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THE SYNAPTOLYTIC EFFECT OF THIAMINE RELATED TO ITS INTERACTION WITH NEUROTRANSMITTERS*

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Abstract—Thiamine interacts *in vitro* with acetylcholine, norepinephrine and serotonin yielding molecular complexes. Such interaction can be related to the physiological effect of thiamine which, in large doses, induces a general synaptolysis.

THE USE of thiamine in large doses was introduced in anesthesia in 1962,[1] but the attempts at explaining the mechanism of this phenomenon have been unsatisfactory so far, including the postulate of a curare-like action of thiamine put forward after pharmacological assays on *in vivo* systems.[2,3] The integrity of the thiamine molecule has been proved necessary for its anesthetic action by chemical modification studies.[4] These studies demonstrated that chemical derivatives of thiamine acting as antivitamin B_1 are capable of a synaptolytic effect and thus showed that such an effect is independent of the enzymatic systems which act as normal acceptors of the coenzyme derived from thiamine.

The present study is based upon the general postulate that a molecule able to affect the central nervous system must interfere at one point or another with the mechanism involved in the synthesis, storage, release and ultimate destruction of central chemical transmitters[5] or must interact directly with them at the level of the synaptic cleft. It is well known that as an action potential sweeps along an axon, the transfer of excitation to the next cell is mediated by the liberation of a chemical transmitter. The most widespread substances recognized as neuro-transmitters are acetylcholine, norepinephrine and serotonin which have been found in the "chemical" synapses of cholinergic, adrenergic and seratoninergic neurons.

In this paper, the study of the direct chemical interaction between thiamine and such chemical transmitters is presented.

MATERIAL AND METHODS

The chemicals used throughout this study have been purchased from FLUKA AG, Buchs SG, and their purity was checked chromatographically and spectroscopically before use.

The formation of molecular complexes of thiamine with the different compounds was monitored by the ultraviolet difference spectroscopy technique. Further evidence for

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complex formation was obtained with conductivity measurements carried out with a Radiometer Type CDM-2 conductivity meter and by thin layer chromatography with the solvent system *n*-butanol:methanol:benzene (2:1:1).

Ultraviolet spectra of solutions at final concentration 1×10^{-4} M were determined with a Cary 14 recording spectrophotometer. Difference spectra were obtained directly using the 0·0–0·2 slide wire and a tandem of two-compartment quartz cells was employed. The sample cuvette contained two separate solutions of thiamine and the substance tested, the reference cuvette contained the mixture in one compartment and water in the other. In such a way it was easy to record the hypochromic effects generally observed as positive peaks of absorbance changes. The ability of the neurotransmitters to act as electron donors was tested by reacting them with chloranil.[6] The charge-transfer band appearance was measured at 530 mμ by using 60% tetrahydrofurane as a solvent according the conditions given by Nogrady and Algieri.[6]

More experimental details are given in caption to figures.

RESULTS

When aqueous solutions of thiamine at a molar concentration not lower than 0·01 M are mixed together with equimolar solutions of either acetylcholine, choline or succinylcholine, the mixtures exhibit non additive conductivity (Table 1) and a marked

TABLE 1. CONDUCTIVITY MEASUREMENTS AND CHROMATOGRAPHIC BEHAVIOUR OF THE THIAMINE-NEUROTRANSMITTERS MIXTURES

	Conductivity milli Siemens	Chromatographic behaviour	
		Fluorescence	R_f
Thiamine	9·8	—	0·36
Acetylcholine	10·7	—	0·15
Serotonin	5·6	+ +	0·60
Norepinephrine	6·2	+ +	0·20
Thiamine–acetylcholine	13·0	+	0·18
Thiamine–serotonin	7·3	+ +	0·23
Thiamine–norepinephrine	7·5	+	0·05

The mixtures are prepared with 0·025 M solutions (1:1) in 0·1 M Tris-HCl, pH 7·4. The chromatography is carried out on silicagel (Eastman sheets 20 × 20, type K 301 R2) with *n*-butanolm–ethanol–benzene (2:1:1).

hypochromic effect with a slight blue shift is observed on the absorption peaks of thiamine at 235 and 265 mμ. Such a spectral change is evidentiated by differential spectro-photometric analysis, as shown in Fig. 1 and the ΔE_{235} or the ΔE_{265} can be taken as an index of complex formation. The molar ratio thiamine–choline derivative influences the amount of complex formed (Fig. 2) and the stability of the complex shows a bell-shaped pH dependency with an optimum around pH 7 (Fig. 3). The spectral difference is temperature dependent showing a 1·5 times increase with a 10° increase of temperature.

