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SHORT COMMUNICATIONS

The effect of local anaesthetics on the osmotic fragility of liposomes

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From among various effects produced by local anaesthetics on biomembranes [1–8], special attention has been drawn to the anaesthetic-induced stabilization and expansion of biological membranes [9, 10]. The membrane stabilizing effect of local anaesthetics is reflected, among others, in the protection of intact red cells from hypotonic haemolysis [9, 10]. Despite numerous studies, the molecular basis of the antihaemolytic action of anaesthetics is far from being clearly understood. In the present study the role of the lipid bilayer in local anaesthetic-induced membrane stabilization is investigated using protein-free model membranes. The elucidation of the molecular mechanisms of biomembrane stabilization by anaesthetics seems to be important in view of the correlation existing between the antihaemolytic properties of these drugs and their ability to perturb the functional state of excitable tissues [10].

Materials and methods

Chromatographically pure egg yolk phosphatidylcholine was prepared by the method of Singleton *et al.* [11]. Dibucaine–HCl, tetracaine–HCl, procaine–HCl, dicetyl phosphate, cholesterol, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and reduced glutathione (GSH) were obtained from Sigma Chemical Co. (St. Louis, MO). Sephadex G-25 was from Pharmacia (Uppsala, Sweden). Multilamellar liposomes were prepared and their osmotic fragility was determined essentially as described previously [12, 13]. Briefly, for liposome preparation phosphatidylcholine : cholesterol : dicetyl phosphate (1 : 1 : 0.1, molar ratio) mixture in chloroform (*ca.* 20 mg lipid) was evaporated until dry. The dried film was then vortexed with 1 ml of suspension containing 100 μmoles DTNB in 0.1 M Tris–HCl buffer, pH 8.0. Untrapped marker (DTNB) was removed by Sephadex G-25 chromatography. In order to determine the osmotic fragility of these liposomes, 0.04 ml aliquots of gel filtrated liposome suspension were rapidly mixed with 2 ml of 0.01 M Tris–HCl buffer (pH 8.0) containing local anaesthetics at the desired concentration. After 10 min incubation in hypotonic buffer the per cent of marker released from liposomes was determined by measuring the absorbance at 420 nm of the reaction product between released DTNB and GSH added. The total amount of the trapped marker was determined after complete liposome disruption by Triton X-100.

Results and discussion

The osmotic fragility of multilamellar liposomes was studied by following the release of trapped marker upon rupture of liposomes in hypotonic buffer. DTNB appeared to be a very convenient marker for such studies [12]. The release of DTNB in hypotonic solution from liposomes of lipid composition used in this study is very fast. The time required for the release of 50% of the marker discharged within 5 min is about 40 sec. After 5 min no more DTNB is released. Several lines of evidence indicate that (as discussed in detail by Alhanaty and Livne [12]) the release in hypotonic buffer of trapped DTNB from multilamellar liposomes is caused primarily by the osmotic rupture of liposomal membranes.

The effect of three local anaesthetics: dibucaine, tetracaine and procaine on the osmotic fragility of multilamellar liposomes is shown in Fig. 1. All anaesthetics tested caused destabilization of liposomes as reflected in increased DTNB release. The destabilizing effect on model membranes was observed at relatively low and pharmacologically relevant concentrations of the drugs and, moreover, it correlated with their local anaesthetic potency [2].

It is well established [10] that all anaesthetics exert a biphasic effect on the stability of red cell membranes. At relatively low concentrations anaesthetics protect erythrocytes against osmotic haemolysis whereas at higher concentrations they destabilize the membranes, leading to erythrocyte lysis. Drug concentrations which reduce osmotic haemolysis by 50%, amount to 1×10^{-4} M, 5×10^{-4} M and 3.5×10^{-2} M for dibucaine, tetracaine and procaine, respectively [9]. Comparison of the effects of local anaesthetics on the osmotic fragility of erythrocytes and liposomes indicates that factors other than direct anaesthetic–lipid bilayer interactions are involved in antihaemolytic action of these drugs. It seems that membrane proteins are required for evoking the stabilizing effect of local anaesthetics. Membrane stabilization may result from direct or lipid-mediated interaction of local anaesthetics with membrane proteins. The second of these possibilities seems to be more likely in view of the high affinity of local anaesthetics to phospholipids [1]. The biphasic effect of local anaesthetics on the stability of red cells may be explained by assuming that at lower concentrations their interaction with protein or lipoprotein plays a dominating

