Biochemical Pharmacology, Vol. 18, pp. 545–547. Pergamon Press. 1969. Printed in Great Britain

On a relationship between ion transport and thiamine in nervous tissue

(*Received* 12 *June* 1968; *accepted* 2 *August* 1968)

FROM A VARIETY of experimental approaches, both in this laboratory and others, a considerable amount of evidence has been accumulating to suggest that thiamine has a specific role in the conduction process in nervous tissue that is independent of its coenzyme function. This evidence may be summarized as follows: 1) electrical stimulation of a variety of preparations of nervous tissue causes the release of the vitamin;[1–3] 2) pyrithiamine, an antimetabolite of thiamine that produces polyneuritis *in vivo*, has a profound and irreversible effect on the electrical activity of isolated nervous tissue *in vitro*.[4–6] This effect has been shown to be due to the ability of the antimetabolite to displace the vitamin from the nerve, rather than to inhibit the TPP*-dependent enzymes in the tissue;[7–8] 3) by fluorescence microscopy, thiamine has been shown to be localized in nerve membranes rather than in the axoplasm;[9,10] 4) TPPase, an enzyme with an appraent absolute specificity for TPP as substrate,[11] is localized specifically in membrane structures;[12] 5) although the bulk of thiamine in nerves is in the form of TPP, about 4–10 per cent is in the form of the triphosphate, TTP. Recently, an enzyme that catalyzes the synthesis of TTP from TPP and ATP has been isolated from brain mitochondria.[13] This phosphotransferase is extremely active, comparable to the activity of the Na–K-ATPase of cerebral microsomes; in addition, it is specifically inhibited by blood, spinal fluid and urine extracts from patients with the neurological disease, subacute necrotizing encephalomyelopathy.[14]

In a further attempt to define the specific function of thiamine in nervous tissue, the following experiments were performed. Large bullfrogs (*Rana catesbiana*) were given two intrathecal injections (0·1 ml), 18 hr apart, of ^{35}S-thiamine (100 μc/μmole). The total amount of radioactivity given was 50 μc. The frogs were sacrificed 15–20 hr after the last injection and the spinal cords were removed and divided sagittally. One-half of the cord was deproteinized with 7% perchloric acid for subsequent determination of the labeled thiamine (*vide infra*) and the other hemi-cord was placed in a lucite chamber and perfused with frog Ringer's solution. The solution was pumped through the chamber at a rate of 1 ml/min. The outflow of the chamber was connected to a Nuclear-Chicago flow cell inserted in a liquid scintillation spectrometer. The spectrometer in turn was connected through a ratemeter to a graphic recorder with integrator. Thus, with an exposed surface of the spinal cord to permit rapid exchange and with this experimental design, it was possible to monitor instantaneously the efflux of labeled thiamine from the nerve preparation. When the effect of drugs on this efflux was tested, the drugs were dissolved in 10 ml of the Ringer's solution and this solution was pumped through the system.

To determine the amount of thiamine compounds in the cord that were labeled, an aliquot of the deproteinized hemi-cord was used for an assay of total radioactivity; total thiamine content was determined by the fluorometric procedure of Fujiwara and Matsui.[15] From this experiment it was calculated that about 90 per cent of the total thiamine derivatives in the cord were labeled. By means of an electrophoretic procedure to separate thiamine and its phosphate esters,[13] it was found that about 77 per cent of the label was TPP, about 5% was TTP, and the remaining radioactivity was TMP and free thiamine (Table 1). This distribution corresponds to the usual pattern of thiamine and its phosphate esters in cells.[16] All the radioactivity could be accounted for as thiamine compounds.

As shown in Fig. 1, crude dose–response curves could be obtained with the flow system. However, the sensitivity of the response varied somewhat from one preparation to another so that, for example, ACh could be demonstrated to release labeled thiamine from the cord at a concentration of 10^{-6} M in 5 experiments, but in 3 experiments the concentration of the neurohumor had to be raised to 5×10^{-6} M to effect a significant release of the vitamin.

* Abbreviations are: TPP, thiamine pyrophosphate; TMP, thiamine monophosphate; TTP, thiamine triphosphate; ACh, acetylcholine; 5-HT, 5-hydroxytryptamine.

TABLE 1. DISTRIBUTION OF LABELED THIAMINE ABD THIAMINE PHOSPHATES*

Thiamine compound	Spinal cord (% ± S.D.)	Effluent (% ± S.D.)
TTP	4·6 ± 1·3	0
TPP	76·7 ± 5·6	4·3 ± 3·7
TMP	4·1 ± 1·6	35·0 ± 0·4
Thiamine	14·6 ± 2·7	60·4 ± 3·7

* Thiamine compounds in the cord were determined by the electrophoretic and fluorometric procedure of Itokawa and Cooper.[13] In the effluent the compounds were adsorbed on charcoal and eluted with 50% EtOH[17] and then separated and assayed as above. Figures represent an average of three experiments.

