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CHANGES OF TAURINE CONTENT IN THE BRAIN TISSUE OF BARBITURATE-DEPENDENT RATS

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Abstract—The concentrations of taurine, γ-aminobutyric acid (GABA), glutamic acid and aspartic acid in the brain of barbiturate-dependent rats were determined. Barbiturate-dependent rats, engendered by long-term administration of feed containing barbital sodium, showed signs of withdrawal, such as spontaneous convulsions and marked loss of body weight. Prolonged administration of barbital sodium caused a significant increase in cerebellar taurine content; soon after withdrawal of the drug the content returned to normal. The taurine content in the cerebral cortex increased 48 hr after the withdrawal. These changes of taurine content occurred in the brain but not in other organs. The GABA content of the cerebral cortex and brain stem decreased significantly during barbiturate administration and returned to normal within 48 hr after withdrawal of barbiturate. A significant decrease of aspartate content in the cerebral cortex was also observed at 48 hr after barbiturate withdrawal, but the glutamate content did not change during or after drug administration.

Convulsions are generally recognized as a sign of the condition which follows withdrawal of barbiturate from human addicts and also from chronically intoxicated experimental animals. Essig [1] showed that amino-oxiacetic acid prevented the convulsions that occurred after abrupt barbiturate withdrawal, and he proposed that the convulsions might be brought about by a deficiency of γ-aminobutyric acid (GABA) in the brain. Recently, Tzeng and Ho [2] showed that prolonged administration of pentobarbital resulted in a decrease of GABA content in mouse brain and they suggested that the GABA system in the central nervous system may be involved in the development of tolerance to barbiturate. In contrast, Crossland and Turnbull [3] and Sutton and Simmonds [4] reported no change of the GABA content in rat brain either during prolonged administration of barbiturate or after its abrupt withdrawal.

Taurine, which resembles GABA in structure, is thought to act as an inhibitory neurotransmitter or neuromodulator in the central nervous system [5–8]. Recent investigations have also shown that the taurine content is reduced at epileptogenic foci in human [9] and experimental animals [10–12], and that administration of taurine reduces epileptic activity [11, 13–17]. However, there is little information on taurine metabolism in the brain, and some workers [18, 19] have questioned the anti-epileptic action of taurine. In general, taurine content in the brain is hardly changed in various conditions [20].

In this study, we examined the effect of prolonged administration of barbital sodium and its abrupt withdrawal on brain taurine content in the rat. We also measured the concentrations of other putative neurotransmitter amino acids, such as GABA, glutamate and aspartate, in the brain of barbiturate-dependent rats.

MATERIALS AND METHODS

Male Sprague–Dawley rats, weighing 90–100 g, were made barbiturate-dependent by long-term administration of barbital sodium in their feed, following the method of Yanaura *et al.* [21]. Animals were housed individually in cages (40 cm × 18 cm × 19 cm) with two feed containers and were fed *ad lib.* on powdered feed containing barbital sodium at various concentrations. The concentrations of drug tested were: 0.5 mg barbital sodium/g of feed (CA-1 powder, CLEA JAPAN, Inc., Tokyo) vs 1 mg/g for 5 days, 1 mg/g vs 2 mg/g for 7 days, 2 mg/g vs 4 mg/g for 7 days, 4 mg/g vs 6 mg/g for 10 days and 6 mg/g vs 8 mg/g for 10 days

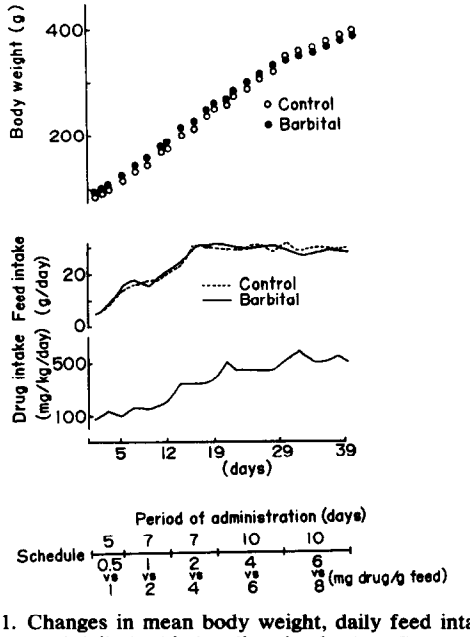

Fig. 1. Changes in mean body weight, daily feed intake and daily barbital sodium intake ($n = 5$).