It must be noted that the difference spectra technique is based on the balancing out of most of the absorber (e.g. thiamine) extinction, thus leaving the absorption of the complex as a major component of what is recorded by the instrument. The exact balance is not achieved however, since the absorber concentration used as a reference should be equal to the free absorber concentration in the sample cell rather than the

total concentration there. This does not allow a calculation of affinity constants and only a comparison of the effects induced by equimolecular amounts of choline derivatives is possible (Fig. 1).

Figure 4 shows the difference spectra obtained by mixing acetylcholine with nicotinamide and thiamine with nicotinamide. Nicotinamide induces exactly the same spectral change as observed with acetylcholine on thiamine absorption spectrum and, on the other hand, acetylcholine induces a hypochromic–hypsochromic shift on the nicotinamide chromophore, which has the absorption maximum at 262 mμ. Figure 5 shows the difference spectra obtained after complexing thiamine with serotonin and norepinephrine. In this case thiamine induces a hypochromic–hypsochromic effect on the absorption peak of serotonin at 278 mμ and norepinephrine, at 280 mμ.

When thiamine is chromatographed (Table 1) in the presence of choline derivatives a slight reduction of thiamine mobility can be observed and the spots corresponding to the thiamine–choline derivatives complexes are fluorescent when the chromatoplate is viewed under u.v. light. The quantum yield of fluorescence of the complexes is similar to the one obtained after oxidation of thiamine to thiochrome.

With chloranil, serotonin, norepinephrine and acetylcholine gave a broad charge-transfer band in the 510–530 mμ region not shown by either compound alone. The

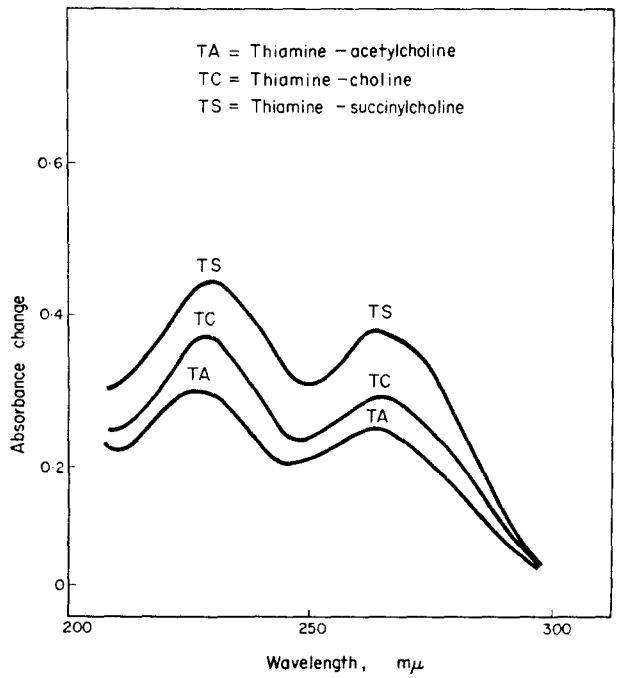


FIG. 1. Difference spectra recorded with a Cary 14 spectrophotometer. The separate solutions of thiamine and choline derivative were in the upper chamber, the mixtures of thiamine–choline derivative (1:1) were in the lower chamber. The mixtures were prepared by mixing equal volume of 0·1 M solutions in 0·1 M Tris-HCl buffer, pH 7·3, ionic strength 0·1, 24°. Prior to reading the solutions were diluted 1:1000 with b.d. water.

chloranil-neurotransmitter stability constants were calculated by means of the Benesi–Hildebrand equation[7] and values of 172, 225 and 233 l. mole. 10^{-6} were found for norepinephrine, acetylcholine and serotonin respectively.

DISCUSSION

The results presented in this paper provide clear evidence that thiamine is capable of a direct interaction *in vitro* with central chemical transmitters to yield molecular complexes which, very likely, are originated also *in vivo* when thiamine is administered in large doses. The binding of the neurotransmitter released from the synaptic vesicles may be thought to prevent the interaction of the free transmitter with its specific receptor and the change in permeability of the postsynaptic membrane responsible for the nervous transmission.

