^1H and ^{13}C nuclear magnetic resonance studies of interaction of 5′-AMP with phenylcarbamate possessing local anaesthetic effect

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Dedicated to Professor DrPH. PhMr. J. Heger, in honour of his 60th birthday

^1H and ^{13}C NMR spectroscopy has been used to study the interaction of 5′-AMP with 1-[2-(2-methoxyphenylcarbamoyloxy)ethyl]piperidinium chloride, a local anaesthetic drug. In 0.1 molal solution ($p^2H = 7.25$) the interaction between 5′-AMP and 1-[2-(2-methoxyphenylcarbamoyloxy)ethyl]piperidinium chloride was demonstrated by the chemical shifts changes in the interacting parts of the two substrates. These changes were interpreted in terms of a molecular complex formation between the aromatic part of local anaesthetic and the 5′-AMP.

Для изучения взаимодействия 5′-АМФ с хлоридом 1-[2-(2-метоксифенилкарбамоилокси)этил]пиперидиния, местным анестетиком, были использованы методы ^1H и ^{13}C ЯМР спектроскопии. В 0,1 моляльном растворе ($p^2H = 7{,}25$) взаимодействие между 5′-АМФ и хлоридом 1-[2-(2-метоксифенилкарбамоилокси)этил]пиперидиния было продемонстрировано на изменении величин химических сдвигов во взаимодействующих частях обоих субстратов. Эти изменения интерпретируются в рамках образования молекулярного комплекса между ароматической частью местного анестетика и 5′-АМФ.

Pharmacological tests [1—6] proved that basic esters of the phenylcarbamic acid exhibit a marked local anaesthetic and antiarythmic activity. These compounds, like other basic-type local anaesthetics, can be characterized by the general scheme: lipophile (aromatic) part—connecting chain—hydrophilic (amino) group.

Despite of the intense research, the molecular mechanism of their reversible blocking of nerve conduction is still not clearly understood. However, a non-

covalent nature of the interaction of the local anaesthetic with the biological receptor is well known. According to *Büchi's* bonding model of procaine [7] the aromatic part of the local anaesthetic is bound to the receptor by means of the electron donor-acceptor forces. *Eckert* and *Mőbus* assumed [8] that the local anaesthetic can form molecular complex with the ATP and in this way influence the normal function of nerve cell. This is because ATP plays an important role at active transport of compounds across the cell membrane. *Agin* [9] described the charge-transfer complexes formed between procaine and ribonucleic acid. *Mlynarčík*, *Denyer*, and *Hugo* [10] have shown that the local anaesthetic phenylcarbamate influences metabolic functions in *Staphylococcus aureus*: *Staphylococcus aureus* cells in the presence of local anaesthetic lose the ability to synthesize ATP. An evidence of the electron donor-acceptor complex formation of the local anaesthetics with diverse acceptors has been demonstrated in [11—13]. Theoretical [14] and experimental [15, 16] investigations of local anaesthetic phenylcarbamates have shown that also those compounds and suitable electron acceptors form electron donor-acceptor complexes.

The present work is a continuation of our spectroscopic studies on molecular complexes of the local anaesthetic phenylcarbamates. Its purpose is a high resolution NMR study of the mixture of 1-[2-(2-methoxyphenylcarbamoyloxy)-ethyl]piperidinium chloride and the 5′-AMP in order to find out whether the aromatic part of the local anaesthetic of this type is able to form the molecular complex with 5′-AMP.

