ROLE OF THE CARBONYL GROUP OF LOCAL ANESTHETICS IN THEIR ANESTHETIC EFFECT

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Intermolecular interaction between anesthetics and chloroacetic acid was demonstrated by absorption spectroscopy; the degree of this interaction correlates with the anesthetic activity of the substances. Interaction between local anesthetics and monocarboxylic acids was shown to take place with the formation of complexes with a 1:1 composition. Determination of the stability constants and enthalpies ($-\Delta H$), the values of which vary between 1.61 and 9.51 kcal/mole, leads to the conclusion that hydrogen bonding participates in the formation of complexes of local anesthetics with carboxylic acids. The study of the infrared spectra of the complexes in chloroform and in matrices with potassium bromide showed that intermolecular interaction between local anesthetics and the acids takes place on account of the carbonyl groups. The data on intermolecular interaction in model systems of anesthetics with electron-acceptor components are regarded as confirmation of the earlier hypothesis that the carbonyl group plays a role in the anesthetic effect and they confirm the importance of the hydrogen bond formed during interaction between the local anesthetic and the active areas of the nerve fiber membrane.

Local anesthetics interfere with the conduction of the impulse along a nerve by depressing sodium permeability and stabilizing the nerve fiber membrane [10, 13]. An important role in the genesis of this effect is ascribed to physicochemical interaction between the local anesthetics and active areas of the nerve fiber membrane [8, 17]. These active areas (receptors) may be carboxyl (serine phosphatides) and phosphate groups of phospholipids, which are regarded as the chief substrate for the action of local anesthetics in the nerve fiber membrane [6, 11, 12].

TABLE 1. Optical Densities and Their Deviations at the Maximum of the Absorption Band of Solutions of Local Anesthetics in the Presence of Chloroacetic Acid

Local anesthetic	Anesthetic activity (EC)	Optical dens. of mix. of anesthetics with chloroacetic acid (D)		
		additive	found experimentally	ΔD
Cinchocaine	0,013	0,425	0,332	0,093
Amethocaine	0,025	0,440	0,525	0,085
Xylocaine	0,10	0,525	0,470	0,055
Benzocaine	0,31	0,525	0,475	0,050
Procaine	0,56	0,590	0,550	0,040
Anesthesin	0,625	0,495	0,512	0,017

It was shown previously [4] that the anesthetic activity of compounds correlates closely with the intensity of intermolecular interaction between the anesthetics and carboxyl and phosphate groups, and the more marked the electron-donor properties of the anesthetic molecules the greater the degree of this interaction. According to Löfgren [15], in anesthetics belonging to the categories of esters and amides of aromatic acids, because of the presence of a polarized carbonyl group in which the oxygen atom is an electron donor, dipole-dipole interaction with the receptors of the membrane can occur. Galinsky et al. [14] found a high degree of correlation in a series of para-substituted derivatives of the diethylaminoethyl ester of benzoic acid between the anesthetic activity of the compounds and the frequency of absorption of the carbonyl group in the infrared spectrum.

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TABLE 2. Thermodynamic Characteristics of Complexes of Local Anesthetics with Some Monocarboxylic Acids (M ± m)

Local anesthetic	Monochloroacetic acid		Formic acid		Acetic acid	
	−ΔH	−ΔS	−ΔH	−ΔS	−ΔH	−ΔS
Cinchocaine	9,5±0,2	24,8±0,4	7,30±0,3	18,4±0,5	6,30±0,1	16,0±0,2
Amethocaine	8,4±0,2	21,2±0,3	6,20±0,2	15,2±0,3	5,70±0,2	14,20±0,3
Xylocaine	7,0±0,1	17,2±0,2	6,0±0,1	15,0±0,2	4,9±0,1	11,8±0,2
Benzocaine	5,7±0,3	13,6±0,1	4,8±0,5	11,7±0,8	3,5±0,6	7,9±1,0
Procaine	4,9±0,1	11,9±0,1	4,0±0,1	9,4±0,1	2,5±0,1	5,8±0,1
Anesthesin	3,9±0,1	9,1±0,2	3,0±0,1	7,4±0,1	1,6±0,2	3,5±0,4

Note. −ΔH) Values of enthalpy (in kcal/mole; −ΔS) entropy values (in entropy units).