The interaction shown by these experiments provide a baseline for pharmacological experiments on thiamine–choline derivatives interaction carried out on animals.[8] A striking parallelism has been obtained with these two different approaches. In fact phenomena such as the succinyl–choline antagonism *in vivo* can be correlated with the higher affinity of succinylcholine for thiamine observed *in vitro*.

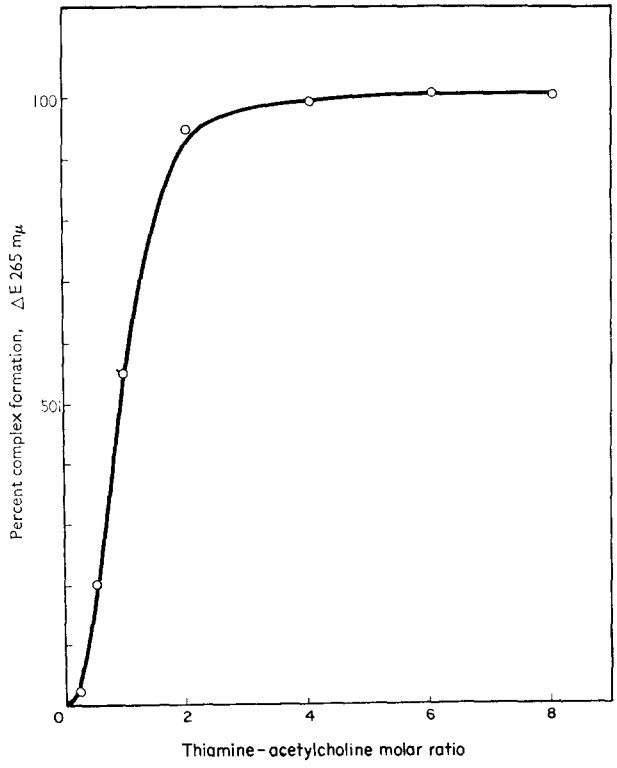


FIG. 2. Effect of molar ratio on the formation of thiamine–acetylcholine complex. The concentration of the absorber was held constant and different amounts of acetylcholine were added with the same conditions given in Fig. 1.

Besides, the abolition of the nicotinic but not of the muscarinic effect of acetylcholine demonstrated on the experimental animal[8] can be paralleled to the circumstantial evidence presented here, which indicates that thiamine binds acetylcholine in its nicotinic or "gauche" configuration.

For interpreting the difference spectra presented here, one must bear in mind their relation to parent spectra which is approximately that of a first derivative. Both the polarity and the polarizability of the chromophores environment have to be considered and the nature of the interactions observed thus depend on the kind of electronic transition, polarity of the solvent, dipole moments in the ground state and excited state and polarizability of the solvent.[9] The thiazolium ring is known for its moderate electron acceptor properties, which have been invoked already to explain its interaction with local anesthetics (e.g. procaine) to form donor–acceptor complexes.[10] If one considers the neurotransmitters ability of donating electrons measured by the charge-transfer obtained with chloranil, a donor–acceptor type interaction neurotransmitter-thiamine should be possible. The pyrimidine moiety, on the other hand, might participate with its inductive and steric effects, the whole molecule giving rise to a donor–acceptor complex. The formation of molecular complexes between thiamine and indole derivatives has been in fact already demonstrated with proton magnetic

