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Chronic Administration of Sulbutiamine Improves Long Term Memory Formation in Mice: Possible Cholinergic Mediation

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MICHEAU, J., T. P. DURKIN, C. DESTRADE, Y. ROLLAND AND R. JAFFARD. *Chronic administration of sulbutiamine improves long term memory formation in mice: possible cholinergic mediation.* PHARMACOL BIOCHEM BEHAV **23**(2) 195–198, 1985.—Thiamine deficiency in both man and animals is known to produce memory dysfunction and cognitive disorders which have been related to an impairment of cholinergic activity. The present experiment was aimed at testing whether, inversely, chronic administration of large doses of sulbutiamine would have a facilitative effect on memory and would induce changes in central cholinergic activity. Accordingly mice received 300 mg/kg of sulbutiamine daily for 10 days. They were then submitted to an appetitive operant level press conditioning test. When compared to control subjects, sulbutiamine treated mice learned the task at the same rate in a single session but showed greatly improved performance when tested 24 hr after partial acquisition of the same task. Parallel neurochemical investigations showed that the treatment induced a slight (+10%) but significant increase in hippocampal sodium-dependant high affinity choline uptake. The present findings and previous results suggest that sulbutiamine improves memory formation and that this behavioral effect could be mediated by an increase in hippocampal cholinergic activity.

Mouse Sulbutiamine Learning and memory Hippocampal cholinergic activity

AS pointed out by several authors much evidence suggests that brain function is dependent on nutritional status, which may lead to alterations in brain neurotransmission. Thiamine deficiency in both man and experimental animals is known to result in Wernicke encephalopathy with characteristic neuropathological manifestations (see [16]) accompanied by regionally selective changes in neurotransmitter function [6] and lesions of a variety of brain structures [26]. Memory dysfunction and cognitive disorders have been described in patients with Wernicke encephalopathy and the well known amnesic Korsakoff syndrome is thought to be caused primarily by thiamine deficiency [30]. Similarly, learning and memory deficits have been reported following thiamine deprivation in animals [17,33] and several observations support the view that impairment of cholinergic function in the central nervous system may be important in the production of neurological symptoms associated with thiamine deficiency. Firstly, symptoms which are reversed by thiamine therapy can also be reversed by administration of cholinomimetics [3–4, 16]. These results, which seem to indicate that metabolic rather than structural changes may be responsible for some of the neurological symptoms, agree with the fact that thiamine plays a key role in the regulation of coenzyme availability for the synthesis of acetylcholine (see [16]). Evidence suggesting an important role of the central cholinergic system in memory mainly comes from pharmacological investigations showing that cholinergic antagonists induced amnesia while cholinergic agonists administered alone or in combination enhanced memory both in normal and deficient subjects (see [5,15]). This hypothesis is also supported by biochemical evaluations showing that changes in cholinergic activity such as reduction in cholineacetyltransferase activity, high affinity choline uptake or muscarinic receptor binding paralleled memory dysfunction in man (see [5]) and animals [18,25]. The possible forementioned relations between thiamine metabolism and cholinergic activity prompted us to study the effects of chronic administration of a thiamine derivative, sulbutiamine (Arcalion®-Laboratoires Servier) on learning and memory in mice and to examine whether any observed effects could be explained by changes in hippocampal cholinergic activity. For this purpose in the present study we chose to use long term retention of a lever press conditioning which was shown in previous experiments, to depend on hippocampal cholinergic activity at the time of training [18,19] and when impaired, could be restored by IV injections of physostigmine [22].