(Fig. 1). This feeding schedule could produce the dependence without severe toxicity. The positions of the feed containers were changed every other day, and feed and water were provided *ad lib*. The animal room was maintained under alternating 12-hr periods of light (6:00 a.m. to 6:00 p.m.) and dark, at constant temperature (22 ± 1°). Rats were decapitated between 10:00 a.m. and 11:00 a.m., since it was found that the taurine content in the brain showed a circadian rhythm [22]. The brain was removed rapidly, put on an ice-cold glass plate, cut into three parts (cerebral cortex, brain stem and cerebellum) and frozen between aluminum plates at −80°. The whole procedure, from decapitation to freezing, required less than 2 min.

Taurine was extracted from tissues with 10% (w/v) cold trichloroacetic acid and purified as described previously [20]. The taurine content was assayed by a modification [23] of the fluorometric method of Udenfriend *et al.* [24]. The concentrations of GABA, glutamate and aspartate in the brain were determined as described by Graham and Aprison [25]. Barbital was determined by the method of Brodie *et al.* [26] using n-heptane for extraction.

The significance of differences was evaluated by Student's *t*-test.

Glutamate dehydrogenase, gutamate oxaloacetate transaminase and malate dehydrogenase were purchased from Boehringer Mannheim. *Pseudomonas fluorescens* (Type II), NAD, NADH and NADP were products of Sigma Chemical Co. Fluorescamine (Fluram) was obtained from Japan Roche Co.

RESULTS

Figure 1 shows changes in mean body weight, daily feed intake and daily barbital sodium intake in the barbiturate-treated and control rats. During administration of barbital sodium, no significant difference in growth or total daily feed intake was observed between the treated and control rats. The daily intake of barbital sodium was about 100 mg/kg/day during week 1 of the experiment, and increased progressively to about 500 mg/kg/day during weeks 4 and 5. The drug-dependent rats exhibited withdrawal signs, such as anorexia, hyperirritability, vocalization, weight loss, jumping and convulsions after drug withdrawal, but they did not die of the convulsions. The maximal decrease in body weight was observed about 48 hr after withdrawal, and then the body weight gradually increased (Fig. 2). Spontaneous convulsions were observed in all the animals at least once within the 48 hr after drug withdrawal, usually from 24 to 48 hr after withdrawal (Fig. 3). The barbital concentrations in the tissues and serum increased gradually on exposure to the drug, and rapidly disappeared after withdrawal (Table 1). The disappearance of the drug from the brain seems to be closely related to the appearance of withdrawal signs (Figs. 2 and 3).

Administration of taurine (2 g/kg/day, i.p., for 10 days before withdrawal of barbital sodium) did not affect the incidence of withdrawal convulsions of barbiturate-dependent rats ($n = 10$, data not shown).

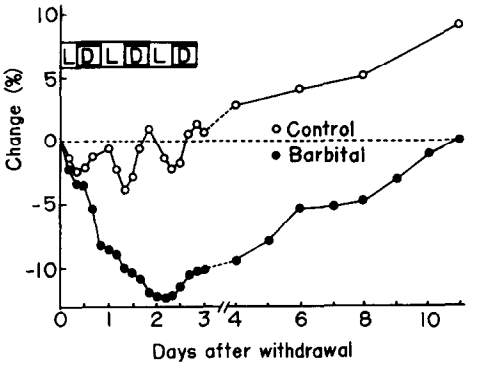

Fig. 2. Changes in mean body weight after withdrawal of barbital sodium ($n = 5$).

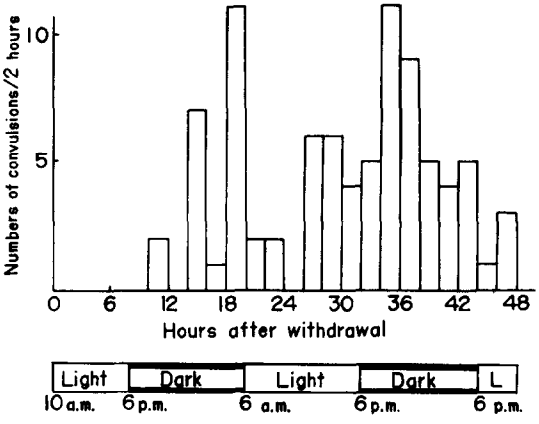

Fig. 3. Spontaneous convulsions of barbiturate-dependent rats after drug withdrawal ($n = 20$).