Experimental

Disodium salt of the adenosine-5′-monophosphoric acid (Fluka, A.G.) was used without further purification and drying. 1-[2-(2-methoxyphenylcarbamoyloxy)-ethyl]piperidinium chloride (LA) was synthesized according to the described method [1]. ^{1}H and ^{13}C NMR spectra were recorded on the Bruker AM 300 spectrometer. Position of the spectral lines in the ^{1}H NMR spectra was determined with accuracy $\pm$ 0.2 Hz. ^{13}C chemical shifts were determined with $\pm$ 0.02 ppm accuracy. Spectra were taken at 295 K. Corresponding amounts of local anaesthetic and 5′-AMP were weighed and dissolved in $^{2}H_2O$. 1 M-$NaO^{2}H$ and 1 M-^{2}HCl were used for $p^{2}H$ adjustment to the value 7.25. The correction of pH with respect to $p^{2}H$ was made according to Ref. [17]. Deuterated sodium 3-trimethylsilyl[$^{2}H_4$]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 structures of the individual structural groups. The assignment of the 5′-AMP signals was taken from Ref. [18]. Chemical shifts of aromatic protons of the local anaesthetic, forming an ABCD system, were determined by comparison of the simulated [19, 20] (LAOCOON III) and the experimental spectra.

Results

^{1}H NMR spectra of 5′-AMP—local anaesthetic

^{1}H NMR spectra of 0.1 molal solution of 5′-AMP, LA, and their mixture (volume ratio = 1 : 1) (Fig. 1) were measured at 300 MHz. Since the samples were prepared in ^2H_2O, protons of the hydroxyl groups on the ribose and the amino groups of 5′-AMP and LA exchanged rapidly with deuterium and we did not observe these resonances. Instead, an HO^{2}H peak was always present in the spectra.

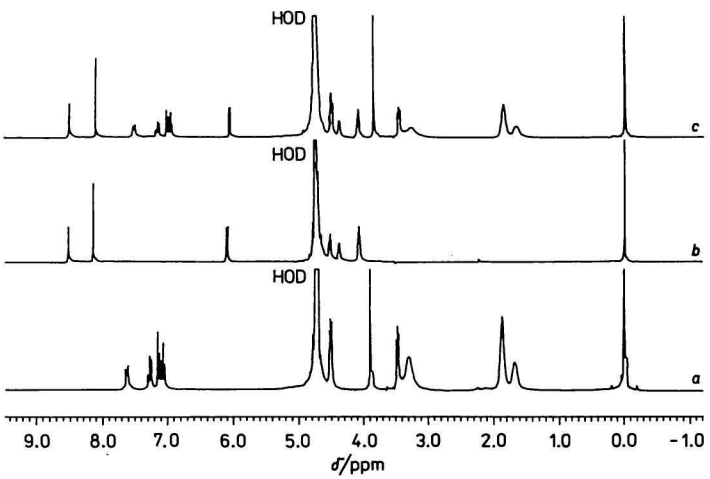


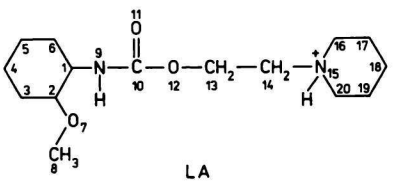
Fig. 1. ^{1}H NMR spectrum of solution with $m_i = 0.1\ \mathrm{mol\,kg^{-1}}$ (i = 1-[2-(2-methoxyphenylcarbamoyloxy)ethyl]piperidinium chloride (*a*), 5′-AMP (*b*)) and mixture of their equal amount of substances (*c*); p^{2}H = 7.25.

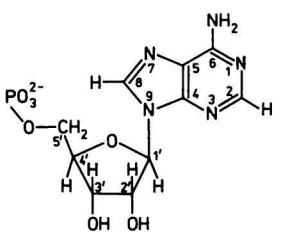
At p^{2}H = 7.25 the 5′-AMP spectrum was well-defined (Fig. 1*b*). The resonance peaks due to the two nonexchangeable base protons appeared in high-frequency part of the spectrum, whereas the resonance peaks of nonexchangeable ribosyl protons appeared in its low-frequency part.

The spectrum of the 1-[2-(2-methoxyphenylcarbamoyloxy)ethyl]piperidinium chloride (LA) is composed of several multiplets having their resonancies at δ/ppm *ca.* 7.2, 4.5, 3.9, 3.5, and 1.8. Peak assignment was made with reference to our previous paper [15].

