The other group treated with pentobarbital, chlorpromazine or naloxone did not show any sign of convulsion nor were there any deaths during a 2 day observation. It is considered, therefore, that the lethal effect would be specific to morphine.

These observations suggest that there is a site which specifically interacts with morphine to induce the convulsion and, when the sensitivity of the site to the drug is unusually increased by poly-D-lysine, the interaction proceeds in such a specific manner so as not to be influenced by naloxone.

More detailed studies are now in progress regarding the mechanism of morphine action in relation to the phenomenon outlined herein.

REFERENCES

- 1) KOIDA, M. AND KANETO, H.: *Folia pharmacol. jap.* **68**, 265P (1972) (in Japanese); 2) NEVO, A., DE VRIES, A. AND KATCHALSKY, A.: *Biochim. Biophys. Acta* **17**, 536 (1955); 3) KATCHALSKY, A., DANON, D., NEVO, A. AND DE VRIES, A.: *Biochim. Biophys. Acta* **33**, 120 (1959); 4) KORN-GUTH, S.E. AND STAHMANN, M.A.: *Cancer Res.* **21**, 907 (1961); 5) RYSER, H. J.-P.: *Science* **159**, 390 (1968); 6) MEHRISHI, J.N.: *Europ. J. Cancer* **5**, 427 (1969); 7) PADAWER, J.: *J. Cell. Biol.* **47**, 352 (1970); 8) MARTIN, W.R.: *Pharmacol. Rev.* **19**, 463 (1967); 9) ADLER, T.K.: *J. Pharmacol. exp. Ther.* **140**, 155 (1963); 10) KANETO, H., SHIMOMURA, K., KAMEI, C. AND NAKANISHI, H.: *Folia pharmacol. jap.* **66**, 487 (1970) (in Japanese)

THIAMINE ACCUMULATION INDUCED BY L-5-HYDROXY- TRYPTOPHAN IN RAT BRAIN

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It has been reported by Iwata et al. that the catecholamine contents in the brain, heart and spleen are increased in thiamine deficient rats, while the blood catecholamine is decreased (1, 2). An increase in the brain serotonin contents and decrease in the liver, stomach and blood serotonin contents have been found in the thiamine deficient rats by Kimura et al. and Itokawa et al. (3, 4). There is a concept that thiamine has a specific function in membrane transport in the nerve which is independent of its coenzyme role (5). Evidence for supporting this concept is the release of thiamine from the nerve preparation following electrical stimulation or application of neuroactive drugs (6). In this paper, effects of L-5-hydroxytryptophan (L-5HTP) on the brain thiamine level were studied and compared to those of L-dihydroxyphenylalanine (L-dopa).

Male Wistar rats, weighing 150–200 g, were maintained on a standard diet (CA-1, CLEA, Japan) with water ad libitum. Rats were administered i.p. L-5HTP (Kyowa Hakko) and L-dopa (Sankyo) in doses of 1, 10 and 100 mg/kg. Drugs were suspended

in saline. The animals were sacrificed one hr after a single injection or one hr after the last of 7 daily injections. The brain and liver were removed and homogenized in 0.4 N perchloric acid for catecholamine assay or in 0.1 N hydrochloric acid for serotonin and thiamine assays. The amount of serotonin was estimated by the method of Bogdanski et al. (7) and catecholamines by the procedure of Anton and Sayre (8, 9). For fluometric determination of thiamine, the alkaline cyanogen bromide method was used as described by Fujiwara and Matsui (10).

Brain serotonin content was significantly increased one hr after a single i.p. injection of L-5HTP 100 mg/kg, but was not affected by L-5HTP in doses of 1 or 10 mg/kg. This elevation in the brain serotonin level was not enhanced by seven daily injections of L-5HTP. Brain thiamine content in the L-5HTP treated rats was increased in a dose-dependent manner. Seven daily injections of L-5HTP slightly enhanced the L-5HTP induced thiamine increase in the rat brain (Table 1). In the liver, L-5HTP induced an increase of serotonin but not thiamine.

TABLE 1. Brain serotonin and thiamine contents in L-5HTP treated rats.

