ON THE GALANTAMINE INTERACTION WITH ARTIFICIAL LIPID MEMBRANES

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Abstract. Using an electrophysiological method, Black Lipid Membrane (BLM), we have studied the modifications induced by galantamine (GAL) on the electric properties of artificial lipid membranes formed either with diphytanoyl-phosphatidylcholine+octadecylamine (PCOA) or with 1,2-Dioleoyl-sn-Glycero-3-[phospho-rac-(1-glycerol)] (DOPG). The observed capacitance and conductance changes of artificial lipid bilayers depended both on the concentration of GAL and on the charge carried by the lipids. Our results suggest that more than one mechanism is responsible for the GAL interaction with the lipid membrane.

Key words: Galantamine, BLM, PCOA, DOPG.

INTRODUCTION

Galantamine (GAL) is an alkaloid obtained from the bulbs and flowers of the Caucasian snowdrop and it is currently approved as an anticholinesterasic drug for the treatment of mild to moderate Alzheimer's disease and other memory impairments [3, 10, 13, 14]. It has an aminic structure (Fig. 1) and a pKa value of 8.2 (prospect Reminyl – Johnson & Johnson) showing thus a tendency to interact differently with positively or negatively charged species. GAL is also known to act directly on nicotinic receptors demonstrated both *in vitro,* on the neuroblastoma derived cell line SH-SY5Y [4] or in hippocampic neurons [12], and *in vivo* (in New Zealand White rabbits [15]. It was shown that galantamine has a modulatory effect on nicotinic receptor activation, by allosteric potentiation [1, 4, 8] and recently it was found that the presence of galantamine modulates the recovery from ACh-induced desensitization, an effect which is concentration dependent and non-linear [9].

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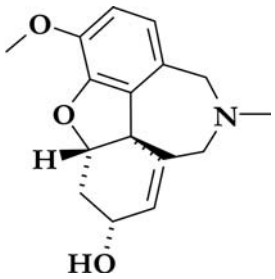

Fig. 1. Galantamine structure.

Although an allosteric binding site for GAL has been identified on the acetylcholine binding protein (ABP) at the interface of non-alpha subunits [6], some of the reported effects of the alkaloid cannot be explained solely by allosteric potentiation. In regard to its nicotinic modulatory effects on desensitization, it was not possible to establish the precise location of the galantamine binding site on the nicotinic receptor and it could not be discriminated to which specific form of the receptor (open / closed / desensitized) galantamine binds [9]. Previous researches (e.g. [5, 7, 11]) have shown that the various charged species are able to influence the membrane-related physiological processes either by modifying the membrane dipole moment or by disorganizing the structure of the lipid bilayer in the vicinity of various proteins embedded in it. Therefore, it was of interest for us to find out if the action of GAL at central nervous system level, and in particular on the nicotinic receptors, could be due not only to specific interactions with the receptors themselves, but also to non-specific interactions with the lipid membrane as well. Such non-specific interactions could be the result of GAL attachment and/or insertion in the lipid bilayer leading to modifications of various membrane parameters (e.g. membrane fluidity, dipole moment, etc.) and influencing thus the functionality of membrane proteins. The subject of the present paper is the effect of GAL on artificial lipid membranes followed up by monitoring the electrical parameters of lipid bilayers consisting of two different lipids carrying opposite electrical charge.

MATERIALS AND METHODS

THE BLM METHOD

The black lipid membranes (BLM), having an area of ca. 10^{-2} cm^2, are formed in a Teflon cuvette, consisting of two compartments, each with a volume of 1.5 mL. The compartments are filled with an appropriate electrolyte solution and, via agar bridges and Ag/AgCl electrodes, each compartment is connected to an external electric circuit that contains a current amplifier, an oscilloscope, a function generator, an electric filter, and a computer for data acquisition. The whole set-up

is placed inside a compact Faraday cage, with the possibility of optional light inside. The stirring of the solutions inside the cuvette was performed by a magnetic stirrer operated by a small magnet placed under the cuvette, which is rotated with variable velocity. By means of a function generator one can measure the electric capacitance and conductance of the membrane. Typical values for those electric characteristics of the black lipid membrane are ca. 400 nF/cm^2 for the specific capacitance and ca. 7 nS/cm^2 for the specific conductance (for further details see [2]).