It is possible to do a comparative study on the spectra of the mixture of 5′-AMP and local anaesthetic because the resonance peaks, with the exception of the region of resonance of ribosyl protons and protons of the —CH_2—CH_2—

connecting chain of LA, did not overlap. In accord with a supposed composition of the complex, we have chosen for the study a mixture (volume ratio = 1 : 1) of 5′-AMP and LA. We have focused our attention to $—OCH_3$, $—CH_2N^+$ and aromatic ring protons of LA and H-2, H-8, and H-1′ protons of 5′-AMP (Scheme 1).





Scheme 1

Table 1 shows chemical shifts of resolved protons of the systems under study. Molecular interaction is in the case of anaesthetic accompanied by marked low-frequency changes of chemical shifts of the aromatic protons and a smaller change of the chemical shifts of the methoxy group protons. On the contrary, the chemical shifts of the $—CH_2N^+$ group protons of LA did not experience substantial changes. Smaller changes were observed in chemical shifts of the H-2 and H-1′ protons of the 5′-AMP. On the other hand, the chemical shift of the H-8 proton of adenine did not experience changes.

We have also investigated the influence of the excess of 5′-AMP on chemical shifts of LA and the influence of the excess of LA on chemical shifts of 5′-AMP. The results of these observations are shown in Table 1. However, the largest changes in chemical shifts of the protons studied were only observed in the mixture 5′-AMP—LA.

^{13}C NMR spectra of 5′-AMP—local anaesthetic

Since the ^1H NMR spectra indicated interactions between 5′-AMP and local

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Table 1

Proton chemical shifts δ and the changes of chemical shifts $\Delta\delta$ ($\Delta\delta = \delta_{i-1} - \delta_i$) of 1-[2-(2-methoxyphenylcarbamoyloxy)ethyl]piperidinium chloride (LA), 5′-AMP and their mixtures in $m = 0.1\ \mathrm{mol\,kg^{-1}}$ solution, $p^2H = 7.25$

i	n(LA)/n(5′-AMP)	$\delta_{H\text{-}6}$	$\Delta\delta_{H\text{-}6}$	$\delta_{H\text{-}5}$	$\Delta\delta_{H\text{-}5}$	$\delta_{H\text{-}4}$	$\Delta\delta_{H\text{-}4}$	$\delta_{H\text{-}3}$	$\Delta\delta_{H\text{-}3}$	δ_{OCH_3}	$\Delta\delta_{OCH_3}$	$\delta_{CH_2—N+}$	$\Delta\delta_{CH_2—N+}$	$\delta_{H\text{-}8}$	$\Delta\delta_{H\text{-}8}$	$\delta_{H\text{-}2}$	$\Delta\delta_{H\text{-}2}$	$\delta_{H\text{-}1'}$	$\Delta\delta_{H\text{-}1'}$
		ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
		Changes of chemical shifts of local anaesthetic																	
0	1:0	7.58	0.09	7.04	0.11	7.24	0.12	7.11	0.14	3.89	0.04	3.47	0.01	—		—		—	
1	1:1	7.49	0.04	6.93	0.05	7.12	0.05	6.97	0.05	3.85	0.01	3.46	0.00	8.52		8.08		6.06	
2	1:2	7.45	0.02	6.88	0.02	7.07	0.04	6.92	0.04	3.84	0.01	3.46	−0.01	8.51		8.03		6.06	
3	1:3	7.43	0.02	6.86	0.03	7.03	0.02	6.88	0.03	3.83	0.00	3.47	−0.02	8.50		8.00		6.05	
4	1:4	7.41		6.83		7.01		6.85		3.83		3.49		8.50		7.98		6.05	
		Changes of chemical shifts of 5′-AMP																	
0	0:1	—		—		—		—		—		—		8.51	−0.01	8.10	0.02	6.09	0.03
1	1:1	7.49		6.93		7.12		6.97		3.85		3.46		8.52	−0.02	8.08	0.01	6.06	0.01
2	2:1	7.53		6.96		7.14		6.99		3.86		3.45		8.53	−0.02	8.07	−0.02	6.05	−0.01
3	3:1	7.59		6.97		7.15		7.00		3.87		3.45		8.55	−0.02	8.09	−0.01	6.06	−0.01
4	4:1	7.63		6.99		7.16		7.01		3.88		3.45		8.57		8.10		6.07	

anaesthetic, we were interested to confirm this result using ^{13}C NMR spectroscopy.

