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Partition of local anesthetic heptacaine homologs between phosphatidylcholine bilayers in unilamellar liposomes and aqueous phase: UV-VIS spectrophotometry study

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Tertiary amines [2-(alkyloxy)phenyl]-2-(1-piperidinyl)-ethyl esters of carbamic acid (CnA, n is the number of carbons in the alkyloxy chain) are local anesthetics [1, 2]. The partition coefficient K_p of C5A, C7A and C9A between egg yolk phosphatidylcholine (EYPC) bilayers in unilamellar liposomes and aqueous phase increases with n [3]. In the present paper, we study this dependence for $n = 3–9$ using more precise methods elaborated earlier [4, 5].

CnAs were prepared as described [1], EYPC was isolated, purified and analyzed [6]. The solvents were from Mikrochem (Bratislava, Slovakia) and the other chemicals from Lachema (Brno, Czech Republic). The solvents except of those of spectral purity were redistilled before use. The Silufol chromatographic plates were from Kavalier (Sázava, Czech Republic). EYPC liposomes were prepared by ultrasonication [3, 4]. The UV-VIS spectra were recorded using the 8452A spectrophotometer (Hewlett-Packard, Palo Alto, USA) at 20 °C. For the pH estimation, the Radelkis OP-930/1 pH-meter (Budapest, Hungary) and Metrohm EA 125 combined glass electrode (Switzerland) were used. The data were evaluated using standard statistical methods. The CnA (0.3 mmol/l) absorption $A(\lambda)$ in the region of $\lambda = 278–294$ nm was estimated in the presence of ultrasonicated unilamellar EYPC liposomes dispersed in the aqueous phase (0.1 mol/l NaCl, pH 4.5, 20 °C) at increasing EYPC concentration in the range $c_l = 0–3$ mmol/l. The effect of light scattering on liposomes on the CnA absorption was eliminated by a numerical subtraction of the turbidance A_T [4, 5].

The partition coefficient K_p defined by using the CnA molar concentrations in the lipid and aqueous phase was obtained by a two-parameter non-constrained non-linear computer fit of

$$\delta A(\lambda) = A(\lambda) : A(\lambda)_0$$
$$= 1 + v_{l,\,mol} c_l [\varepsilon_{al}(\lambda) : \varepsilon_{aw}(\lambda)] : (K_p^{-1} + v_{l,\,mol} c_l)$$

to experimental data, where $A(\lambda)_0$ is the absorption measured at $c_l = 0$, $\varepsilon_{al}(\lambda) : \varepsilon_{aw}(\lambda)$ is the ratio of CnA molar extinction coefficient located in the lipid phase to that in the aqueous phase, and $v_{l,\,mol}$ is the molar volume of EYPC in liposomes calculated by using the partial specific volume of EYPC [7] and the EYPC molecular weight obtained from the EYPC acyl chain composition [8]. The equation above was derived [4, 5] under the premiss that total volumes occupied by lipid and anesthetic molecules are very small compared to the volume of sample which is fulfilled in our experiments. The following values of K_p were obtained from the fit: 184 ± 38 (C3A), 502 ± 167 and 354 ± 92 (C4A), 1040 ± 365 and 921 ± 284 (C5A), 936 ± 126 and 974 ± 97 (C6A), 1234 ± 122 and 2025 ± 417 (C7A), 2576 ± 115 (C8A) and 4692 ± 229 (C9A). Simultaneously, the following $\varepsilon_{al}(\lambda):\varepsilon_{aw}(\lambda)$ ratios were obtained at $\lambda = 290$ nm: 1.94 ± 0.21 (C3A), 2.08 ± 0.25 (C4A), 3.18 ± 0.31 (C5A), 3.08 ± 0.32 (C6A), 3.40 ± 0.23 (C7A), 2.97 ± 0.36 (C8A) and 2.70 ± 0.18 (C9A). The change of Gibbs free energy due to one alkyloxy CH_2 group transfer from aqueous phase into EYPC bilayer calculated from the K_p data is -0.83 ± 0.17 RT (R – gas constant, T – absolute temperature) for C3A–C5A homologs. This is equal to -0.89 ± 0.02 RT calculated from the CnA partition coefficients between the n-octanol and the aqueous phase [9]. The value of -0.54 ± 0.03 RT calculated for C6A–C9A is lower because of electrostatic interactions of CnA cations (CnA $pK_a = 8.86 \pm 0.02$ in H_2O [9]) located in the aqueous phase with the bilayer surface charge which builds-up by CnA cation intercalation into the bilayer. This electrostatic effect is small for C3A–C5A because of substantially lower K_p values.

The CnA anesthetic potency progressively increases with increasing n up to a maximum at $n = 7$ beyond which a decrease (cut-off effect) is observed [2]. It is evident from the measured K_p values, that the partition equilibrium between the aqueous and lipid phases cannot be solely responsible for the cut-off effect. The dependence of $\varepsilon_{al}(\lambda) : \varepsilon_{aw}(\lambda)$ ratio on n indicates two different locations of short- and long-chain CnA molecules in the lipid bilayer. This is in agreement with results of spin label ESR [10], ^{31}P NMR [11], and neutron [12] and synchrotron X-ray [11, 13] diffraction studies of the CnA – lipid bilayer interaction. Different locations of short- and long-chain CnA molecules in the bilayer can be an important factor in their effects on membrane transport systems, in their transfer to their sites of action and in their interactions with these sites. All these effects can contribute to the above-mentioned quasi-parabolic dependence of the CnA potency on n.

Acknowledgements: This study was supported by the VEGA grant 1/7704/2000. The authors thank Prof. J. Čižmárik for providing local anesthetics.

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Received November 19, 2001
Accepted March 16, 2002

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