Fluorescence and Membrane-Action of Tetracaine

J. G. R. Elferink

Laboratory for Medical Chemistry, University of Leiden

(Z. Naturforsch. **32 c**, 135–136 [1977]; received November 29, 1976)

Tetracaine, Fluorescence, Erythrocyte Membrane

The fluorescence of tetracaine depends on the microenvironment of the molecule and increases with increasing hydrophobicity. The presence of erythrocyte membranes strongly enhances tetracaine fluorescence. The results support the view that the alkyl chain and aromatic part of tetracaine is embedded in apolar regions of the lipid or protein phase of the membrane.

In a study concerning the membrane action of anesthetics we observed that the fluorescence of the local anesthetic tetracaine is highly dependent on the polarity of its environment. In phosphate buffered saline (pH 7.4) tetracaine has an excitation maximum at 315 nm and an emission maximum at 372 nm. In mixtures of buffer (pH 7.4) with organic solvents like ethanol, methanol or dioxane a strong increase of fluorescence, concomitant with a small blue shift of the maximum, was observed when the ratio of organic solvent to water was increased. In 95% ethanol (10 mM Tris-HCl, pH 7.4) the increase is twenty fold as compared with tetracaine in buffer, and the maximum shifts from 372 to 364 nm. For each single series of buffer-solvent mixtures the fluorescence was found to increase with increasing hydrophobicity. However, attempts to correlate the fluorescence increase in different solvents with their Z values – an empirical scale for solvent polarity [1] – were not successful. This may be due to the fact that tetracaine is a tertiary amine, which can be present in a charged or an uncharged form, the ratio being partly dependent on the nature of the solvent. In a medium of given polarity tetracaine fluorescence depends on the pH: In buffer at pH 10 (>95% tetracaine base) the fluorescence was 30% higher than at pH 7 (>95% tetracaine in ionized form).

Table. Fluorescence emission of tetracaine in the presence of membranes or membrane components. The values are relative to tetracaine without addition = 100.

	Ghosts	Isolated ghost protein	Liposomes
Without Ca^{2+}	203 ± 6	131 ± 7	149 ± 7
With 5×10^{-3} M Ca^{2+}	116 ± 3	105 ± 2	115 ± 2

10^{-5} M tetracaine in 20 mOsm Tris-HCl buffer pH 7.4. Concentrations of ghosts and isolated ghost protein: 60 μg protein/ml; liposomes: 60 μg lipid/ml. The values represent the relative fluorescence emission at 370 nm, corrected for background fluorescence of ghosts, protein or liposomes.

Requests for reprints should be sent to Dr. J. G. R. Elferink, Laboratory for Medical Chemistry, Wassenaarseweg 72, *Leiden*, The Netherlands.

Fluorescence measurements were used to study the location of the tetracaine molecule in the erythrocyte membrane. Ghosts were prepared from pig erythrocytes according the method of Dodge [2]. Ghost proteins were obtained by removal of the lipids from the ghosts by the butanol-extraction method of Rega [3], followed by extensive dialysis to remove the butanol. The lipids of the membrane were isolated according to the procedure of De Gier and Van Deenen [4]. The lipids were converted into liposomes by sonicating them for 15 min, under a nitrogen atmosphere and in ice, in 15 mOsm phosphate buffer (pH 7.4). The addition of ghosts to a tetracaine solution results in a strong increase of fluorescence (see Table). The presence of 5×10^{-3} M Ca, which is known to displace the membrane-bound tetracaine [5–7], results in a strong diminution of the fluorescence increase. Both isolated ghost protein and liposomes of ghost lipids added to a tetracaine solution in corresponding quantities caused an increase in fluorescence, but to a lesser degree than with ghosts.

The binding percentage of 2×10^{-5} M tetracaine to ghosts (240 μg protein per ml) was $19 \pm 2\%$. About the same percentage was found for binding to isolated ghost protein and liposomes in corresponding concentrations: $20 \pm 2\%$ for binding to ghost protein and $19 \pm 2\%$ for binding to liposomes. The observed increase in fluorescence is due to the tetracaine molecules which are bound. The results suggest that in the intact membrane a part of the tetracaine molecule is in an apolar region of the membrane. The same applies also to the isolated membrane components, but to a much lesser degree. This supports previous findings that a change in binding pattern may result from disruption of the interaction between the protein and lipid phase in the intact membrane [8].

Feinstein [9] investigated the reaction of tetracaine with phosphatidylserine, an important membrane component as regards drug action [10]. This author found that a 1 : 2 molar complex was formed by these compounds. He suggested that the interaction of tetracaine with anionic phospholipids may be based on a two point electrostatic attachment of the two electropositive centers existing in the tetracaine molecule to the negatively charged phosphate groups of two phospholipid molecules. One of the two is the

positively charged alkylamine nitrogen, the other is the aromatic amino group which derives a partly positive charge from mesomeric effects. Accordingly the chromophoric part of the tetracaine molecule resides in the polar region of the bilayer. Our results suggest an apolar environment; a two-point attachment may not be present in the intact erythrocyte membrane. The results do not conflict with the presence of tetracaine as a free base in the membrane, but several investigators have shown that it is more probable that tetracaine exists in the membrane in its charged form [11–13]. It seems therefore likely that in the membrane the tetracaine molecule is located with its charged alkyl amine group in the polar part of either the protein or lipid phase whereas its aromatic part with the aliphatic chain is directed into the apolar region of the corresponding phase. This idea is also consistent with the findings of Koblin *et al.* [14], who showed that tetracaine quenches the fluorescence of membrane-incorporated dyes, for which process a penetration of the tetracaine molecule into the hydrocarbon interior of the membrane is necessary.

The author would like to thank Prof. Dr. H. L. Booij and Dr. J. C. Riemersma for their advice and editorial assistance.

[1] E. M. Kossower, J. Amer. Chem. Soc. **80,** 3253–3260 [1958].
[2] J. T. Dodge, C. D. Mitchell, and D. J. Hanahan, Arch. Biochem. Biophys. **100,** 119–130 [1963].
[3] A. F. Rega, R. I. Weed, G. G. Berg, and A. Rothstein, Biochim. Biophys. Acta **147,** 297–312 [1967].
[4] J. De Gier and L. L. M. Van Deenen, Biochim. Biophys. Acta **49,** 286–296 [1961].
[5] H. Hauser and R. M. C. Dawson, Biochem. J. **109,** 909–916 [1968].
[6] P. Seeman, Pharmacol. Rev. **24,** 583–655 [1972].
[7] D. Papahadjopoulos, Biochim. Biophys. Acta **265,** 169–186 [1972].
[8] J. G. R. Elferink, Biochem. Pharmacol., in press.
[9] M. B. Feinstein, J. Gen. Physiol. **48,** 357–374 [1964].
[10] M. P. Sheetz and S. J. Singer, Proc. Nat. Acad. Sci. U.S. **71,** 4457–4461 [1974].
[11] T. Narahashi, M. Yamada, and D. T. Frazier, Nature **22,** 748–749 [1969].
[12] T. Narahashi, D. T. Frazier, and M. Yamada, J. Pharmacol. Exp. Ther. **171,** 32–44 [1970].
[13] F. Piccinini, A. Chiarra, and F. Villan, Experientia **28,** 140–141 [1972].
[14] D. D. Koblin, W. D. Pace, and H. H. Wang, Arch. Biochem. Biophys. **171,** 176–182 [1975].