The 75.47 MHz spectra of solution with $m(5'\text{-AMP}) = 0.1\,\text{mol kg}^{-1}$, $m(\text{LA}) = 0.1\,\text{mol kg}^{-1}$ and their mixture (volume ratio = 1 : 1) are seen in Fig. 2.

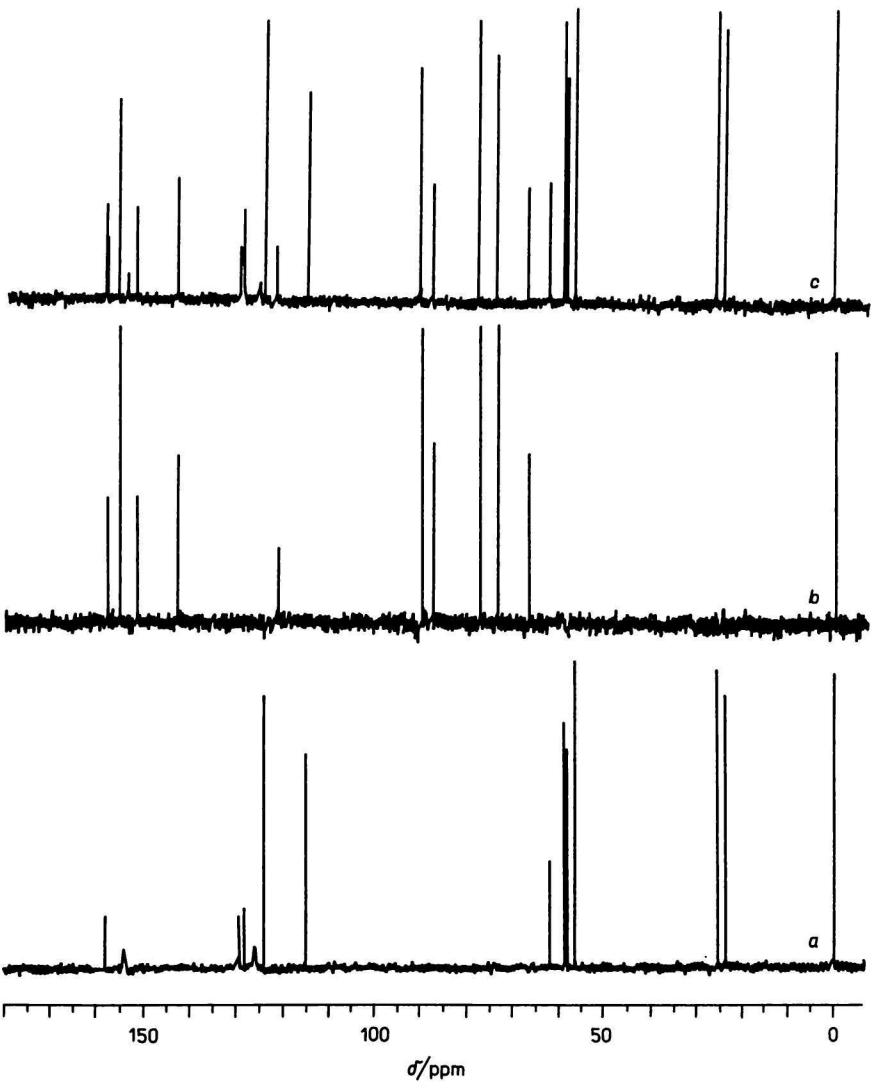


Fig. 2. ^{13}C NMR spectrum of solution with $m_i = 0.1\,\text{mol kg}^{-1}$ (i = 1-[2-(2-methoxyphenylcarbamoyloxy)ethyl]piperidinium chloride (*a*), 5′-AMP (*b*)) and mixture of their equal amount of substances (*c*); $p^2H = 7.25$.