METHOD

Animals

The subjects were 52 male mice of the BALB/c strain 14–16 weeks old at the time of the experiments. At 10 weeks

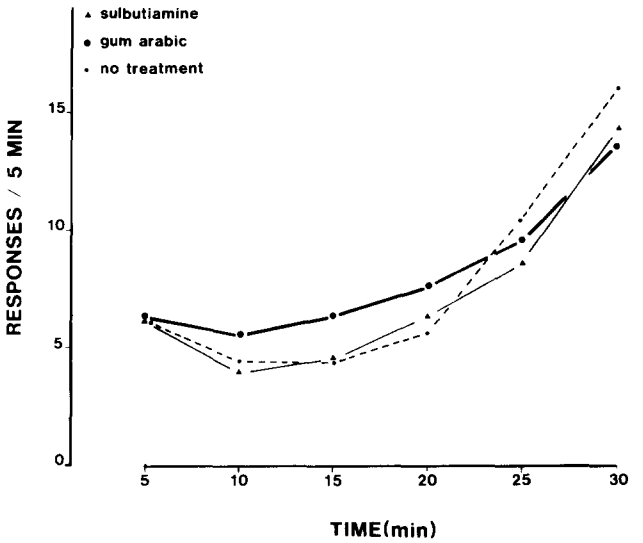

FIG. 1. Effects of sulbutiamine treatment on performance observed during a 30-min continuous acquisition session of CRF conditioning.

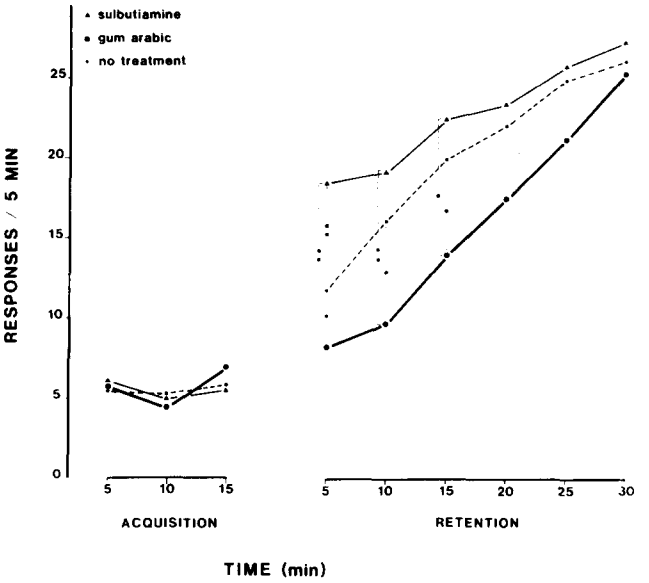

FIG. 2. Effects of sulbutiamine treatment on scores obtained during the 24 hr-delayed retention after partial acquisition of CRF conditioning. Statistical comparisons: $*p<0.05$; $**p<0.01$.

of age they were housed individually with ad lib access to food and water in a room which was maintained at a constant temperature (21°C) and a light-dark cycle (12 hr-12 hr).

Drug Administration

Sulbutiamine was administered orally by intragastric intubation. Each animal of the experimental group received 0.2 ml of a gum arabic emulsion (15% v/w) containing the drug at a dose of 300 mg/kg daily. The treatment was administered for 10 days and stopped the day before either behavioral or neurochemical investigations. Mice receiving only gum arabic (0.2 ml) or no treatment except handling served as controls.

Behavioral Testing

The apparatus, previously described elsewhere [9] was a Skinner box in which the lever and the food cup were separated by a small partition. All lever-presses were reinforced by a 6 mg pellet (continuous reinforcement) and registered on a pen-recorder. The procedure used was the same as that described in previous papers [10,21]. A deprivation schedule was initiated 4 days before testing so that throughout the course of conditioning all subjects were maintained at 81–84% of their ad lib body weights. Mice belonging to each of the three groups (Sulbutiamine: N=18; gum arabic: N=17 and no treatment: N=19) were randomly divided into two subgroups assigned to two experimental test procedures. The first test procedure was aimed at testing the influence of treatments on the speed of acquisition in a single session of 30 min (see Fig. 1). The second test procedure consisted of a 15 mm acquisition session followed by a retention session of 30 min 24 hr later (see Fig. 2). These two procedures allowed us to distinguish between a possible effect of the drug on learning rate and on long term memory (see [20]).

