ARTICLE IN PRESS
BBAMEM-81803; No. of pages: 9; 4C: 3, 4, 5, 6, 8

Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Effects of neurosteroids on a model membrane including cholesterol: A micropipette aspiration study

Daniel Balleza [b], Mattia Sacchi [a], Giulia Vena [c], Debora Galloni [a], Giulia Puia [c], Paolo Facci [d], Andrea Alessandrini [a,b,*]

[a] Dipartimento di Scienze Fisiche, Matematiche e Informatiche, Via Campi 213/A, 41125 Modena, Italy
[b] CNR — Istituto Nanoscienze, S3, Via Campi 213/A, 41125, Italy
[c] Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia, Via Campi 287, Modena, Italy
[d] CNR — Istituto di Biofisica, Via De Marini 6, 16149 Genova, Italy

ARTICLE INFO

Article history:
Received 30 August 2014
Received in revised form 14 January 2015
Accepted 23 January 2015
Available online xxxx

Keywords:
Neurosteroids
Micropipette aspiration
Allopregnanolone
Giant unilamellar vesicle (GUV)

ABSTRACT

Amphiphilic molecules supposed to affect membrane protein activity could strongly interact also with the lipid component of the membrane itself. Neurosteroids are amphiphilic molecules that bind to plasma membrane receptors of cells in the central nervous system but their effect on membrane is still under debate. For this reason it is interesting to investigate their effects on pure lipid bilayers as model systems. Using the micropipette aspiration technique (MAT), here we studied the effects of a neurosteroid, allopregnanolone (3α,5α-tetrahydroprogesterone or Allo) and of one of its isoforms, isoallopregnanolone (3β,5α-tetrahydroprogesterone or isoAllo), on the physical properties of pure lipid bilayers composed by DOPC/bSM/chol. Allo is a well-known positive allosteric modulator of $GABA_A$ receptor activity while isoAllo acts as a non-competitive functional antagonist of Allo modulation. We found that Allo, when applied at nanomolar concentrations (50–200 nM) to a lipid bilayer model system including cholesterol, induces an increase of the lipid bilayer area and a decrease of the mechanical parameters. Conversely, isoAllo, decreases the lipid bilayer area and, when applied, at the same nanomolar concentrations, it does not affect significantly its mechanical parameters. We characterized the kinetics of Allo uptake by the lipid bilayer and we also discussed its aspects in relation to the slow kinetics of Allo gating effects on $GABA_A$ receptors. The overall results presented here show that a correlation exists between the modulation of Allo and isoAllo of $GABA_A$ receptor activity and their effects on a lipid bilayer model system containing cholesterol.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

An increasing number of works in biophysics and in cell biology are nowadays devoted to the relevant role that lipid components of the biological membrane can have on the activity of membrane proteins, by both specific [1] and non-specific mechanisms that consider the bilayer as a continuum [2–4]. The non-specific roles of lipids are related to both their mechanical properties that are relevant in the conformational transitions of membrane proteins (mechanical spring constants of the bilayers and lateral pressure profile) and to the lateral heterogeneity of membranes. The latter aspect represents an issue that is not new [5, 6], but it has received a strong burst after the so called "*raft* hypothesis" was introduced in the biological community [7] and it is far from being resolved. In fact, previous hypothesis on the basic role of the lipid bilayer in affecting the membrane protein activity relied mainly on evidence from model systems. In the first introduced models of the biological membrane, the lateral heterogeneity in the organization of the membrane regarded mainly the possibility of phase segregation or the presence of different phase domains such as in the case of solid ordered domains (S_o, also gel phase) coexisting with the liquid disordered phase (L_d, also liquid crystalline). The raft hypothesis shifted the attention to the possibility of another phase separation in biological membranes. The new idea is related to the possibility of a phase separation between two liquid domains in the biological membrane, the liquid ordered (L_o) phase and the L_d one [8]. In this case, relevant roles are played by the presence of sterols such as cholesterol in the membrane and by their preferred interactions with specific lipids. Many membrane proteins seem to have the tendency to segregate into one of the two phases and the segregation of different proteins in the same domain is considered as the basic mechanism by which many signaling pathways could be activated [8–10]. In this scenario, any mechanism able to produce an alteration of the bilayer phase properties could be relevant for the normal operation of a biological membrane [11]. However, the detection of phase separation between different liquid phases in biological membranes remains still elusive [12]. The elusive character of these domains

