods are shown in Table II. Analyses of variance revealed no significant treatment effect on any of the amino acids measured in the cerebellum, striatum, cortex, and olfactory bulb. In the midbrain-thalamus region, however, significant changes due to treatment were detected for the concentration of GABA, glutamate, and aspartate. When compared to control group data, GABA level of midbrain-thalamus was significantly reduced at 9 weeks. Glutamate was significantly reduced at both the 2 and 9 week recovery periods. Aspartate was significantly reduced at 2 but not 9 weeks. Within the hypothalamus region, a significant treatment effect was detected for glutamine but post hoc tests failed to demonstrate any significant control vs PTD differences at either the 2 or 9 week periods. Glutamine content within pons-medulla region of the PTD group was significantly elevated at the 2 but not at the 9 week period.

Length of recovery had significant (ANOVA) effects on amino acid content of several brain regions of both control and PTD treated animals. The most striking effects were observed in the cerebellum, in which all amino acids were significantly higher after 9 weeks compared to 2 weeks of nutritional therapy. The levels of glutamine in cortex and striatum and glycine in cortex were also significantly higher at 9 weeks in both treatments. By contrast, glutamate, GABA, and glycine levels were significantly lower at 9 weeks in olfactory bulb and pons-medulla of the control group. In addition, glutamine and aspartate levels in olfactory bulb declined significantly from the 2nd to 9th week of recovery.

DISCUSSION

Behavioral Measures. Like the animals treated with a similar bout of PTD (5), the current group of PTD rats was significantly impaired on the 24 h retention of a passive avoidance task. A significant difference between control and PTD animals on the T1 but not the T2 latency may have been due to a ceiling effect since retention trials were terminated after 600 s if an animal had not broken both photocells.

Monoamines and Metabolites. The present results confirm observations from our two previous studies of increased NE content of cerebellum following recovery from an acute bout of PTD induced thiamine deficiency in the rat (5, 8). An effect of PTD treatment on cortical NE is demonstrated by its significant increase from the 2 to 9 week period of PTD but not control animals. This observation supports our hypothesis that a severe bout of PTD induced thiamine deficiency is followed by recovery of cortical NE levels. On the other hand, the large depletion of cortical NE expected in the early stage of recovery was not observed. The NE reduction (23%) observed after 2 weeks of recovery is considerably less than the 35% reduction observed following five months of recovery in our original study (5). Furthermore, no significant reduction was observed in cortical NE concentration at the 9 week recovery period. The current data, therefore, have failed to demonstrate a significant reduction of cortical NE similar to that observed in the original study (5). These discrepancies cannot be attributed to a difference in severity of treatment. Length of time in opisthotonos, the acute stage of this treatment, was similar to the original study. Both the previous (5) and current groups of PTD rats were impaired on retention of passive avoidance. Furthermore, 40% of the 2 week PTD animals were anorectic at the time of sacrifice. Examination of cortical NE levels in these 2 week anorectic animals revealed no differences from animals that quickly regained self feeding. The present finding of normal cortical NE concentration is consistent with the ob-

Table III. Summary of Present and Previous Changes in Regional Brain Serotonin Measures in Recovered PTD Treated Rats

Source	Length of recovery	Percent change from control group			
		5-HT		5-HIAA	
		Midbrain-thalamus	Striatum	Midbrain-thalamus	Striatum
Brain Res. 360: 273–284, 1985	7 months	+55%	+34%	+24%	+21%
Brain Res. 421: 140–149, 1987	5 months	+19%	+13%	+11%	+13%
Present study	9 weeks	+35%	+37%	+24%	+33%

servations of unchanged immunohistochemical staining of cortical tyrosine hydroxylase and the absence of lesions in regions of noradrenergic cell bodies (locus coeruleus) or ascending fibers (periventricular and periacqueductal grey) in a third group of PTD animals treated in this study (Armstrong-James et al., unpublished observations).

An alternative explanation for these discrepancies is suggested by the fact that in the original study demonstrating a large cortical NE loss (5), animals were 7 months old when subjected to PTD treatment. In both the current and most recently published (8) studies demonstrating no cortical NE change, rats were 7 weeks old when treatment was administered. Age at time of treatment may be important since recent studies have shown that the brain catecholamine systems of older animals are more susceptible to the effects of metabolic and neurotoxic insult (18, 19).