Table 2 shows the taurine content in various regions of the brain in barbiturate-dependent and control rats. The taurine content in the cerebellum increased significantly on prolonged administration of barbital sodium and rapidly returned to the normal value after drug withdrawal. In the cerebral cortex, the taurine content was significantly increased at 48 hr after withdrawal, although it did not change during administration of barbital sodium. On day 11 after withdrawal, withdrawal signs had completely disappeared and the brain taurine content of the dependent rats was the same as that of the control. In contrast, the taurine content in the brain stem did not change during or after administration of barbital sodium. Table 3 shows the taurine content in various tissues of barbiturate-dependent rats. In the liver and kidney, there was no significant change of taurine content during or after the administration of barbital sodium. Taurine content in the heart increased markedly at 48 hr, but not at 24 hr, after drug withdrawal. However, this increase in the heart is probably due to anorexia after the withdrawal, since a separate experiment showed that the taurine content in the heart, but not in the brain, was increased by fasting for 48 hr (unpublished observations).

The concentrations of various amino acids other than taurine in the brain of the barbiturate-dependent and control rats are shown in Table 4. The

Table 1. Barbital concentration in tissues (μg/g wet weight) and serum (μg/ml)*

	Period of administration (days)		Days after withdrawal		
	10	20	0	1	2
Serum	107 ± 8 (5)	243 ± 15 (5)	241 ± 9 (8)	7 ± 1 (5)	0 (5)
Brain			158 ± 19 (5)	19 ± 3 (5)	0 (5)
Heart			129 ± 10 (5)	30 ± 6 (4)	10 ± 4 (5)
Kidney			474 ± 19 (5)	274 ± 18 (5)	22 ± 3 (4)
Liver			189 ± 16 (5)	39 ± 7 (5)	8 ± 1 (4)

* Values are expressed as means ± S.E. of values for the number of animals shown in parentheses.

Table 2. Brain taurine content of barbiturate-dependent and control rats*

	No. of animals	Days after withdrawal	Taurine content (μmoles/g wet weight) Cerebral cortex	Brain stem	Cerebellum
Control	15		5.8 ± 0.2	4.3 ± 0.2	5.6 ± 0.3
Barbiturate-dependent	9	0	6.3 ± 0.2	4.9 ± 0.5	7.3 ± 0.5†
	5	1	6.4 ± 0.9	5.0 ± 1.0	5.3 ± 0.8‡
	10	2	7.6 ± 0.5†‡	4.8 ± 0.3	5.8 ± 0.5‡
	10	11	6.3 ± 0.5	4.4 ± 0.4	5.5 ± 0.4‡

* Values are expressed as means ± S.E.
† $P < 0.01$, compared with control value.
‡ $P < 0.05$, compared with value at 0 time after drug withdrawal.

Table 3. Taurine content of various tissues of barbiturate-dependent and control rats*

	Days after withdrawal	Taurine content (μmoles/g wet weight) Whole brain	Heart	Kidney	Liver
Control		4.9 ± 0.2 (5)	23.5 ± 0.7 (15)	6.7 ± 0.5 (5)	6.8 ± 0.3 (5)
Barbiturate-dependent	0	5.8 ± 0.3† (8)	24.5 ± 0.9 (9)	7.3 ± 0.4 (5)	5.6 ± 0.7 (5)
	1		24.1 ± 1.0 (5)		
	2	5.9 ± 0.4† (5)	27.9 ± 1.0‡§ (10)	6.9 ± 0.3 (10)	6.0 ± 0.4 (10)
	11		25.6 ± 1.1 (10)	6.6 ± 0.3 (5)	

* Values are expressed as means ± S.E. of values for the numbers of animals shown in parentheses.
† $P < 0.01$, compared with control value.
‡ $P < 0.05$, compared with control value.
§ $P < 0.05$, compared with value at 0 time after drug withdrawal.

GABA content in the cerebral cortex and brain stem, but not the cerebellum, decreased significantly during the administration of barbital sodium and returned to the normal value at 48 hr after withdrawal. The aspartate content in the cerebral cortex decreased after, but not during, administration of barbital sodium. The glutamate content was not affected by prolonged administration or withdrawal of the drug.

DISCUSSION

In this work we observed significant changes in the concentrations of taurine, GABA and aspartate, but not glutamate, in some regions of the brain during prolonged administration or withdrawal of barbital sodium. The barbiturate-dependent rats in this study were obtained by the procedure of Yanaura *et al.* [21]. As these workers pointed out, this method is better than previous ones for producing physical dependence on the drug, because it causes a higher degree of dependence and less toxicity.* The high degree of barbiturate dependence of the rats in this study was evidenced by the high incidence of spontaneous convulsions and marked weight loss of the animals.