* Corresponding author at: University of Modena and Reggio Emilia, Department of Physics, Informatics and Mathematics, Via Campi 213/A, 41125 Modena, Italy. Tel.: +39 059 2055297; fax: +39 059 2055651.
E-mail address: andrea.alessandrini@unimore.it (A. Alessandrini).

http://dx.doi.org/10.1016/j.bbamem.2015.01.017
0005-2736/© 2015 Elsevier B.V. All rights reserved.

Please cite this article as: D. Balleza, et al., Effects of neurosteroids on a model membrane including cholesterol: A micropipette aspiration study, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamem.2015.01.017

ARTICLE IN PRESS

in biological membranes is now ascribed to their small lateral dimensions (in the order of a few tens of nanometers) and to their dynamic aspects (lifetime in the order of milliseconds) [13]. However, the *raft* hypothesis stimulated a plethora of studies trying to elucidate if specific membrane proteins could be considered raft-associated. In cases where this association is possible, it can be concluded that any modification of the bilayer affecting the thermodynamics of raft domains can also affect protein function.

A very interesting breakthrough was the realization that specific thermodynamic properties could play important roles in the organization and consequently in the activity of a biological membrane. In particular, the proximity, in physiological conditions, of the biological membrane to a critical point or to several critical points emerged as an intriguing possibility in this research field [14,15]. Around physiological temperature, the membrane is thought to be, due to its lipid composition, just above its critical condition [16]. In this situation, fluctuations in lipid composition could explain both the small lateral dimensions of the domains and their dynamic organization. Moreover, it was demonstrated that lipid bilayer model systems composed of three different lipid types, one low-melting lipid type, one high-melting lipid type and cholesterol, could be considered representative of much more complex bilayers in eukaryotic cells [17] and useful information could be obtained studying these model systems.

The role of thermodynamics in the behavior of a membrane brings about considerations also on the possibility that a dopant in the bilayer could alter the thermodynamics of the system and impair the regular formation of domains. This aspect could be particularly relevant in the case of proximity of the membrane to a critical point or in general to a miscibility border.

Accordingly, the longstanding discussion on the interactions between drugs and lipids in the membrane [18] was enriched with a new viewpoint related to the thermodynamical aspects of lipid bilayers near critical points [19]. In fact, the simple insertion of a drug could change the bilayer position in the phase diagram affecting the domain organization as a consequence of a changed distance from the miscibility border and could consequently affect the activity of membrane proteins. Even if a mechanistic view of the process leading from the presence of the drug in the membrane to changes in its functions is not completely clear, it is evident that further studies of the interaction of drugs with membranes deserve great attention. This is particularly true for highly lipophilic drugs such as some anesthetics and neurosteroids.

In this work we studied the effects of neurosteroids on the physical properties of a lipid bilayer composed by DOPC, bSM and cholesterol. To do this, we exploited the micropipette aspiration technique (MAT), concentrating on giant unilamellar vesicle (GUV) model systems. The neurosteroids that we concentrated on are allopregnanolone (in the following Allo), an endogenous highly lipophilic molecule [20], known to modulate $GABA_A$ receptor activity [21,22] and one of its isoform isoallopregnanolone (in the following isoAllo). In particular, Allo potentiates GABA-evoked currents mediated by $GABA_A$ receptor activation at low nanomolar concentrations and is able by itself to activate the $GABA_A$ receptor at higher concentrations [23]. Many studies report on the possible interaction of Allo with the lipid bilayer [24], although a well-established understanding on this aspect is still lacking. For example, studies on the gating behavior of Allo found that the activation of $GABA_A$ receptors occurred with a slow kinetics and this behavior was hypothesized to derive from the slow accumulation of the neurosteroid in the plasma membrane [23]. However, the possible effects of Allo on the mechanical parameters characterizing the membrane have not yet been considered, neglecting the possible contribution of lipid bilayer properties on the behavior of the receptors. Instead, isoAllo is known to be a non-competitive antagonist of Allo with regard to $GABA_A$ receptors [25]. Thus, the two isoforms represent a very interesting test case to investigate if their different pharmacological properties at the level of a membrane protein could be somehow also related to differential effects on the lipid components of the membrane. In the past, the different effects of Allo and isoAllo on lipid bilayers have already been considered, albeit at higher concentrations than what we used in the present work [26,27]. Considering that the neurosteroid concentrations that we used are functionally relevant for the $GABA_A$ receptor, investigating their effect on pure lipid bilayers is worthwhile.