Table 2

^{13}C Chemical shifts δ/ppm of 1-[2-(2-methoxyphenylcarbamoyloxy)ethyl]piperidinium chloride (LA), 5′-AMP, and n(LA) : n(5′-AMP) = 1 : 1 mixture in $m = 0.1\ \mathrm{mol\,kg^{-1}}$ solution, $p^2H = 7.25$

	Local anaesthetic (LA)												
	C-1	C-2	C-3	C-4	C-5	C-6	C-8	C-10	C-13	C-14	C-16 C-20	C-17 C-19	C-18
*δ/ppm	128.24	153.69	114.75	129.11	123.84	125.62	58.69	157.93	58.29	61.89	56.42	25.50	23.82
**δ'/ppm	128.14	153.18	114.38	128.65	123.64	124.70	58.61	157.65	58.19	61.88	56.37	25.46	23.83
$\Delta\delta$/ppm = $(\delta - \delta')$/ppm	0.10	0.51	0.37	0.46	0.20	0.92	0.08	0.28	0.10	0.01	0.05	0.04	−0.01
	5′-AMP												
	C-2	C-4	C-5	C-6	C-8	C-1′	C-2′	C-3′	C-4′	C-5′			
*δ/ppm	155.38	151.54	121.11	158.03	142.77	89.79	77.34	73.41	87.27[a]	66.54[a]			
**δ'/ppm	155.26	151.39	121.11	157.95	142.61	89.86	77.36	73.29	87.14	66.45			
$\Delta\delta$/ppm = $(\delta - \delta')$/ppm	0.12	0.15	0.00	0.08	0.16	−0.07	−0.02	0.12	0.13	0.09			

* Original chemical shifts.

** Chemical shifts of n(LA) : n(5′-AMP) = 1 : 1 mixture.

a) In the experimental spectrum peaks of these atoms are split into doublets due to coupling with the phosphorus nucleus.

Chemical shifts assignments of 5′-AMP were made according to Ref. [18, 21]. Because the ^{13}C NMR spectrum of 1-[2-(2-methoxyphenylcarbamoyloxy)ethyl]-piperidinium chloride was not described so far, the chemical shifts of this compound we assigned (Table 2) on the basis of a series of ^{13}C NMR spectra with the selective decoupling of protons. By known assignment of ^{1}H NMR spectra it was possible in this way to assign unambiguously all signals in ^{13}C NMR spectra.

The spectrum of the mixture of these compounds is well separated into discrete regions, thus allowing unambiguous spectral interpretation. As regards the 5′-AMP the largest changes of chemical shifts towards lower frequencies were observed with the carbon atoms C-8, C-4, C-2, C-6, C-3′, C-4′, and C-5′ of adenine and ribosyl parts, respectively. The C-1′ carbon exhibited marked high-frequency shift.

In the case of local anaesthetic the significant changes of the chemical shifts of aromatic carbons and the carbon of the carbamate group were observed. Since the reproducibility of the data was in the order $\pm$ 0.02 ppm the changes in chemical shifts of the remaining carbon atoms were insignificant.

Discussion

Significant changes in the ^{1}H and ^{13}C chemical shifts of 5′-AMP and 1-[2-(2--methoxyphenylcarbamoyloxy)ethyl]piperidinium chloride due to their interaction were observed in their mixture (volume ratio = 1 :1) ($m = 0.1\ \mathrm{mol\,kg^{-1}}$).

In the ^{1}H spectra of the local anaesthetic the largest changes of the chemical shifts towards lower frequencies were observed for the aromatic protons. Besides other factors, this is also the result of the ring current effect of the interacting systems with aromatic ring [22]. In the case of 5′-AMP changes of the proton chemical shifts due to the interaction with LA were, with respect to the accuracy of their determination ($\pm$ 0.2 Hz), insignificant. Observed shifts of the H-3, H-4, H-5, and H-6 protons (Scheme 1) of the local anaesthetic towards lower frequencies may be interpreted by the formation of molecular complex between the aromatic part of LA and 5′-AMP.