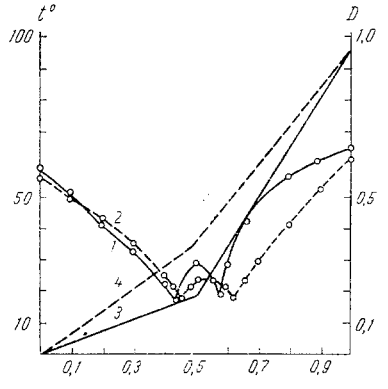

Fig. 1. Fusibility and optical density curves of cinchocaine and procaine. Fusibility of the systems of cinchocaine (1) and procaine (2) with chloroacetic acid; optical density of complexes of cinchocaine (3) and procaine (4) with chloroacetic acid. Abscissa, ratio between components (anesthetic−acid); ordinate, left: temperature in °C; right: optical density.

However, the possibility that the carbonyl group participates in the formation of a complex between local anesthetics and electron-acceptor components and the character of the bond thus formed has not been investigated experimentally. An attempt was made to repair this omission.

EXPERIMENTAL METHOD

Interaction in an aqueous medium between the local anesthetics cinchocaine, amethocaine, xylocaine, benzocaine, procaine and anesthesin (as bases) with chloroacetic acid was investigated by absorption spectroscopy in the UV part of the spectrum. Details of the method have been described previously [4]. The concentration of the acid was 2.5×10^{-2} M and of the anesthetics 1.75×10^{-4} M. The composition of the complexes formed were studied by a visual polythermic method [1] in the solid phase and by the isomolar series method [2] in solution. The composition of complexes of anesthesis, procaine, xylocaine, and cinchocaine with chloroacetic acid were investigated by the visual-polythermic method; amethocaine and benzocaine were investigated with trans-cinnamic acid, for these two local anesthetics give thermically unstable systems with the acids mentioned above. To determine the composition of the complexes by the isomolar series method, equimolar solutions of chloroacetic acid and of the local anesthetic were mixed in different proportions by volume; the optical density of the systems was measured at wavelengths corresponding to the maximum of absorption.

To determine the thermodynamic characteristics of the complexes, the constants of stability (K) of the complexes between anesthetics and a series of aliphatic acids [16] were determined at temperatures of 10, 20, 30, and 40°C and the values of the enthalphy (ΔH) and entropy (ΔS) were calculated from the graph of RlnK as a function of 1/T.

The character of the bond in the complexes was also studied by infrared spectrography. The infrared spectra were recorded on an IR-20 instrument with NaCl (for the region 1500-1600 cm^{-1}) and LiF (in the region 1600-3600 cm^{-1}) prisms. Chloroform was used as the solvent. Spectra of insoluble complexes were obtained in matrices with potassium bromide [3].

The anesthetic activity was studied in experiments on guinea pigs by the method of Bülbring and Wajda [9]. It was characterized by the EC_{50} value, determined graphically.

EXPERIMENTAL RESULTS AND DISCUSSION

Absorption curves in systems of chloroacetic acid−local anesthetic were found not to coincide with the additive curves calculated on the basis of Beer's law (Table 1). This is evidence of intermolecular interaction between the chloracetic acid and the local anesthetic [7]. It is significant that the anesthetic activity increases in the same order as the intensity of intermolecular interaction (ΔD).