CHEMICALS AND PROTOCOLS

The lipid film forming solution contained PCOA and DOPG as follows: (1) PCOA: 1.5% (w/v) diphytanoyl-phosphatidylcholine (Avanti Polar Lipids, USA) and 0.025% (w/v) octadecylamine in n-decane (Fluka, Sigma-Aldrich Germany, > 98%) 2) DOPG: 1,2-Dioleoyl-sn-Glycero-3-[phospho-rac-(1-glycerol)] (Avanti Polar Lipids, USA). The lipid solutions were prepared from stock solutions diluted (under nitrogen flow, in order to avoid peroxidation) in nDecan (Fluka, 99%). Before painting the lipid film the cuvette hole was impregnated with a solution of 0.5% (w/v) corresponding lipid in hexane (Fluka, Sigma-Aldrich Germany, > 98%). The artificial lipid membranes were formed in the presence of 20 mM HEPES (Sigma) and 100 mM NaCl (Merck, 99%) in both compartments of the cuvette. All the experiments were carried out at pH = 7 and at room temperature (22–24 ºC). The pH was adjusted with TRIS (Sigma) and before use the solutions were filtered through a cellulose-acetate filter 0.2μm (Sartorius). Galantamine (Galantamine hydrobromide, Sigma) was prepared as 10 mM, 50 mM and 500 mM stock solutions in ultrapure water. The desired galantamine (GAL) concentrations in the cuvette were obtained by adding bilaterally the appropriate amount from the stock solutions. Galantamine was added on both sides of the lipid membrane after checking its stability, i.e. the electrical capacitance C and conductance G measured three times within a 15 minutes interval remained unchanged. The same protocol was used throughout the whole experiment after each addition of galantamine. For each measurement the final value of the electrical parameters was the average of the three recorded values, normalized to the values of capacitance and the conductance of the lipid bilayer, C_0 and G_0 respectively, recorded before the addition of GAL. All the experiments were performed at room temperature (22–24 ºC). For each of the studied lipids, data were averaged for 2–3 different membranes. The statistical data processing was performed by using the OriginPro 7.0 software.

RESULTS

We present here the results concerning the modifications of the electric properties of the artificial lipid membrane in the presence of various concentrations of galantamine by means of the BLM technique. The lipids used for the formation

of the lipid film were PCOA, positively charged due to the added octadecylamine, and DOPG, negatively charged. Figure 2 depicts the evolution of the lipid bilayer capacitance at increasing GAL concentrations. For both lipids, the values of the capacitance are oscillating around its initial value at GAL concentrations below 100 μM, while after 200 μM GAL a slight increase of the capacitance, with a similar profile was found (Fig. 2A). A more detailed comparison of the lipid bilayer capacitance at small GAL concentrations (below 20 μM) (Fig. 2B) reveals a symmetrical evolution of capacitance values for the two lipids, namely, while at 0.5 μM GAL the capacitance has a maximum value for PCOA, in the case of DOPG it has a minimum value.

