The resting level of the NEFA-like substance in perfusate obtained from the anterior or posterior hypothalamus was 0.42 ± 0.18 µmol/30 min, and this level failed to change significantly in the cooled monkey. However, the amount of NEFA contained in the anterior hypothalamic perfusate obtained from the heated monkey increased significantly ($t = 3.66$; $df = 7$; $p < 0.01$) to 0.78 ± 0.34 µmol/30 min, and remained elevated for 1–2 h after heating had been terminated. The Table presents values of the NEFA-like substance in the perfusate obtained from monkeys during heating, cooling, and control periods. The changes in blood levels of NEFA were not significantly correlated with the changes in the content of NEFA in hypothalamic perfusate.

From these experiments, it is evident that at least 2 substances are released reciprocally within the hypothalamus of the warm or cold monkey. When the animal is cooled, the release of 5-HT from the neurons in the anterior region increases. This apparently activates the heat production pathway because the monkey shivers and maintains its normal temperature in the cold environment. During heating, the release of 5-HT does not change since heat production is not required. The increase in the NEFA-like substance in the effluent collected from the anterior hypothalamus of the heated monkey may reflect an increase in noradrenaline release within the anterior, pre-optic region. This monoamine could act, in functional opposition to 5-HT, as a transmitter which blocks the heat production system and activates the heat loss pathway[8,9]. Thus far, we have failed to detect an appreciable amount of noradrenaline in the hypothalamic perfusates probably because of the rapid re-uptake and subsequent inactivation following noradrenaline's release at the synapse.

Taken together with the fact that hyperthermia is caused by 5-HT micro-injected directly into the anterior hypothalamus of the conscious monkey[9,10], these results indicate that this indole amine could well be the synaptic transmitter delegated to the reflex regulation of heat production. For 5-HT, 3 of the criteria outlined by McLennan[11] for a substance to be considered as a chemical transmitter in the central nervous system are now fulfilled: (1) the natural occurrence of 5-HT in the anterior hypothalamus as reflected by its resting output in the 'push-pull' perfusate; (2) the pharmacologically stimulating properties of 5-HT in evoking hyperthermia when locally applied to the anterior hypothalamus; and (3) the evoked elevation in the rate of release of 5-HT in response to cold environmental stimulation.

Zusammenfassung. Unterkühlungsexperimente mit Affen zeigen einen Anstieg des 5-HT-Gehaltes in der Durchströmungsflüssigkeit des vorderen Hypothalamus. Erwärmung der Tiere ergab 2–4fachen Anstieg eines NEFA-ähnlichen Stoffes.

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[8] W. Feldberg and R. D. Myers, J. Physiol. *177*, 239 (1965).
[9] R. D. Myers and T. Yaksh, J. Physiol. *202*, 483 (1969).
[10] R. D. Myers, Adv. Pharmac. *6*, 318 (1968).
[11] H. McLennan, *Synaptic Transmission* (W. Saunders Co., Philadelphia 1963).
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The Effects of Intracerebrally Injected Oxythiamine on Free Thiamine and Thiamine Phosphates Content of Rat Brain

Oxythiamine (OTh), when orally or parenterally administered to rats, does not modify the brain total thiamine (Th) content[1–3], nor does it call forth the neuromuscular signs of Th deficiency[4,5] or affect the activity of thiamine diphosphate (ThDP)-dependent cerebral enzymes[6–8]. On the other hand, OTh is a weak competitive inhibitor of cerebral thiaminekinase, by which also it seems to be phosphorylated[9]. It is well known that OTh diphosphate is, in vitro, a powerful inhibitor of ThDP-dependent enzymes[5,10]. Since OTh cannot penetrate the blood-brain barrier[11,12], its failure to affect the brain in vivo is likely to be due to its inability to enter this organ and to be there phosphorylated, producing the changes that one could expect from OTh diphosphate activity in vitro[5,10]. Therefore, we decided to introduce OTh directly into the brain of rats, in order to investigate its effects in vivo on free and phosphorylated Th cerebral content. A brief account of the results we obtained is here reported.

