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Effects of propofol and other GABAergic phenols on membrane molecular organization

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ABSTRACT

$GABA_A$ receptor is the main inhibitory receptor of the central nervous system. The phenols propofol and thymol have been shown to act on this receptor. $GABA_A$ is an intrinsic protein, the activity of which may be affected by physical changes in the membrane. Taking into account the lipophilicity of phenols, their interaction with the membrane and a consequent non-specific receptor modulation cannot be discarded. By using Langmuir films, we analyze the comparative effects on the molecular properties of the membrane exerted by propofol, thymol and other related compounds, the activities of which on the $GABA_A$ are under investigation in our laboratory. All the compounds were able to expand phospholipid films, by their incorporation into the monolayer being favored by less-packed structures. Nonetheless, they were able to be incorporated at lateral pressures above the equilibrium pressure estimated for a natural membrane. Epifluorescence images revealed their presence between phospholipid molecules, probably at the head-group region. Hence, all results indicated that the phenols studied were clearly able to interact with membranes, suggesting that their anesthetic activity could be the combined result of their interaction with specific receptor proteins and with their surrounding lipid molecules modulating the supramolecular organization of the receptor environment.

1. Introduction

The $GABA_A$ receptor (GABA-R), the main inhibitory receptor of the central nervous system, is a ligand-gated ion channel that mediates fast synaptic inhibition in brain and spinal cord. GABA-R is unique among neurotransmitter receptors in the number of allosteric ligands that modulate its function [1,2]. The GABA-R ligands include drugs other than the GABA neurotransmitter, such as benzodiazepines, barbiturates, anesthetics, neurosteroids, ethanol and the convulsant picrotoxinin, which behave as allosteric modulators or channel blockers. This wide spectrum of drugs modifies GABA-R function by directly interacting either with these binding sites or with other as yet not well-described sites, present in the receptor complex [3,4]. The phenols propofol and thymol have been shown to act on this receptor as positive allosteric modulators or as direct agonists, according to the concentration assayed. These activities are mediated by their interaction with a specific site in the GABA-R [5,6].

GABA-R is a membrane intrinsic protein whose activity may be affected by surface-active compounds and by physical changes in the membrane [7–11]. Thus, given the lipophilicity of thymol and propofol, their interaction with the membrane region surrounding the receptor and a consequent non-specific receptor modulation cannot be discarded.

Recently we determined several lipophilic parameters for two phenol derivatives (PDs) with known GABAergic activity (thymol and propofol), and another three (carvacrol, eugenol and chlorothymol) that are structurally related with the former. The results obtained, based on the octanol–water partition coefficient ($\log P_{o/w}$), retention data in high performance liquid chromatography (HPLC) using C18 and immobilized artificial membrane (IAM) columns at different temperatures, and partition coefficients determined in phospholipid liposomes, demonstrated the high capacity of all the compounds assayed to interact with membrane phases. In addition, this supported the possible participation of some kind of alteration of the GABA-R lipid environment as part of the receptor modulation exerted by phenolic compounds [12].

The interaction between surface active compounds and phospholipids has been extensively studied in several model membrane systems, including liposomes and Langmuir monolayers [13,14]. In the present study, using Langmuir dpPC films, we analyze the comparative effects of these five PDs on the molecular properties of the membrane. Langmuir films constitute an informative and convenient membrane model because they permit subtle control

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of the membrane molecular packing. Three types of experimental approaches were used in the work: (i) surface pressure–molecular area isotherms, (ii) compound penetration capacity at different lateral surface pressures, and (iii) topographic film analysis through epifluorescence microscopy imaging.

2. Materials and methods

2.1. Materials

Propofol (2,6-bis(isopropyl)-phenol), thymol (5-methyl-2-isopropyl-phenol), carvacrol (2-methyl-5-isopropyl-phenol), eugenol (2-methoxy-4-prop-2-enyl-phenol) and chlorothymol (5-methyl-4-chloro-2-isopropyl-phenol) were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and used without further purification. 1,2-Dipalmitoyl-phosphatidylcholine (dpPC) was from Avanti Polar Lipids (Alabaster, USA) and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate ($DiIC_{18}$) was from Molecular Probes (Invitrogen, Argentina). Water was bidistilled in an all-glass apparatus (pH 6.5 ± 0.3). Other drugs and solvents used were of analytical grade. The compounds were dissolved in DMSO at 400× concentration, so that the final concentration of DMSO in the testing solution was 0.25% (v/v). Controls contained the same amount of DMSO, when so required.

2.2. Surface pressure–molecular area isotherms

Monomolecular layers were prepared and monitored essentially according to Garcia and Perillo [15]. The equipment used was a Minitrough II (KSV, Finland). A constant volume (15 μl) of chloroformic solution of dpPC (1 mg/ml) was spread over an aqueous surface; about 5 min were allowed for the evaporation of chloroform. Lateral surface pressure (π) was measured by the Wilhelmy plate method. Reproducibility was within ±0.01 nm^2 and ±0.001 mN/m for molecular area (A) and π, respectively.