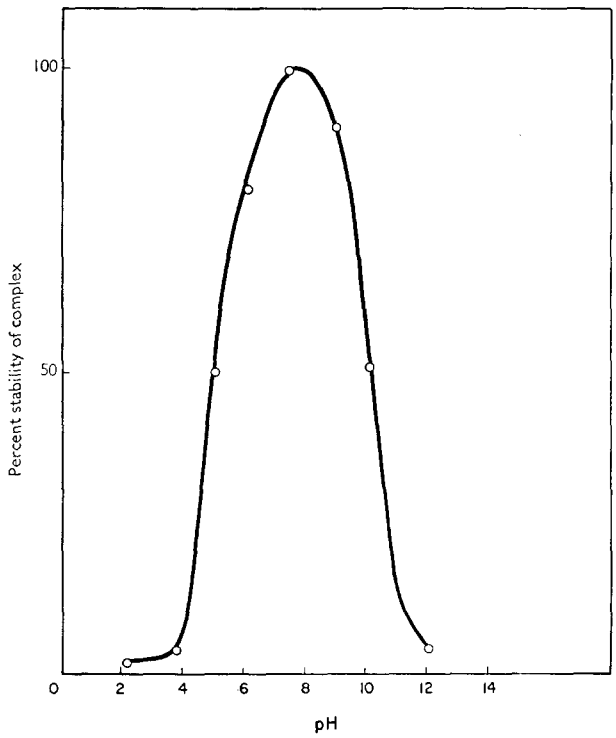


FIG. 3. Stability of the thiamine–acetylcholine complex (1:1) brought to different pH values with standard acid or alkali after its formation at pH 7·3. The ionic strength was held at a constant value of 0·1 with KCl. The final dilution prior to reading was done with b.d. water.

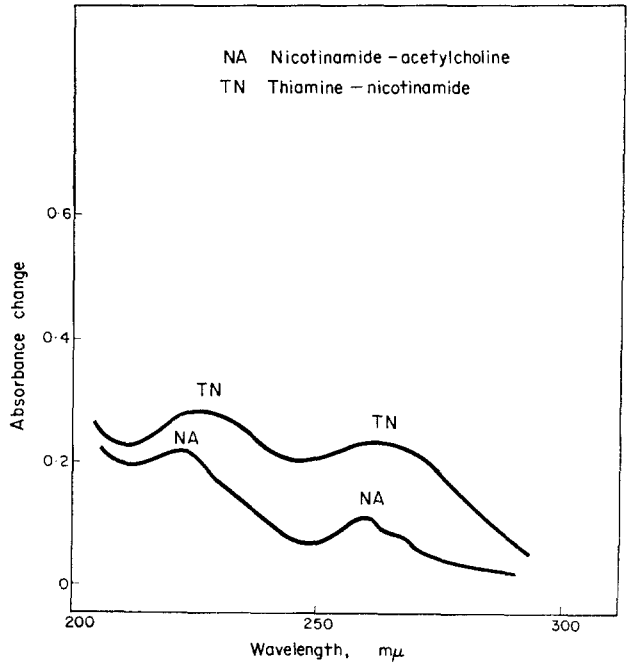
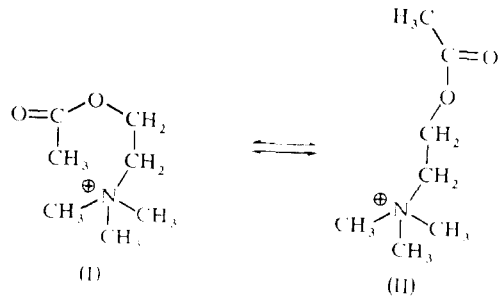


FIG. 4. Difference spectra of acetylcholine–nicotinamide (1:1) and thiamine–nicotinamide (1:1). The mixtures were prepared under the conditions described for the thiamine–choline derivatives complexes. The upper chamber of spectrophotometer contained the separated solutions, the lower chamber contained the mixtures.

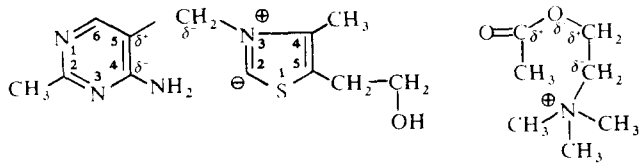
resonance studies[11] suggesting a primary role of the pyrimidine moiety in the formation of the complexes.

The interaction of choline derivatives which induces a spectral shift on thiamine absorption identical to the one obtained with nicotinamide is understandable if the structural analogy between nicotinamide and the *cis* configuration of acetylcholine is considered. In fact an equilibrium exists in solution between a configuration *cis*(I) and *trans*(II) of the acetylcholine molecule:



The *cis* form has been associated with nicotinic activity and the *trans* form with the muscarinic activity.[12]

There is a good complementarity between the atoms in position 2, 3 of the thiazole ring, the methylene group and the atoms 4, 5 of the pyrimidine ring with the atoms of the acetylcholine molecule in its nicotinic form.