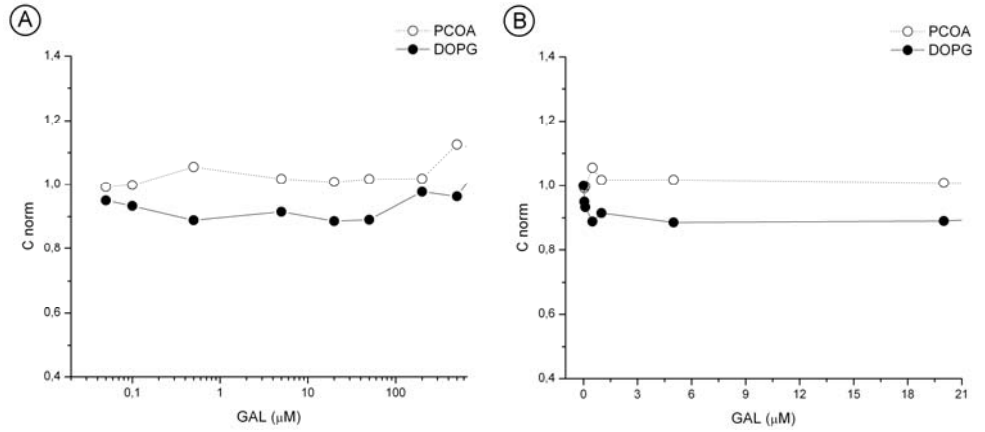

Fig. 2. Comparative evolution of membrane capacitance for two different lipids, PCOA and DPOG, in the presence of various GAL concentrations. A. The GAL concentrations, represented on a logarithmic scale, varied between 0.05 μM – 500 μM. B. Membrane capacitance at concentrations below 20 μM. The buffer contained 20 mM HEPES and 100 mM NaCl at pH = 7.

The lipid bilayer conductance has a similar evolution for both PCOA and DOPG, up to 5 μM GAL (Fig. 3), i.e. the conductance initially decreases and then, starting with a concentration of 100 nM GAL, a plateau is reached. Over 5 μM GAL the behavior of the two lipids shows a symmetrical evolution. The conductance of the PCOA lipid bilayer increases again toward its initial value as the GAL concentration increases, while the conductance of DOPG bilayer decreases mirroring that of PCOA. In both cases a plateau is reached at GAL concentrations higher than 50 μM.

The modifications of the electrical parameters of the artificial membrane in the presence of GAL indicate an interaction of the substance with the lipids of the bilayer. The mechanisms underlying this interaction, as suggested by the experimental results, could be possibly related either to GAL insertion in the lipid bilayer, leading to changes in the membrane capacitance, or/and to interfacial lipid-solution phenomena (attachment of GAL to the lipid membrane).

GAL has a slightly lipophilic character (the partition coefficient n-octanol/water: log P = 1.09, according to the prospects of the producer company Reminyl, Janssen Pharmaceutica for the commercial form, undertaken by Johnson & Johnson), therefore it is to be expected a degree of insertion of the substance in the bilayer. The modifications of the electrical capacitance following the GAL addition support the hypothesis of GAL insertion in the bilayer.

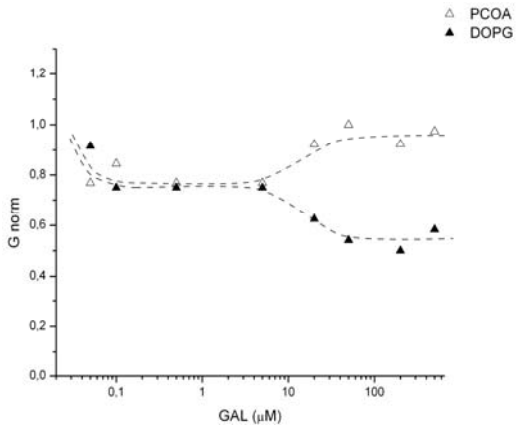

Fig. 3. Comparative evolution of membrane conductance for two different lipids, PCOA and DPOG, in the presence of various GAL concentrations. The triangles represent the experimental values and the dashed lines are orientative in order to guide the eye. The buffer solutions contained, beside GAL, 20 mM HEPES and 100 mM NaCl at pH = 7.

Nevertheless, the PCOA membrane capacitance variation is minimal, oscillating around the initial value, except for a small increase at 0.5 μM GAL. This small increase of capacitance in PCOA membrane is mirrored by the evolution of capacitance for the DOPG membrane and, at the same time, a more significant decrease of DOPG bilayer capacitance can be noticed, possibly due to the increase of the membrane thickness following GAL insertion. The symmetrical evolution of the capacitance in DOPG as compared to that in PCOA could be correlated with the different electrical charge carried by the two lipids. While PCOA is positively charged, due to added octadecylamine, DOPG carries a negative charge. This observation suggests a better insertion of GAL in a negatively charged membrane which is to be expected taking into account the aminic structure of GAL (Fig. 1) and its pKa value of 8.2 (prospect Reminyl – Johnson & Johnson). The fact that the capacitance peak value is encountered at small GAL concentrations, together with the symmetrical profiles of evolution for lipids carrying opposite electrical charge, suggests that at GAL concentrations below 1 μM the electrical interactions between galantamine and lipid headgroups, leading to the modification of the dipole moment of the membrane, are dominating over the insertion of GAL in the bilayer.