Neurochemical Analysis

Neurochemical analyses were conducted on 6 subjects of each group treated in exactly the same way as mice used for behavioral testing, animals being sacrificed in place of being trained in the Skinner box. Sodium-dependent high affinity choline uptake (SDHACU) kinetics were measured in aliquots of resuspended crude synaptosomal (P2) pellets of hippocampi from the different groups of animals. The procedure, based on that of ATWEH *et al.* [2] consists of measuring the difference in the amount of methyl-^{3}H-choline (0.25 μM) taken up by the synaptosomal aliquots over a 4 min period in parallel incubations in sodium-free and normal sodium Krebs ringer and has been described previously [13].

RESULTS

Behavioral

As can be seen in Fig. 1, there was no between groups differences in the performance registered during the 30 min of the uninterrupted session, $F(2,22)=0.64$, n.s.; the speed of acquisition was not different among the three groups (trend analysis = $F(10,110)=0.83$, n.s.) which reached comparable levels of performance at the end of the session. By contrast, results summarized in Fig. 2 show that the 24 hr interval interposed between the 1st (partial acquisition) and 2nd (retention) session induced significant between group differences. Thus, as for the uninterrupted 30 min session, no differences were observed during the 15 min of partial acquisition, $F(2,26)=0.10$, n.s., but performance on the whole retention session significantly differed among the three groups, $F(2,26)=3.89$, $p<0.05$, notably at the start (first 5 min) of the session, $F(2,26)=11.26$, $p<0.001$. Between groups comparisons during this first 5 min block show that gum arabic treatment induced an impairment, $F(1,17)=5,28$, $p<0.05$, while sulbutiamine dramatically improved retention

TABLE 1

EFFECT OF SULBUTIAMINE TREATMENT ON HIGH AFFINITY SODIUM DEPENDENT CHOLINE (Ch) UPTAKE IN HIPPOCAMPUS

	No treatment	Gum arabic	Sulbutiamine
SDHACU			
p.moles Ch/	18.72	18.31	*20.16
4 min/mg protein	0.32	0.63	0.23
S.D.			

*See text for statistical comparisons.

performance, $F(1,17)=15.11$, $p<0.01$, even when compared to that of the untreated group, $F(1,18)=8.84$, $p<0.01$.

Neurochemical

Results are summarized in Table 1. An analysis of variance showed that treatments significantly modified the rate of SDHACU in dorsal hippocampus, $F(2,15)=4.70$, $p=0.025$. Daily gum arabic administration had no effect (−2.2%, n.s.) while sulbutiamine induced a slight (+10.1%) but significant increase in the rate of SDHACU, $F(1,10)=7.68$, $p<0.025$.

DISCUSSION

Our results showed that daily administration of a high dose of sulbutiamine (300 mg/kg) for 10 days induced subtle but highly significant behavioral and neurochemical effects. Behavioral analysis demonstrates that sulbutiamine treatment improves 24 hr delayed retention of a partially learned operant lever press conditioning on continuous reinforcement. This improvement seems to be specific of long-term memory formation since no effects were observed when learning was uninterrupted. Moreover, additional behavioral testing reported elsewhere [23] showed that the treatment had no effect on either motivation for food, locomotor and exploratory activity (hole-board test) or emotionality (open-field). The same observations remain valid for subjects treated with placebo (gum arabic solution) which did not exhibit any behavioral differences to untreated mice except for performance on retention which was impaired (see Fig. 2). We have at present no explanation for this deficit which however most likely results from the repeated stress associated with daily gastric intubation than from gum arabic ingestion.