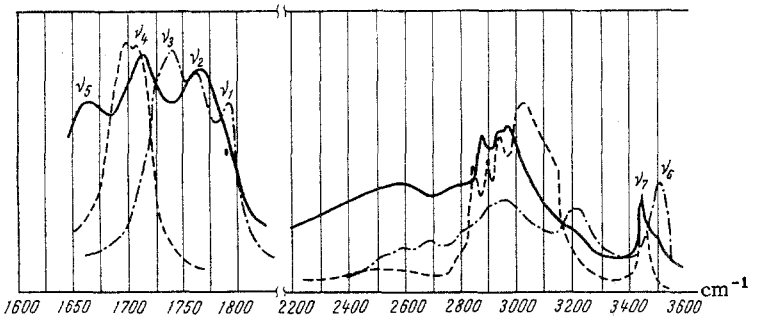

Fig. 2. Spectra of amethocaine, trichloroacetic acid, and a mixture of the two: 1) amethocaine; 2) trichloroacetic acid; 3) mixture of amethocaine and trichloroacetic acid.

Fusibility curves of systems of procaine and cinchocaine with chloroacetic acid are shown in Fig. 1. It is clear that the fusibility curves consist of three branches of crystallization: two branches of pure components and a branch of crystallization of an incongruently fused complex of composition 1 : 1. Curves of the optical density of the same systems in petroleum ether as the solvent are also shown in Fig. 1. These curves also have an "outstanding point" for a ratio of 1 : 1 between the components, indicating that the complex formed in this case preserves its composition in dilute solutions.

Similar results were obtained for the other systems investigated. The results of these experiments confirm the earlier conclusion that local anesthetics are electron donors which form a 1 : 1 complex with monocarboxylic acids.

Enthalpy and enthropy values of complexes of local anesthetics with acids obtained in petroleum ether having an inert solvent are given in Table 2. They show that the enthalpy values ($-\Delta H$) of the complexes formed lie within limits corresponding to energies of the hydrogen bond [5]. The stability of the complexes decreases in a series of electron-donor components in the following order: cinchocaine > amethocaine > xylocaine > benzocaine > procaine > anesthesin (Table 2), which is exactly the same order as the electron-donor activity of these compounds determined from depression of optical density in aqueous solutions, and it correlates with their anesthetic activity (Table 1).

Both oxygen and nitrogen can act theoretically as electron-donor atoms in the local anesthetics studied. Investigation of the infrared spectra of the complexes enables an unequivocal answer to be given to this question.

As Fig. 2 shows, significant differences compared with the spectra of chloroform solutions of each of the components are observed in the spectra of solutions of triple systems (amethocaine–trichloroacetic acid–chloroform) in the region of valency oscillations of the C = O and O–H (N–H) groups. For instance, the band of $\nu_{C=O}$-molecules of amethocaine (ν = 1696-1703 cm^{-1}) in the triple systems is shifted into the region of lower frequencies (ν = 1665 cm^{-1}). The band of $\nu_{C=O}$-bound oscillations of trichloroacetic acid is also slightly displaced toward the low-frequency side.

In the region of $\nu_{C=O}$-unbound oscillations of the acid (ν_1 = 1790 cm^{-1}; ν_2 = 1762 cm^{-1}) only certain changes in the shape of the absorption band are observed, evidently because of a change in the polarity of the medium on the addition of amethocaine to the solution.

Clearer changes in the spectrum of triple systems were observed in the region of the $\nu_{OH(NH)}$-valency oscillations. It is clear that on the addition of amethocaine to a solution of trichloroacetic acid the decrease in intensity of the ν_{OH} band of the monomer molecules (ν_6 = 3515 cm^{-1}) is accompanied by displacement of the center of gravity of the combined band of the associated molecules of acid from ν_{max} 2950 to ν_{max} 2600 cm^{-1}, whereas the intensity and position of the band of the monomer amethocaine molecules (ν = 3440 cm^{-1}) are virtually unchanged.

Similar changes in the spectra were obtained in the experiments with anesthesin, xylocaine, cinchocaine, and procaine. All these changes unequivocally show that a $Cl_3C_2O_2H \cdot$ amethocain complex is formed, and that the hydroxyl group of the acid and carbonyl group of the anesthetic participate in the mechanism of association.

The results of these investigations of intermolecular interaction in model systems of local anesthetics with electron-acceptor components confirm the previously postulated role of the carbonyl group in the anesthetic effect and they also indicate the importance of the hydrogen bond formed during interaction of the local anesthetic with the active areas of the nerve fiber membrane.

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