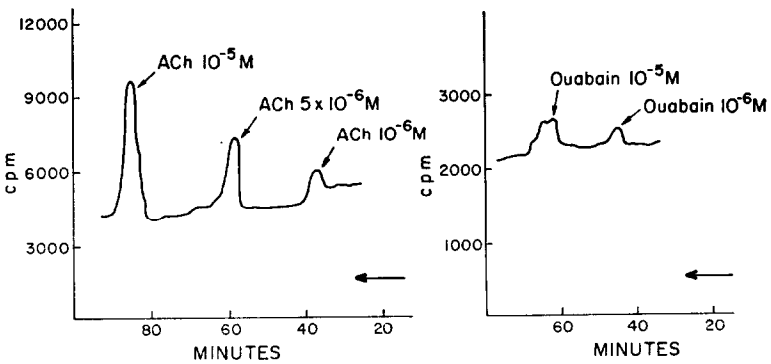

FIG. 1. Dose–response curves for ACh and ouabain in promoting the efflux of labeled thiamine from the frog spinal cord. Details given in text. The system had the following parameters: flow rate 1 ml/min; flow cell volume, 4 ml; ratemeter time constant, 40 sec; chart speed, 3 in./hr.

TABLE 2. AGENTS THAT WERE TESTED FOR THEIR ABILITY TO RELEASE ^{35}S-THIAMINE COMPOUNDS FROM THE FROG SPINAL CORD*

Effective		Ineffective	
Drug	Conc (M)	Drug	Conc (M)
ACh	10^{-6}	Choline	10^{-4}
Carbachol	10^{-5}	NaCl	5×10^{-2}
Ouabain	10^{-6}	Noradrenaline	10^{-4}
EDTA	10^{-3}		
5-HT	5×10^{-6}		
Xylocaine	10^{-2}		
KCl	10^{-2}		
DFP	10^{-5}		
Tetrodotoxin	3×10^{-8} (0·01 μg/ml)		

* Drugs were perfused through the system at the rate of 1 ml/min for 10 min. Effective agents produced a peak height of radioactivity of at least 300 cpm above background. All drugs were tested a minimum of three times.

The specificity of the drugs that promote the efflux of labeled thiamine from the hemi-cord can be noted in Table 2. It is clear from this table that agents which are known to affect ion movements in nervous tissue possess the ability to release thiamine. We have previously shown that electrical stimulation of the spinal cord also releases the vitamin.[3] In contrast, agents such as choline and NaCl, which are ineffective in promoting or inhibiting ion fluxes, also are without effect in this system. The exception to the above statement is in the case of noradrenaline; even at a concentration of 10^{-4} M, this neurotransmitter was without effect. This situation may reflect the finding of Tebecis and Phillis,[18] who observed a marked excitatory effect of 5-HT when applied to a toad spinal cord at concentrations as low as 10^{-7} to 10^{-9} M, but only a minimal effect of noradrenaline even when the concentration was raised to 10^{-3} to 10^{-4} M.

The effects noted above do not appear to apply only to amphibia, since preliminary experiments with the rat spinal cord that was preloaded with labeled thiamine and perfused similarly to the frog preparation also disclosed that ACh at 10^{-6} M released labeled thiamine.

This apparent relationship between ion movements and thiamine has a further dimension in that, as mentioned earlier, although about 80 per cent of the vitamin in nervous tissue is in the form of TPP, about 5 per cent is TTP and the remainder is TMP and thiamine, the released material is primarily in the form of TMP and free thiamine (Table 1). This is also true after electrical stimulation of a nerve preparation.[2,3] This observation suggests that the transport of ions is related to a dephosphorylation of TPP (or perhaps TTP, although the two esters are interconverted very rapidly and there is a specific phosphatase for TPP). This dephosphorylation may reflect a displacement of TPP from some bound form where it is then subject to hydrolytic attack.

Whether thiamine in some form is serving as a carrier for the passive transport of ions or functions to alter membrane structure remains to be determined.

Acknowledgement—This work was supported by Grant No. NB 05121 of the United States Public Health Service.

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REFERENCES

1. B. Minz, *C.r. Seanc. Soc. Biol.* **127**, 1251 (1938).
2. H. P. Gurtner, *Helv. physiol. pharmac. Acta*, **Suppl. XI** (1961).
3. J. R. Cooper, R. H. Roth and M. M. Kini, *Nature, Lond.* **199**, 609 (1963).
4. H. A. Kunz, *Helv. physiol. pharmac. Acta* **14**, 411 (1956).
5. C. J. Armett and J. R. Cooper, *J. Pharmac. exp. Ther.* **148**, 137 (1965).
6. C. J. Armett and J. R. Cooper, *Experientia* **21**, 605 (1965).
7. J. R. Cooper and J. H. Pincus, *Thiamine Deficiency: Biochemical Lesions and Their Clinical Significance*, Ciba Foundation Study Group No. 28, p. 112. Churchill, London (1967).
8. J. R. Cooper, *Biochim. biophys. Acta* **156**, 368 (1968).
9. A. von Muralt, *Pflügers Arch. ges. physiol.* **247**, 1 (1943).
10. C. Tanaka and J. R. Cooper, *J. Histochem. Cytochem.* **16**, 362 (1968).
11. J. R. Cooper and M. M. Kini, in preparation.
12. S. Tanaka, R. J. Barrnett and J. R. Cooper, in preparation.
13. Y. Itokawa and J. R. Cooper, *Biochim. biophys. Acta* **158**, 180 (1968).
14. J. R. Cooper, Y. Itokawa and J. H. Pincus, submitted for publication.
15. M. Fujiwara and K. Matsui, *Analyt. Chem.* **25**, 810 (1953).
16. G. Rindi and L. de Giuseppe, *Biochem. J.* **78**, 602 (1961).
17. D. Siliprandi and N. Siliprandi, *Biochim. biophys. Acta* **14**, 52 (1954).
18. A. K. Tebecis and J. W. Phillis, *Comp. Biochem. Physiol.* **23**, 553 (1967).