SPECTROSCOPIC STUDY OF THIAMINE–PHENYLCARBAMATE COMPLEXES POSSESSING LOCAL ANAESTHETIC EFFECT

Milan REMKO[a], Tibor LIPTAJ[c], Juliana VESELOVSKÁ[b] and Jozef ČIŽMÁRIK[b]

[a] *Faculty of Pharmacy, Comenius University, 832 32 Bratislava*
[b] *Chemical Institute, Comenius University, 832 32 Bratislava, and*
[c] *Central* NMR *Laboratory, Faculty of Chemical Technology, Slovak Institute of Technology, 880 37 Bratislava*

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UV and ^{1}H NMR spectroscopy were used to study the interaction of thiamine with 1(2-(4-methoxyphenylcarbamoyl)ethyl) piperidinium chloride, a local anaesthetic drug. UV spectroscopy did not bring a direct evidence of the existence of a local anaesthetic–thiamine complex. On the contrary, the upfield shifts of certain protons in the mixtures local anaesthetic with thiamine were interpreted as a result of a π-complex formation between the aromatic nucleus and the pyrimidine ring of thiamine.

Basic esters of phenylcarbamic acid belong to the group of compounds exhibiting a marked local anaesthetic[1,2] and antiarhytmic activity[3]. These compounds might be characterized using a general scheme: aromatic part – polar group – connecting chain – amino group. The molecular mechanism of their reversible blocking of neural transmission is so far unknow. However, the non-covalent nature of the interaction of local anaesthetics with the biologic receptors is well established.

In our previous papers, we have studied the stereochemistry and the electronic distribution of several basic esters of phenylcarbamic acid and its *o*-, *m*-, and *p*-methoxy derivatives[4–6] by means of the quantum chemical methods. We have shown[7,8] on the basis of these calculations that the polar groups of these anaesthetics can form hydrogen-bonded complexes of varied stability both with phospholipids and lipoprotein polar groups of the nerve cell. The *ab initio* calculated electrostatic potentials[9] for the aromatic parts of the local anaesthetic belonging to the carbamate, amide, and carboxyl types in the planes parallel to the benzene ring showed that the potential maps are characterized by large regions of negative potentials resulting from the superposition of the aromatic π-electrons and the substituent electrons. Therefore, these parts of the local anaesthetics might act as electron donors in the interaction with the suitable electron acceptors. The ability of 1(2-(2-alkoxyphenylcarbamoyloxy)ethyl)piperidinium chlorides to form such complexes with chloranil was confirmed by UV spectrophotometry[10].

The goal of this work was the study of the interaction of a local anaesthetic of the

carbamate type – 1(2-(4-methoxyphenylcarbamoyloxy)ethyl)piperidinium chloride – with thiamine in order to find out whether the aromatic part of the local anaesthetic of this type is able to form complexes with the complementary sites of the biologic receptor. These studies were done by UV spectrophotometry and by ^{1}H NMR spectroscopy.

EXPERIMENTAL

Thiamine dichloride (Merck, Darmstadt) was used for UV and NMR measurements. 1(2-(4-Methoxyphenylcarbamoyloxy)ethyl)piperidinium chloride was synthetized according to the described method[11]. UV spectra were recorded on a Specord UV/VIS spectrophotometer (Zeiss, Jena). ^{1}H NMR spectra were obtained at the observing frequency 99·6 MHz using a Jeol FX-100 spectrometer operating in the FT mode. Spectra were taken at 22°C. Chemical shifts were determined with 0·2 Hz accuracy. Appropriate amounts of local anaesthetic and thiamine were weighted and dissolved in distilled water. p(^{2}H) was adjusted to the value 7 by 4M-NaO^{2}H. The correction of pH with respect to p^{2}H was made according to ref.[12]. Deuterated sodium 3-trimethylsilyl[$^2H_4$]propionate (Merck, Darmstadt) was used as an internal standard. ^{1}H NMR signals were assigned on the basis of their characteristic chemical shifts and multiplet shapes of the individual structural groups. The assignment of thiamine methyls was taken from the ref.[13]. Chemical shifts of aromatic protons of the local anaesthetic, forming an AA'BB' system, were determined by comparison of simulated (ref.[14,15]) and experimental spectra.

RESULTS AND DISCUSSION

Contrary to the complexes of phenylcarbamate–chloranil type, where the complex formation is accompanied by a new absorption band in the UV spectra[10], the complex formation in the studied system phenylcarbamate–thiamine was not proved by UV spectrum analysis. (Neither new absorption band formation nor bathochromic shift of the absorption bands due to the interacting components were observed).

π-Complex formation in systems containing aromatic rings is accompanied by changes in chemical shifts of protons in the functional groups participating in these interactions[16]. Therefore, the study of this system was made by ^{1}H NMR spectroscopy.