Many studies show positive effects of taurine on epilepsy induced by cobalt [11], ouabain [12],

* Dr. S. Yanaura and Dr. E. Tagashira, personal communication.

Table 4. Concentration of GABA, glutamate and aspartate in the brain of barbiturate-dependent and control rats*

	Concentrations of amino acids (μmoles/g wet weight)		
	Cerebral cortex	Brain stem	Cerebellum
		GABA	
Control (13)	1.7 ± 0.2	1.8 ± 0.1	4.9 ± 0.4
Barbiturate-dependent (13)	1.1 ± 0.2†	1.4 ± 0.1†	5.5 ± 0.2
48 Hr after withdrawal (12)	1.3 ± 0.2	1.8 ± 0.1‡	4.2 ± 0.4
		Glutamate	
Control (3)	9.9 ± 0.6	7.3 ± 0.2	18.5 ± 1.2
Barbiturate-dependent (5)	10.7 ± 0.5	6.9 ± 0.6	20.1 ± 0.1
48 Hr after withdrawal (5)	10.7 ± 0.7	7.3 ± 0.7	17.5 ± 2.0
		Aspartate	
Control (3)	3.2 ± 0.2	1.4 ± 0.2	3.3 ± 0.1
Barbiturate-dependent (5)	2.9 ± 0.3	1.4 ± 0.1	4.2 ± 0.4
48 Hr after withdrawal (5)	2.4 ± 0.1§	1.4 ± 0.1	3.3 ± 0.1

* Values are expressed as means ± S.E. of values for the number of animals shown in parentheses.
† $P < 0.05$, compared with control value.
‡ $P < 0.05$, compared with value at 0 time after withdrawal.
§ $P < 0.01$, compared with control value.

pentylenetetrazol [13, 17], penicillin G [15], strychnine [15], hyperbaric oxygen [14] and alumina [16]. Negative results of this amino acid on epilepsy also have been reported [19]. In this study, we failed to detect the anticonvulsive effect of taurine. This negative result seems to reflect the difference in character between drug-induced epilepsy and withdrawal convulsions of barbiturate-dependent rats.

Prolonged administration of barbital sodium induced a significant increase of taurine content in the cerebellum, but not in the cerebral cortex and brain stem. It is unlikely that the increase in cerebellar taurine is related to the barbital level in the brain, since acute administration of barbital sodium (250 mg/kg, i.p., 1.5 hr) did not change taurine content in any region of the brain (data not shown). On the other hand, withdrawal of barbital sodium caused distinct changes of taurine content in each region of the brain: increase in the cerebral cortex, decrease in the cerebellum. Thus, taurine content in the cerebellum, but not in other regions of the brain, increases with the development of tolerance to barbital and soon after withdrawal the increased taurine content rapidly returned to normal. Furthermore, other tissues besides brain have a high taurine content, but only the content in the brain was affected by the administration or withdrawal of barbital. These results suggest that taurine metabolism in some regions of the brain may be related to the barbital dependence or the withdrawal signs. In the present work, however, the cause of the changes in taurine content in the brain is unknown. The important problem of how the changes of brain taurine content are related to barbital dependence or withdrawal still remains.

In this study we also found that the GABA content in the brain stem and cerebral cortex decreased by 22 and 33 per cent, respectively, on prolonged administration of barbital sodium, and that the content returned to the normal value within 48 hr after drug withdrawal. This finding may support the idea of Essig[1] that the convulsions may be caused by a deficiency of GABA in the brain. However, aspartate in the cerebral cortex of the barbiturate-dependent rats also decreased after drug withdrawal; moreover, the concentrations of acetylcholine [27] and catecholamines [28] in some regions of the brains of barbiturate-dependent rats have recently been shown to change after drug withdrawal. Thus, concentrations of many neurotransmitter candidates in the brain are changed during dependence to barbiturates or during withdrawal. These findings strongly suggest that the mechanisms of barbiturate dependence and withdrawal are much more complex than hitherto realized. Further studies are required to detect more specific changes of neuronal activity in some regions of the brains of barbiturate-dependent animals.

It should be noted that taurine content was markedly changed only in the cerebellum with the development of barbiturate dependence and appearance of withdrawal signs. Recently McBride *et al.* [29] have found that taurine is present in a high concentration in stellate cells in the cerebellum cortex of the rat and that microiontophoretic application of taurine depressed the firing rate of Purkinje cells. Furthermore, they proposed that taurine might be the inhibitory transmitter released from the stellate cells in the cerebellar cortex. The present findings should be a clue to obtaining further information on the metabolism and physiological roles of taurine in the cerebellum.

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