The neurochemical evaluation shows that in parallel with its facilitative effect on memory sulbutiamine significantly increased hippocampal sodium dependent high affinity choline uptake (SDHACU). However, the mechanisms underlying such biochemical changes and the possible causal relationship between these changes and memory facilitation remains to be established. Two non exclusive hypotheses could account for the observed increased choline uptake by cholinergic neurons. Thus on one hand, thiamine is involved in the production of acetyl-CoA providing the acetyl radical for acetylcholine (ACh) synthesis; though less than 1% of acetyl-CoA is convected into ACh, ACh synthesis is highly sensitive to conditions which decrease cerebral metabolism and is reduced in all models of thiamine deficiency [29, 31–32]. On the other hand, it has been shown that thiamine acts on cholinergic synaptic transmission [12,14] and it has been suggested that thiamine-deficient animals cannot increase acetylcholine release under conditions of increased physiological demand [27,28]. Finally, the presently observed increase in choline uptake by hippocampal neurons could be the result of an increase in synthesis and release of acetylcholine by septohippocampal neurons. The fact that several acute treatments which increased hippocampal choline uptake also improves long-term retention in the same learning situation (Galey *et al.*, unpublished results) argues in favor of a link between sulbutiamine administration, cholinergic mechanisms and memory formation. It has recently been shown that treatments aimed at enhancing cholinergic activity by pharmacological intervention at the receptor site were much more efficient when combined with treatments aimed at increasing presynaptic transmitter synthesis than when administered alone [15]. In line with this finding the question arises of whether the chronic administration of a choline-sulbutiamine mixture would be more efficient and at much lower doses than either choline (see [11]) or sulbutiamine administered alone. Such a possibility and the underlying cholinergic hypothesis are presently under investigation in normal and experimental memory deficient animals.

REFERENCES

1. Antuono, P., S. Sorbi, L. Bracco, T. Fusco and L. Amaducci. A discrete sampling technique in senile dementia or the Alzheimer type and alcoholic dementia: study of the cholinergic system. In: *Aging of the Brain and Dementia, Aging, Vol 13,* edited by L. Amaducci *et al.* New York: Raven Press, 1980, pp. 151–158.
2. Atweh, S., J. R. Simon and M. J. Kuhar. Utilization of sodium-dependent high affinity choline uptake *in vitro* as a measure of the activity of cholinergic neurones *in vivo*. *Life Sci* **17:** 1535–1544, 1975.
3. Barclay, L. L., G. E. Gibson and J. P. Blass. The string test: an early behavioral change in thiamine deficiency. *Pharmacol Biochem Behav* **14:** 153–157, 1981.
4. Barclay, L. L., G. E. Gibson and J. P. Blass. Definition of the cholinergic lesion in early thiamine deficiency. *Soc Neurosci Abstr* **8:** Part 1, 55–14, 1982.
5. Bartus, R. T., R. L. Dean, B. Beer and A. S. Lippa. The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217:** 408–417, 1982.
6. Butterworth, R. F. Neurotransmitter function in thiamine-deficiency encephalopathy. *Neurochem Int* **4:** 449–464, 1982.
7. Cheney, D. L., C. J. Gubler and A. W. Jaussi. Production of acetylcholine in rat brain following thiamin deprivation and treatment with thiamin antagonists. *J Neurochem* **14:** 37–40, 1969.
8. Destrade, C. and B. Cardo. Effects of post-trial hippocampal stimulation on time-dependent improvement of performance in mice. *Brain Res* **78:** 447–454, 1974.
9. Destrade, C., B. Soumireu-Mourat and B. Cardo. Effects of post-trial hippocampal stimulation on acquisition of operant behavior in the mouse. *Behav Biol* **8:** 713–724, 1973.
10. Doerge, D. R., M. G. McNamee and L. L. Ingraham. Some neurochemical properties of thiamin. *Ann NY Acad Sci* **378:** 422–434, 1982.
11. Drachman, D. A., G. Glosser, P. Fleming and G. Longenecker. Memory decline in the aged: treatment with lecithin and physostigmine. *Neurology* **32:** 944–950, 1982.
12. Dunant, Y., J. Corthay and L. Eder. Acetylcholine synthesis and release: some unsolved questions. In: *Alzheimer Disease: a report of progress, Aging, Vol 19,* edited by S. Corkin *et al.* New York: Raven Press, 1982, pp. 345–349.