π values were measured at different molecular areas of the phospholipid, in the absence or presence of each assayed phenol, at different concentrations in the subphase. For these experiments, we used a rectangular trough fitted with two barriers that move synchronously by electronic switching. The signal corresponding to the surface area (automatically determined by the Minitrough according to the relative position of the two compression barriers) and the output from the surface pressure transducer (measured automatically by the Minitrough with a platinized Pt foil 5 mm wide × 20 mm long × 0.025 mm thick) were fed into a personal computer through a serial interface using a specific software. Before each experiment, the trough was rinsed and wiped with 70% ethanol and several times with bidistilled water. The absence of surface-active compounds in the pure solvents and in the subphase solution (bidistilled water) was checked before each run by reducing the available surface area to less than 10% of its original value after enough time was allowed for the adsorption of possible impurities that might be present in trace amounts. The monolayer was compressed at a constant low rate of 20 mm^2/s at 28 ± 0.5 °C.

The onset of phase transition points was identified from a minimum and π_c from a maximum in the variation of the compressibility modulus (K) vs. molecular area plot. For this, K values were calculated from π–A isotherm data by applying Eq. (1):

$$K = -(A_\pi)\left(\frac{\partial \pi}{\partial A}\right)_\pi \quad (1)$$

where A_π is the molecular area at the indicated surface pressure.

From the π–A isotherms, the interfacial concentration of phenol expressed as mass per unit area (Γ) was calculated according to the following equation derived from the Gibbs surface tension equation [16]:

$$\Gamma = \frac{c}{RT} \cdot \frac{\partial \gamma_{lip}}{\partial c} \quad (2)$$

where c is the PD concentration in the subphase; γ_{lip} is the surface tension of dpPC monolayer (calculated from: $\gamma_{lip} = \gamma_w - \pi$, with γ_w being the surface tension of water at 25 °C) at a particular molecular area. An ideal behavior of drug solution was assumed, so phenol activity coefficient was equal to one. PD concentration in the subphase varied from 0 to 500 μM.

2.3. Penetration rates of phenol derivatives in lipid monomolecular layers at the air–water interface

In penetration experiments we used a circular Teflon trough (4.5 cm diameter and 0.5 cm depth). The subphase (8.5 ml, 15.9 cm^2 of surface area) was under continuous stirring with a miniature Teflon-coated rod spinning at 150–250 rpm. These experiments were performed at constant surface area but at different initial π (π_i), in order to measure the increment in π induced by PD penetration into the dpPC monolayer as a function of time ($\Delta\pi_t$), after the injection of 100 μM of each compound in the subphase. Accordingly, the compound penetration rate at each π_i was determined as the maximal value of the first derivative calculated from these π–time curves. In all penetration experiments, the injection of each PD in the subphase was made after the stabilization of the π_i (between 5 and 10 min approximately).

2.4. Epifluorescence microscopy of monolayers

DPPC was dissolved in chloroform (1 mg/ml), into which a small volume of a concentrated solution of $diIC_{18}$ in methanol was added to a final concentration of 1 mol%. The lipid mixture was dispersed onto an aqueous subphase and compressed in the same conditions detailed in point 2.2, and observed with an inverted epifluorescence microscope. Briefly, a KSV Minisystems surface barostat was mounted on the stage of a Nikon Eclipse TE2000-U (Tokyo, Japan) microscope, which was supplied with 20× long-working distance optics and with a fluorescence filter (excitation range: 520–553 nm, and emission range: 578–633 nm). The Teflon trough used had a 35 mm diameter quartz window at its base, which allowed the observation of the monolayer through the trough. Each PD was injected in the subphase at 100 μM final concentration, and images were taken at different π with a color Nikon DS-5 M video camera with a supported resolution up to 2560 × 1920 pix (capture). The images were then analyzed using the public domain Java image-processing program, ImageJ (National Institutes of Health, Bethesda, USA).

3. Results and discussion

3.1. Surface pressure–area isotherms

It is important to note that none of the PDs studied was able to form stable monomolecular layers by itself (results not shown); consequently the changes in the dpPC monolayers induced by each of these compounds were interpreted directly as its interaction with the phospholipid monolayer.

Fig. 1A–E shows π–A compression isotherms of dpPC in the absence (Control) or in the presence of each assayed PD present at different concentrations (20, 100 and 500 μM) in the aqueous subphase. Control isotherms obtained in the presence of DMSO 0.25% (v/v) were not different from those performed at 0% DMSO.

All compounds expanded the dpPC π–A isotherm in a concentration dependent manner. Although the well-known dpPC