Increased levels of both 5-HT and 5-HIAA were detected in midbrain-thalamus, striatum, and to a lesser extent within hypothalamus after 9 weeks of thiamine replenishment. These observations are in close agreement with our previous studies of recovered PTD rats receiving behavioral testing prior to sacrifice (5, 8). These data are summarized in Table III and provide strong evidence that PTD treatment produces significant increases of serotonin synthesis and metabolism within midbrain-thalamus and striatum. These serotonin changes are independent of the post-recovery training history and may be involved in the behavioral deficits observed in this model of Korsakoff's disease. The normal levels of 5-HT and 5-HIAA observed in all other brain areas agree with earlier studies in which thiamine administration reversed PTD induced serotonin alterations in these same brain areas (20, 21).

The factor(s) responsible for these serotonin changes cannot be determined from the present study. One possible mechanism is suggested by the simultaneous observations of decreased GABA in midbrain-thalamus and increased serotonin and 5-HIAA concentrations within midbrain-thalamus and striatum. Immunocytochemical (22), electrophysiological (23), and biochemical (24) observations have demonstrated a significant GABA-mediated tonic inhibition of serotonergic midbrain raphe nuclei. A major pathway involved in this GABA inhibition originates in the lateral habenula (25) and courses through those areas of thalamus consistently damaged by PTD treatment in the rat (5, 6, 14).

Amino Acid Changes. In the present study, significant decreases in GABA and glutamate content of midbrain-thalamus were observed in recovered PTD treated rats. These findings are consistent with the report of an irreversible decrease in the activity of glutamate decarboxylase (GAD) (13) within thalamus of recovered PTD rats. These observations suggest permanent alterations in the synthesis and/or utilization of GABA and glutamate within this brain area. The present finding of unchanged amino acid concentrations in all other brain regions agree with an earlier study of recovered PTD animals in which midbrain-thalamus was not examined (26) and underscore the regional specificity of PTD induced amino acid alterations. Furthermore, these observations provide strong evidence that the GABA and glutamate changes are the direct result of PTD treatment and not due to altered dietary intake. This conclusion is further supported by the similarities in body weights of the PTD and control groups at the time of sacrifice and the absence of marked differences in amino acid levels between self-feeding and anorectic animals. The significant changes in regional brain amino acids observed in the control group from the 2 to 9 week recovery period emphasize the necessity of using pair-fed animals in studies of the long-term biochemical consequences of thiamine deficiency.

The reductions in midbrain-thalamus levels of glutamate and GABA of the PTD groups were greater at the 9 week compared to 2 week recovery period. In addition, a significant decrease in aspartate was observed at the 2 week but not 9 week recovery period. These observations suggest a complex series of biochemical and physiological events within the midbrain-thalamus during the weeks and months following recovery from an acute bout of PTD induced thiamine deficiency. The mechanisms responsible for the reduced GABA and glutamate concentrations within midbrain-thalamus are unknown. The study of Thompson & McGeer (13) suggested that PTD treatment produces a structural loss of GABAergic systems within the thalamus, a brain region frequently damaged by this treatment in the rat (4). This possibility is further supported by our observations that PTD treatment produces highly reproducible lesions within the intralaminar nuclei of the thalamus (6, 14) a region densely innervated by GABAergic fibers originating from the n. reticularis thalami (27, 28). Pathological destruction of these areas were observed in another group of animals treated along with those used in the cur-

rent study and allowed to recover for 6–8 weeks (Armstrong-James et al., unpublished observations).

An alternative explanation for the selective reductions of GABA and glutamate is suggested by reports that the thiamine pyrophosphate dependent enzyme transketolase (29) but not the pyruvate dehydrogenase or 2-oxoglutarate complexes (29–33) is irreversibly lowered in recovered PTD animals. Transketolase is an important regulatory enzyme for the activity of the hexose monophosphate shunt (HMP), a metabolic pathway purported to play an important role in the synthesis of GABA and glutamate neurotransmitters within the brain (34). Treatment of rats with 6-aminonicotinamide, a selective inhibitor of the HMP shunt, results in a selective decrease of brain glutamate and GABA (35, 36). In addition, 6-aminonicotinamide produces neuropathological changes (37) and behavioral symptoms (34) resembling those observed in PTD treated animals. Although these observations suggest that reduced HMP shunt activity may be involved in PTD induced long-lasting reductions of GABA and glutamate, the reason for their selective reduction in midbrain-thalamus remains unclear.

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