This complementarity is more evident if one considers the polarizability of thiamine and its ability to react as a carbanion.[13] An examination of the calculated electronic charges approximated by a linear, combination of the atomic orbitals–molecular orbitals theory for thiamine and acetylcholine[14] suggests regions of potential charge complementarity within these molecules which are consistent with an overlap between

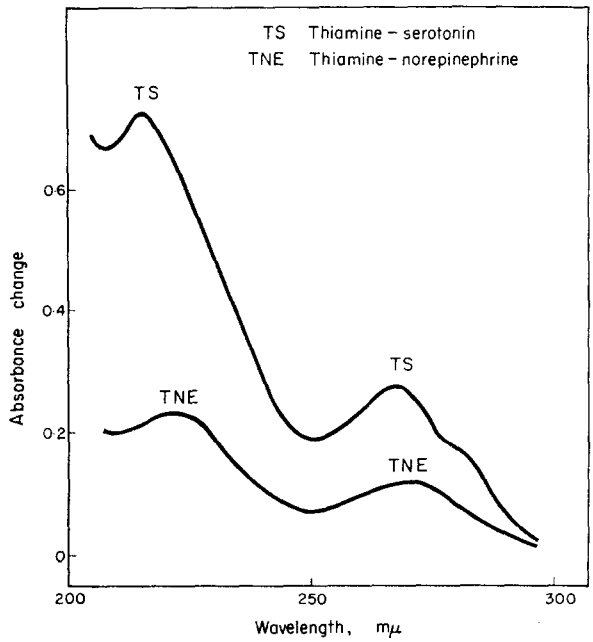


FIG. 5. Difference spectra of thiamine–serotonin (1:1) and thiamine–norepinephrine (1:1). The conditions of mixtures preparation was the same as for the experiments of Figs. 1 and 4, e.g. pH 7·3, ionic strength 0·1, molarity 0·1, temperature 24°, final dilution 1:1000 with b.d. water.

thiamine and the nicotinic form of acetylcholine because they are nearly all oppositely polarized and sizeable. The coplanarity of the overlapping regions is well documented by the close fit observed with molecular models. The thiochrome-like fluorescence of the thiamine–choline derivatives complexes compared with the non fluorescence of the thiamine molecule supports this model.

The thiamine–norepinephrine interaction can also be explained according to the proposed model, by considering the O—C—C—N^+ system in the norepinephrine molecule which has been shown to occur in the "gauche" configuration[15] as in the majority of choline derivatives.

The main effect observed in this study, e.g. the small shift of the electronic absorption band of thiamine toward shorter wavelength and the marked hypochromic effect can be associated to an n-π^* blue-shift phenomenon, which can be hydrogen bond dependent or not according to the type of molecule bound by thiamine. The phenomenon studied here, namely the interaction thiamine–acetylcholine, shows some characteristics which are very close to the ones observed spectroscopically after changing the dielectric constant of the solvent of unsaturated molecules.[16] Further physicochemical studies of this aspect of the problem are in progress in our laboratory.

An electrostatic model based on dipole–dipole interaction seems the most suitable for explaining the formation of molecular complexes between thiamine and chemical transmitters, since hypochromic effects and band broadening can be often justified by this type of interaction.[17]

The interaction thiamine-chemical transmitter should be increased in the quasi-hydrophobic medium of the neuron system and has a precise physiological meaning which can explain the general synaptolytic effect of thiamine. The order of magnitude and the similarity of the electron donor properties of the three neurotransmitters tested is consistent with such a general effect. In fact large doses of thiamine can saturate the neuron system and modify the dielectric constant characteristics of the synaptic cleft.[18]

In this way the large array of chemical transmitter molecules necessary to produce the electric field through the synapse upon which depends the chemical transmission[1] is substituted by a large array of molecular complexes. The stacking of thiamine and neurotransmitter molecules in fact creates a new electric field that is no more anchorable to the postsynaptic membranes and thus does not allow the ionic driving over the junction barrier which is responsible for the chemical synaptic transmission.

CONCLUSION

The synaptolytic effect of thiamine is related to its ability of forming molecular complexes with acetylcholine, norepinephrine and serotonin. Such complexes hinder the interaction of the free chemical transmitter with the postsynaptic membrane, thus blocking the nervous transmission. Acetylcholine interacts with thiamine in its nicotinic, e.g. "gauche" configuration and also other choline derivatives are able of interaction, probably with a similar mechanism. The integrity of thiamine molecule which has been proved necessary for its synaptolytic action is understandable according to the present model where the interaction involves both the pyrimidine and the thiazolic moiety.

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