13. Durkin, T., H. Hashem-Zadeh, P. Mandel and A. Ebel. A comparative study of the acute effects of ethanol on the cholinergic system in hippocampus and striatum of inbred mouse strains. *J Pharmacol Exp Ther* **220:** 203–208, 1982.
14. Eder, L., Y. Dunant and F. Loctin. Thiamine and cholinergic transmission in the electric organ of Torpedo. II. Effects of exogenous thiamine and analogs on ACh release. *J Neurochem* **35:** 1287–1296, 1980.
15. Flood, J. F., G. E. Smith and A. Cherkin. Memory retention: potentiation of cholinergic drug combinations in mice. *Neurobiol Aging* **4:** 37–43, 1983.
16. Gibson, G., L. Barclay and J. P. Blass. The role of the cholinergic system in thiamin deficiency. *Ann NY Acad Sci* **378:** 382–403, 1982.
17. Irle, E. and H. J. Markowitsch. Widespread neuroanatomical damage and learning deficits following chronic alcohol consumption or vitamin-B (thiamine) deficiency in rats. *Behav Brain Res* **9:** 277–294, 1983.
18. Jaffard, R. and C. Destrade. Learning and memory processes as related to genotypic or experimental variations of hippocampal cholinergic activity in inbred strains of mice. In: *Genetics of the Brain,* edited by I. Lieblich. Amsterdam: Elsevier Biomedical Press, 1982, pp. 300–321.
19. Jaffard, R., C. Destrade, T. Durkin and A. Ebel. Memory formation as related to genotypic or experimental variations of hippocampal cholinergic activity in mice. *Physiol Behav* **22:** 1093–1096, 1979.
20. Jaffard, R., C. Destrade, B. Soumireu-Mourat and B. Cardo. Time-dependent improvement of performance on appetitive tasks in mice. *Behav Biol* **11:** 89–100, 1974.
21. Jaffard, R., A. Ebel, C. Destrade, T. Durkin, P. Mandel and B. Cardo. Effects of hippocampal electrical stimulation in long term memory and on cholinergic mechanisms in three inbred strains of mice. *Brain Res* **133:** 277–289, 1977.
22. Micheau, J., C. Destrade and R. Jaffard. Effects of physostigmine on memory deficits induced by pretraining electrical stimulation of the dorsal hippocampus in mice. *Soc Neurosci Abstr* **9:** part 2, 239.9, 1983.
23. Micheau, J., C. Destrade, Y. Rolland and R. Jaffard. Sulbutiamine improves memory formation in BALB/c mice. Paper presented at the Third European Winter Conference on Brain Research, Les Arcs, France, 1983.
24. Nordberg, A., C. Larsson, E. Perdahl and B. Winblad. Changes in cholinergic activity in human hippocampus following chronic alcohol abuse. *Pharmacol Biochem Behav* **18:** 397–400, 1983.
25. Rauca, C. H., E. Kammerer and H. Matthies. Choline uptake and permanent memory storage. *Pharmacol Biochem Behav* **13:** 21–25, 1980.
26. Ron, M. A. Syndromes of alcohol related brain damage. *Br Med Bull* **38:** 91–94, 1982.
27. Sacchi, O., S. Consolo, G. Peri, I. Prigioni, H. Ladinsky and V. Perri. Storage and release of acetylcholine in the isolated superior cervical ganglion of the rat. *Brain Res* **151:** 443–456, 1978.
28. Sacchi, O., H. Ladinsky, I. Prigioni, S. Consolo, G. Peri and V. Perri. Acetylcholine turnover in the thiamin-depleted superior cervical ganglion of the rat. *Brain Res* **151:** 609–614, 1978.
29. Speeg, K. V., D. Chen, D. W. Candless and S. Schenker. Cerebral acetylcholine in thiamin deficiency. *Proc Soc Exp Biol Med* **134:** 1005–1009, 1970.
30. Thomson, A. D. Alcohol-related structural brain changes. *Br Med Bull* **38:** 87–91, 1982.
31. Vorhees, C. V., D. E. Schmidt and R. J. Barret. Effects of pyrithiamin and oxythiamin on acetylcholine levels and utilization in rat brain. *Brain Res Bull* **3:** 493–496, 1978.
32. Vorhees, C. V., D. E. Schmidt, R. J. Barrett and S. Schenker. Effect of thiamine deficiency on acetylcholine levels and utilization *in vivo* in rat brain. *J Nutr* **107:** 1902–1908, 1977.
33. Witt, E. D. and P. S. Goldman-Rakic. Intermittent thiamine deficiency in the rhesus monkey. II. Evidence for memory loss. *Ann Neurol* **13:** 396–401, 1983.