Material and method. 0.15 or 0.6 µmoles of OTh (Sigma, St. Louis, Mo., USA), approximately corresponding to 10 and 40 times the total Th content of the brain, dissolved in 10 µl of saline (0.9% NaCl), were injected, with an Agla micrometer syringe (Burroughs Wellcome and Co., London), into the brain of female albino rats (Wistar strain, 130–140 g body wt.), following the technique of Valzelli[13] slightly modified. Contemporaneously, 10 µl of saline were similarly injected into the brain of control

[1] L. De Caro, G. Rindi, V. Perri and G. Ferrari, Int. Z. Vitaminforsch. *26*, 343 (1956).
[2] L. De Caro, G. Rindi, V. Perri and G. Ferrari, Int. Z. Vitaminforsch. *28*, 252 (1958).
[3] H. P. Gurtner, cited by A. von Muralt in Ann. N.Y. Acad. Sci. *98*, 499 (1961).
[4] A. von Muralt, Ann. N.Y. Acad. Sci. *98*, 499 (1961).
[5] G. Rindi, Boll. chim.-farm. *102*, 363 (1963).
[6] C. J. Gubler, J. biol. Chem. *236*, 3112 (1961).
[7] C. J. Gubler, Int. Z. Vitaminforsch. *38*, 287 (1968).
[8] M. Brin, J. Nutrition *78*, 179 (1962).
[9] L. R. Johnson and C. J. Gubler, Biochim. biophys. Acta *156*, 85 (1968).
[10] E. Steyn-Parvé, *Thiamine Deficiency: Biochemical Lesions and their Clinical Significance* (Ed. G. E. W. Wolstenholme and M. O'Connor; Churchill Ltd., London 1967), p. 26.
[11] G. Rindi, L. de Giuseppe and U. Ventura, J. Nutrition *81*, 417 (1963).
[12] Yu. Ostrovsky, Vop. med. Khim. *11*, 95 (1965).
[13] L. Valzelli, Medna exp. *11*, 23 (1964).

rats. After the injection, the animals were singularly housed and fed a Th-deficient diet[11]. They were killed by decapitation at different time intervals from the injection, together with their controls. The brain was rapidly removed, weighed in cold 5% trichloroacetic acid and homogenized. After centrifugation at 16,000 *g* in a refrigerated centrifuge, the separation of free Th from Th phosphates was made according to SHARMA and QUASTEL[14], with minor modifications. Th was determined fluorometrically as thiocrome[15] (when necessary, after enzymatic dephosphorylation of its phosphates), using a Beckman spectrophotometer mod. DU. In some preliminary experiments where OTh was added to the homogenized rat brain, no interference with Th determination could be demonstrated.

Results and discussion. The behaviour of control rats was never affected by saline injection into the brain, also when a hypertonic solution (1.6% NaCl) was injected. On the other hand, immediately after OTh intracerebral injection, sometimes the rats became lightly spastic, and constantly they were unable to perform coordinate movements for 8–15 min. Successively, most of the rats treated with 0.6 μmoles of OTh remained restless and irritable so that acoustic or tactile stimuli could elicit violent fits. A few of them died during the fits and were not used for Th determination. All the rats receiving 0.15 μmoles of OTh were apparently normal.