Fig. 1 depicts the chemical shift changes of protons of local anaesthetic and thiamine in dependence on the mixture composition. As follows from the analysis of NMR spectra of various mixtures local anaesthetic–thiamine, chemical shifts of protons H_a and H_b of the local anaesthetic experienced marked dependence on the thaimine concentration; small changes only were observed for the methoxyl or CH_2 bonded to nitrogen protons. The chemical shift of CH_2 group attached to oxygen in the local anaesthetic also did not experienced substantial changes. Similarly, when studying the mixtures thiamine–local anaesthetic, large changes in chemical shifts of protons H_1 and H_2 and small ones in chemical shifts of both thiamine methyl groups were found. Marked upfield shifts of protons H_a, H_b, H_1, and H_2

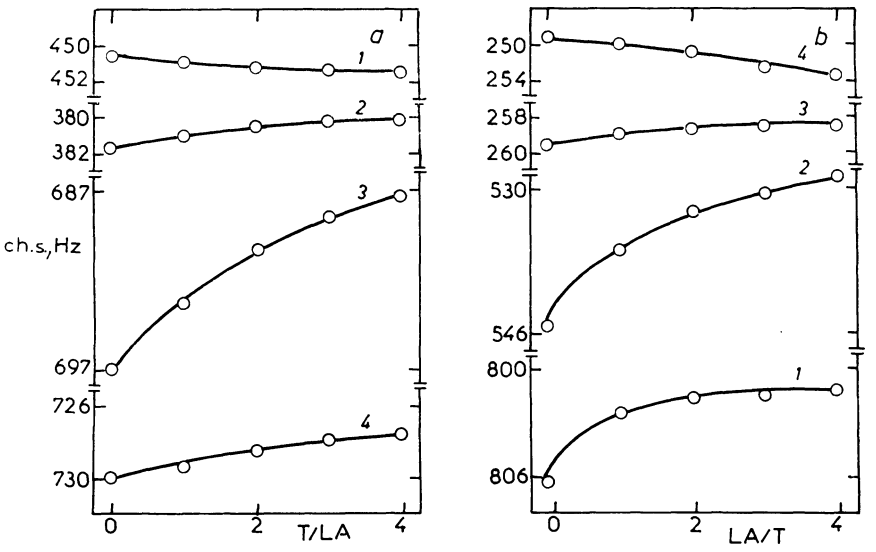

FIG. 1

Dependence of proton chemical shifts of 0·12 mol/kg solution of local anaesthetic (LA) 1(2-(4-methoxyphenylcarbamoyl)ethyl)piperidinium chloride and thiamine (T) on the mixture composition; *a* molar ratios thiamine–local anaesthetic, *1* H_a, *2* H_b, *3* H_c, *4* H_d *b* molar ratios local anaesthetic–thiamine. *1* H_1, *2* H_2, *3* H_3, *4* H_4. For the signal assignment, see Fig. 2

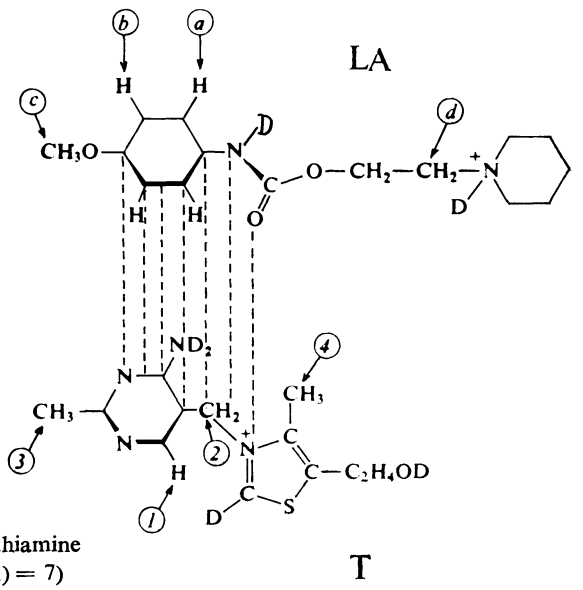

FIG. 2

Model of the local anaesthetic–thiamine complex (in deuterium oxide at $p(^2H) = 7$)

can be attributed only to a π-complex formation between the aromatic part of the local anaesthetic and the heterocyclic part of thiamine.

Fig. 2 shows an approximate shape of the local anaesthetic–thiamine complex. It was constructed both using the results of NMR measurements on the mixtures of local anaesthetic with thiamine and with the help of Dreiding models. According to this model, the protons H_1, H_2, H_a, and H_b are situated in the space where the direction of the induced magnetic field of the aromatic part is opposite to that of the applied magnetic field.

Our results indicate that aromatic parts of local anaesthetic can form complexes with the planar sites of receptors. This event might be a part – or a key – to the complementary nature of the drug–receptor interaction. It is probably the first step in the mechanism of action of a drug that is necessary to place the polar groups at right places. These groups then disrupt the hydrogen-bonding network of the membrane what eventually leads to the disconnection of the conducting system of the nerve cell.

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