The biphasic profiles of electrical conductance evolution at increasing GAL concentrations suggest the existence of two concurrent mechanisms of interaction, depending on galantamine concentration. A first mechanism, responsible for the initial decrease of bilayer conductance as the GAL concentration increases, is present at concentrations up to 5 μM GAL for both lipids studied. Below 100 nM GAL the conductance decreases, possibly due to GAL insertion in the bilayer and afterwards saturation is observed in the concentration range of 100 nM – 5 μM. A second mechanism, responsible for the increase of conductance in PCOA bilayer (positively charged) and the decrease of conductance for DOPG bilayer (negatively charged), dominates at GAL concentrations above 5 μM, where the electrical charge of the lipid bilayer seems to significantly influence the evolution of membrane electrical conductance. At concentrations higher than 5 μM GAL an additional attachment of galantamine to the DOPG bilayer by an electrical interaction could take place, leading to an increased thickness and, consequently, to a decreased conductance of the membrane. At the same concentration range the capacitance value remains nearly constant, possibly due to the fact that the modifications in one direction induced by the increase of membrane thickness are compensated by modifications in the opposite direction of membrane electrical permittivity and of dipole moment. In the case of PCOA bilayer, on applying GAL concentrations above 5 μM, taking into account that the positively charged membrane is already saturated due to its lower affinity for GAL, the membrane could possibly become partially disorganized leading to an increase of its conductance.

Figure 4 comparatively depicts the modifications of membrane capacitance and conductance for each of the two lipids and the significant phenomena responsible for the modification of bilayer electrical parameters.

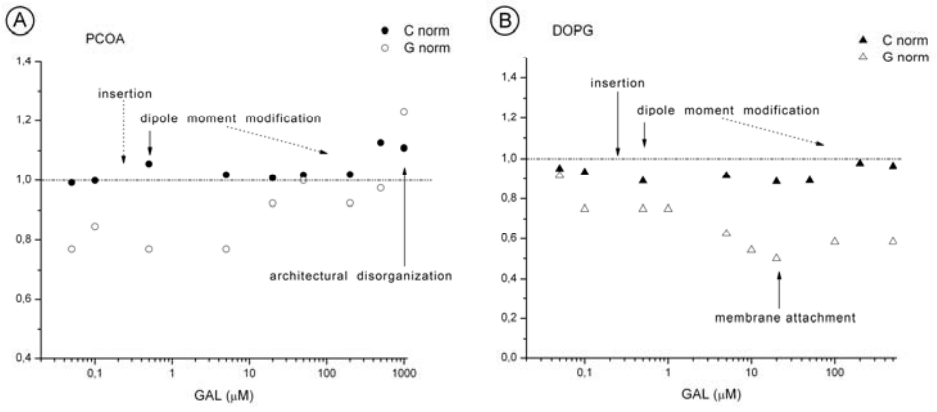

Fig. 4. Comparative profiles of the electrical capacitance and conductance of the artificial lipid membranes as a function of GAL concentration for PCOA (A) and DOPG (B). In the figure there are also depicted the main mechanisms responsible for the electrical parameters modification (insertion, attachment, dipole moment modification, architectural disorganization) related to the concentration ranges where they are dominating.

CONCLUSIONS

Due to its lypophilic character, galantamine inserts itself in the lipid bilayer, the insertion being more significant in the negatively charged lipids (DOPG) as compared to the positively charged ones (PCOA). The modifications of the electrical parameters of the membrane in the presence of GAL are not due exclusively to GAL insertion in the bilayer but other mechanisms could be involved as well, e.g.: i) interactions at the interface lipid-GAL-containing solution, leading to modifications in membrane architecture and/or dipole moment; ii) the attachment of GAL molecules to the lipid bilayer, more important for the negatively charged lipids.

Thus, it is possible that GAL effects at cellular level, reported in the literature (e.g. [9]), are due also to a non-specific interaction at the level of cell membrane. Nevertheless, taking into account the fact that the most abundant lipid in natural plasma membranes is phosphatidylcholine (PC) which is zwitterionic, comprising a negatively charged phosphate group and a positively charged ammonium group, this non-specific interaction might be not too important.

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