As can be seen in Figures 1 and 2, both doses of OTh produced only small and irregular changes of free Th content. However, 0.15 μmoles of OTh caused a moderate (about 10%), but statistically significant ($p < 0.05$) decrease of cerebral Th phosphates only at fourth and seventh day after injection. Conversely, already 24 h after the intracerebral injection, 0.6 μmoles of OTh induced a statistically significant ($p < 0.001$) higher decrease (about 20%) of Th phosphates, which lasted for the following days. However, the Th phosphates levels so reached in brain were far above those causing the onset of beri-beric neuromuscular signs in rats[2]. Actually, during all the experiments, the animals showed symptoms rather different from those of athiaminic polyneuritis. On the whole, the effects of OTh on Th phosphates brain content were not only modest, but also rather slow to begin. We cannot completely rule out a close relationship between the small Th phosphates decrease in brain and the irritability of rats receiving the higher dose of OTh, but it is likely that OTh could otherwise affect the neuronal metabolism. It may be that OTh, introduced into the brain, could be there phosphorylated, as JOHNSON and GUBLER[9] showed in vitro, probably to diphosphate. This, in turn, could inhibit cerebral ThDP-dependent enzymes, without greatly affecting Th phosphates content, since these latter are produced by thiaminekinase, which is very poorly inhibited only by OTh[9]. Thus, the observed symptoms could derive not so much from the modest decrease of Th phosphates as from the inhibition of ThDP-dependent enzymes (specially transketolase)[16], so important in brain metabolism. In this connection it is noteworthy that OTh is able to lower, in an unknown way, both the acetylcholine content of rat brain and its in vitro acetylcholine-synthetizing power[7].

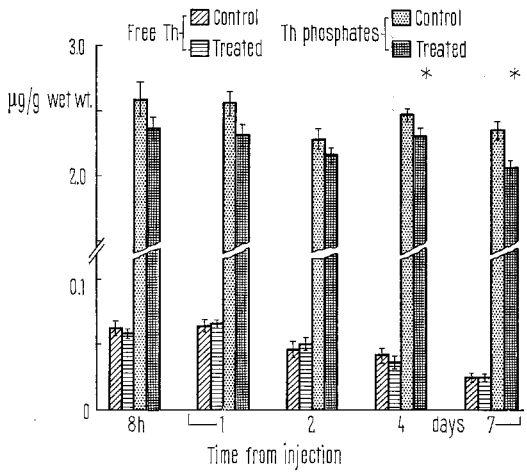

Fig. 1. Free thiamine and thiamine phosphates content of rat brain after intracerebral injection of 0.15 μmoles of oxythiamine. Each value is the mean of at least 6 animals. Th, thiamine; *, statistically significant at $p < 0.05$.

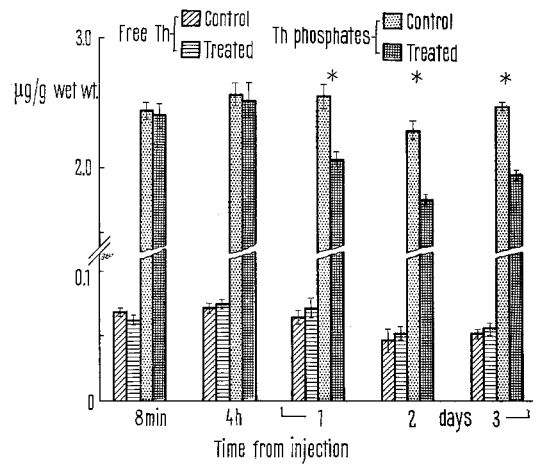

Fig. 2. Free thiamine and thiamine phosphates content of rat brain after intracerebral injection of 0.6 μmoles of oxythiamine. Each value is the mean of at least 6 animals. Th, thiamine; *, statistically significant at $p < 0.05$.

Riassunto. L'iniezione intracerebrale di 0,6 μmoli di OTh nel ratto causa duraturi sintomi di ipereccitabilità e, dopo 24 ore, una modesta diminuzione del contenuto cerebrale di Th fosforilata. L'iniezione di 0,15 μmoli causa soltanto una molto lieve diminuzione del contenuto che compare al quarto giorno dall'iniezione.

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[14] K. SHARMA and S. H. QUASTEL, Biochem. J. *94*, 790 (1965).
[15] K. FUJIWARA and K. MATSUI, Anal. Chem. *25*, 809 (1953).
[16] A. G. DATTA and E. RACHER, J. biol. Chem. *236*, 617 (1961).