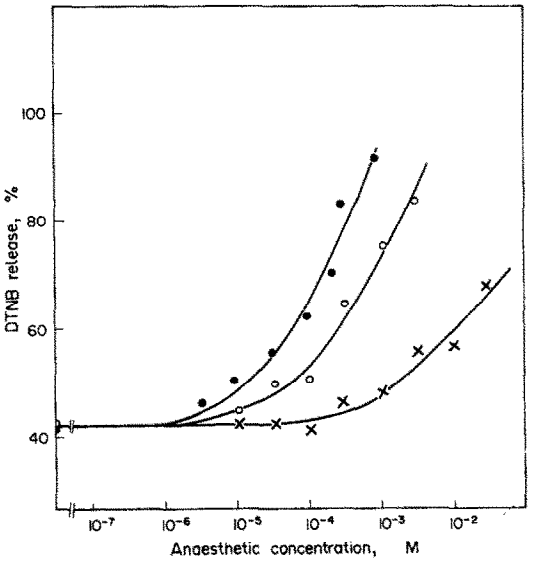

Fig. 1. Effect of local anaesthetics on the release of DTNB from liposomes in hypotonic solution. (●) Dibucaine, (○) tetracaine, (X) procaine. Liposomes were prepared from the phosphatidylcholine : cholesterol : dicetyl phosphate (1 : 1 : 0.1) mixture. Each point represents the mean of 5–7 experiments.

role, leading to membrane stabilization. At higher concentrations lytic interaction of the drugs with membrane lipids promotes hypotonic haemolysis in red cells.

Besides the drugs tested in this study, similar results were obtained previously for phenothiazine derivatives [12] and propranolol [13]. It seems, therefore, that the above proposed mechanism of drug-induced stabilization and destabilization of biological membranes may be applicable to all cationic local anaesthetics. This model does not account, however, for the action of all antihaemolytic compounds. Fatty acids and some hashish components have been shown to stabilize both erythrocytes and liposomes [12] indicating that direct lipidic interactions are responsible for the antihaemolytic effects of these substances.

In summary, the local anaesthetics dibucaine, tetracaine and procaine increase the osmotic fragility of multilamellar liposomes. Comparison of the effects of these drugs on red cells and model lipid membranes suggests that the crucial role in local anaesthetics-induced stabilization of biological membranes is played by the protein component.

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Comparative effects of ketanserin, a novel serotonergic receptor antagonist, on 5HT-induced shape change and 5HT uptake in rat and human platelets

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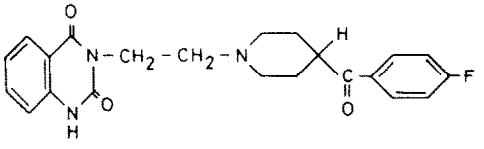
The serotonergic receptor antagonist 3-{2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl}-2,4-(1H, 3H) quinazolinedione, ketanserin (see structure below), has recently



been characterized [1–4]. It antagonizes 5-hydroxytryptamine (5-HT)-induced effects in several pharmacological tests [5, 6]. In particular it inhibits 5HT-induced contractions in the isolated rat caudal artery and other blood vessels [2].

Receptor-binding studies in brain tissues [1] indicate that ketanserin specifically combines with a subpopulation of 5HT receptors indicated as '$5HT_2$ receptors' [1, 4, 7].

5HT induces a shape change in platelets from different animal species and brings about aggregation of human platelets [8, 9]. It is actively taken up by platelets which release it in response to various stimuli [10]. It has been observed [11] that the stimulatory effect of 5HT on platelet shape change and aggregation is unrelated to its active transport and that at least two separate 'receptors' for 5HT are present on rat [12] and human platelets [13, 14].

More recently the presence of functional $5HT_2$ receptors on human platelets different from those involved in its uptake was postulated by De Clerck *et al.* [15]. These authors have shown that ketanserin inhibits 5HT-induced and amplified platelet aggregation in human platelets ($IC_{50} = 1.66 \times 10^{-8}$ M) but does not affect its active uptake at concentrations below 5×10^{-6} M.

In the present study we compared the inhibitory effects of ketanserin on 5HT-induced shape change and 5HT uptake in human and rat platelets. The relative potency of ketanserin against the two platelet functions examined was compared to methysergide, a potent antagonist of 5HT-induced platelet shape change [12, 14] and chlorimipramine, a potent inhibitor of 5HT uptake by platelets [12, 14].

Materials and methods

Blood was collected from healthy donors of both sexes (20–30 years old) and from 250–300 g CD-COBS male rats (Charles River, Calco, Italy), on 3.8 and 3.1% trisodium citrate (ratio of anticoagulant/final sample volume 1:10) respectively, and processed as previously described [16] to obtain platelet-rich (PRP) and platelet-poor plasma (PPP).