Treatment	Serotonin		Thiamine	
	Single inj.	Daily inj. 7 days	Single inj.	Daily inj. 7 days
None	0.50±0.02 (5) [†]		3.13±0.06 (17)	
L-5HTP 1 mg/kg	0.46±0.03 (5)	0.56±0.02 (5)	3.17±0.15 (5)	3.35±0.07 (5)
L-5HTP 10 mg/kg	0.58±0.03 (5)	0.56±0.02 (5)	2.95±0.20 (5)	3.73±0.10 (5)**
L-5HTP 100 mg/kg	1.83±0.07 (5)**	1.91±0.15 (5)**	3.69±0.35 (5)*	4.12±0.07 (5)**

[†] Mean±S.E. (number of rats)

Significant difference from control value using the Student's *t*-test; **P*<0.05, ***P*<0.01

TABLE 2. Brain catecholamines and thiamine contents in L-dopa treated (daily injection 7 days) rats.

Treatment	Dopamine	Noradrenaline	Thiamine
None	0.86±0.04 (9) [†]	0.40±0.04 (4)	3.13±0.06 (17) μ g/g
L-dopa 1 mg/kg	1.82±0.29 (4)**	0.52±0.03 (5)*	2.84±0.25 (5)
L-dopa 10 mg/kg	1.83±0.23 (5)**	0.48±0.09 (5)	3.13±0.11 (4)
L-dopa 100 mg/kg	3.52±0.33 (11)**	0.49±0.02 (5)*	2.67±0.11 (17)**

[†] Mean±S.E. (number of rats)

Significant difference from control value using the Student's *t*-test; **P*<0.05, ***P*<0.01

The accumulation of brain dopamine without that of noradrenaline was observed in the L-dopa treated rats. On the other hand, the brain thiamine content was slightly decreased in this group of rats (Table 2). Liver catecholamines were markedly increased only in the L-dopa 100 mg/kg treated rats among the three groups and the thiamine content remained unchanged.

The free and total thiamine in brain and liver of the 5HTP treated rats were also determined. The ratio of free thiamine to total thiamine in the brain of the L-5HTP treated rats and the control rats was 5.5% and 5.3% respectively. Ratio in the liver was 3.6%

and 3.5%. It is therefore suggested that most of the increased thiamine induced by L-5HTP is the phosphorylated form and presumably binds to tissue protein as the usual tissue thiamine.

Possible interpretations for a dose-dependent increase of brain thiamine induced by L-5HTP are; 1) an increase of thiamine uptake into the brain, 2) acceleration of the synthesis of thiamine pyrophosphate from thiamine and the binding of thiamine to tissue protein, 3) an inhibition of thiamine pyrophosphatase which converts thiamine pyrophosphate to free thiamine. Since increase of liver thiamine was not induced by L-5HTP injection to rats, it is most probable that the uptake process of thiamine into the brain is accelerated by L-5HTP itself, serotonin converted from L-5HTP or serotonin metabolites.

On the other hand, a slight decrease in brain thiamine induced by L-dopa may be a non-specific effect as this decrease is small and not dose-dependent. A full paper including data is in preparation.

REFERENCES

- 1) IWATA, H., WATANABE, K., NISHIKAWA, T. AND OHASHI, M.: *Europ. J. Pharmacol.* **6**, 83 (1969); 2) IWATA, H., FUJIMOTO, S., NISHIKAWA, T. AND HANO, K.: *Experientia* **24**, 378 (1968); 3) KIMURA, M., IKEDA, H., ITOKAWA, Y. AND TANAKA, C.: *Vitamins* **45**, 1 (1972); 4) ITOKAWA, Y., TANAKA, C. AND KIMURA, M.: *Metabolism* **21**, 375 (1972); 5) VON MURALT, A.: *Nature* **154**, 767 (1944); 6) ITOKAWA, Y., SCHULZ, R.A. AND COOPER, J.R.: *Biochim. Biophys. Acta* **266**, 293 (1972); 7) BOGDANSKI, D.F., PLETSCHER, A., BRODIE, B.B. AND UDENFRIEND, S.: *J. Pharmacol. exp. Ther.* **117**, 82 (1956); 8) ANTON, A.H. AND SAYRE, D.F.: *J. Pharmacol. exp. Ther.* **145**, 326 (1964); 9) ANTON, A.H. AND SAYRE, D.F.: *J. Pharmacol. exp. Ther.* **138**, 360 (1962); 10) FUJIWARA, M. AND MATSUI, K.: *Analyt. Chem.* **25**, 810 (1953)