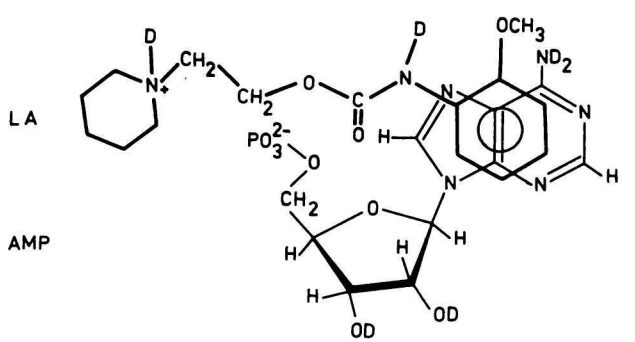
Further information about the type of interaction of these two compounds provided ^{13}C NMR spectra, since in all cases it was possible to determine the chemical shifts of all carbon atoms. More significant changes of the chemical shifts due to the mutual interaction were observed for the carbons of LA. From these the largest low-frequency shifts were found for *ortho* and *para* aromatic carbons. On the contrary, the chemical shifts of the piperidine ring carbons do not practically change. With the 5′-AMP the changes in chemical shifts of all carbon atoms, with the exception of C-5, were observed.

The found changes in ^{13}C NMR spectra of the mixture of the 5′-AMP—LA confirmed the results obtained from the ^{1}H NMR spectra. According to these studies the local anaesthetic interacts preferentially with 5′-AMP by means of its aromatic part.

Experimentally it was found [23] that nucleotides self-associate in aqueous solutions, where the stacking type of interaction is characteristic of bases. The results of our NMR study of the system 5′-AMP—LA indicate that similar interaction between the aromatic part of anaesthetic and adenine of 5′-AMP may take place. The observed proton and carbon induced chemical shifts may be interpreted as a result of the molecular association between the 5′-AMP and LA. The interaction between associated components may have hydrophobic and/or charge-transfer nature.

Since drugs interact with receptors by means of their electron clouds [24], some information about the nature of the interaction between the 5′-AMP and local anaesthetic may be obtained also by *ab initio* SCF calculations of the electron distribution in these compounds. *Clementi* and *Corongiu* [25] carried out *ab initio* calculations of the fragments of DNA. They found out that the overall electronic structure of those fragments is characterized by strongly negative region due to phosphates, electron deficiency region of the saccharide units, and negative charge region of the bases. Similarly, *ab initio* SCF calculations of the cation of the 1-[2-(2-methoxyphenylcarbamoyloxy)ethyl]piperidinium chloride showed [26] that while the hydrophobic aromatic group (including —NHCOO— and $—OCH_3$ groups, respectively) possesses large portion of the negative charge (about $-0.5e$), the hydrophilic piperidine part of LA carries considerable positive charge (about $+0.5e$).

If we consider that in the investigated complex 5′-AMP and LA mutually interact by means of their electron clouds on the basis of complementarity, we can construct the approximate shape of this complex. Scheme 2 shows a model



Scheme 2

of such molecular interaction. The complex was constructed using the results of NMR measurements of the mixtures of the local anaesthetic with 5′-AMP, the *ab initio* SCF data about the electron distribution of these compounds taken from Ref. [25, 26] and with the help of Dreiding models. According to the proposed model, it is assumed that the aromatic ring of anaesthetic interacts with the adenine part of 5′-AMP by means of stacking interaction. This interaction induced ring current effect which was observed in the spectra as a low-frequency changes of chemical shifts of the relevant protons. Observed significant changes of chemical shifts indicate that hydrophobic, charge-transfer, and electrostatic forces may eventually play an important role in complex formation.

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