Here, besides measuring the kinetics of Allo and isoAllo uptake by a DOPC/bSM/cholesterol pseudo-ternary lipid bilayer ("pseudo-ternary" refers to the fact that bSM is already a mixture, even if largely composed by 18:0 acyl chains) in the form of GUVs by the MAT [28,29], we also studied their effects on the mechanical properties of the bilayer. We found that at nanomolar concentrations Allo produces an increase of the overall lipid bilayer area which is coupled to a decrease of the bilayer mechanical parameters. The kinetics of Allo uptake and the obtained time constant have been found to be in the same order of magnitude as the time constant observed for the gating effect of Allo on the $GABA_A$ receptors at similar concentrations. At variance with Allo, we found that isoAllo produced a decrease of the lipid bilayer area and a negligible variation of the mechanical properties. The obtained results are also interpreted on the basis of what is obtained when a supported lipid bilayer of the same lipid composition is studied by atomic force microscopy (AFM) as a function of Allo and isoAllo concentrations to which it is exposed [30]. Finally, we discuss the effects of the neurosteroid on the lipid bilayer properties in light of a general mechanism of action of lipophilic molecules on biological membranes [18].

2. Material and methods

2.1. Lipids

Lipids 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), sphingomyelin (Brain, Porcine) (bSM) and cholesterol were purchased from Avanti Polar Lipids (Alabaster, USA) and were used without further purification. Specific lipid mixtures were prepared by mixing chloroform lipid solutions in the desired amount (the proportions used in this work are molar proportions). Allopregnanolone was purchased from Sigma-Aldrich and isoallopregnanolone was a generous gift from Dr A. Guidotti (see ref. [21] for details on isoAllo) (see Scheme 1 for their structure).

2.2. GUV preparation

GUVs were prepared by the electroformation method [31] with minor modifications. Briefly, lipid mixtures were suspended in chloroform and small drops (2–3 μL, 0.2 mg/mL total lipid) of the lipid mixture were deposited on two opposing Pt wires inside a PTFE chamber. Phospholipid compositions in GUVs are expressed as mole ratios: e.g., DOPC/SM/Chol (1:1:1) denotes an equimolar ternary mixture. Chloroform was removed initially by exposing the Pt wires to a nitrogen flux and then by using a vacuum chamber (10^{-2} mbar) for 2 h. Two Pt wires were then connected to a wave form generator to produce a sinusoidal voltage potential difference. The PTFE chamber was then filled with a 100–200 mM sucrose solution and sealed using glass coverslips and vacuum grease. The applied electroformation protocol was as follows: (1) 10 Hz, 3.0 V_{p-p} for 45 min; (2) 5 Hz, 2.5 V_{p-p} for 20 min; and (3) 2 Hz, 1.5 V_{p-p} for 15–20 min. As the final step we applied a square wave at 5 Hz in order to promote vesicle detachment from the wires. After formation, GUVs were gently extracted from the PTFE chamber and resuspended in a 95–250 mM glucose solution. This procedure assures an increased contrast in Differential Interference Contrast (DIC) images acquired with an inverted optical microscope (Olympus IX70) and a conserved internal volume, at least on a short time scale (a few minutes).

Please cite this article as: D. Balleza, et al., Effects of neurosteroids on a model membrane including cholesterol: A micropipette aspiration study, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamem.2015.01.017

Download English Version:

https://daneshyari.com/en/article/10796647

Download Persian Version:

https://daneshyari.com/article/10796647

Daneshyari.com