Agomelatine strongly interacts with zwitterionic DPPC and charged DPPG membranes

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ABSTRACT

Depression is one of the most common psychiatric diseases in the population. Agomelatine is a novel antidepressant drug with melatonin receptor agonistic and serotonin 5-HT$_{2C}$ antagonistic properties. Furthermore, being a melatonergic drug, agomelatine has the potential of being used in therapeutic applications like melatonin as an antioxidant, anti-inflammatory and antiapoptotic drug. The action mechanism of agomelatine on the membrane structure has not been clarified yet. In the present study, we aimed to investigate the interaction of agomelatine with model membranes of dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylgylcerol (DPPG) by Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). We found that agomelatine interacts with the head group in such a manner that it destabilizes the membrane architecture to a large extent. Thus, agomelatine causes alterations in the order, packing and dynamics of the DPPC and DPPG model membranes. Our results suggest that agomelatine strongly interacts with zwitterionic and charged membrane phospholipids. Because lipid structure and dynamics may have influence on the structure of membrane bound proteins and affect the signal transduction systems of membranes, these effects of agomelatine may be important in its action mechanism.

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1. Introduction

Depression is a complex and incapacitating health problem that may represent a significant burden to patients, their families and to society [1]. Drugs related to the uptake process of biogenic amines, e.g. monoaminergic antidepressants [tricylics, serotonin reuptake inhibitors (SSRIs), serotonin noradrenalin reuptake inhibitors (SNRs)] have been used in the treatment of depression since the middle of the 1950s [2]. However, they do not show their clinical benefit quickly, or do not even provide any benefit at all for some people. Thus, there is considerable interest in new non-monoaminergic approaches for a potentially effective treatment of depression. Currently, targeting melatonin and melatonergic receptors has a key role in synchronizing circadian rhythms, which are known to be disturbed in depressed individuals [3]. Agomelatine, the first melatonergic antidepressant, is a novel drug and has both agonistic activity on melatonin receptors and antagonistic activity on serotonine 5-HT$_{2C}$ receptors [4].

Neuronal membrane and neuroplasticity have a key role in the clinical effectiveness and action mechanisms of antidepressant drugs. A neuroplasticity hypothesis involved in the action mechanism of antidepressants has gained importance. According to this hypothesis, membrane connected elements such as receptor binding targets, ion channels, signal transduction cascades and their interaction with drug molecules are essential for the action of antidepressant drugs [5,6]. Thus, in order to provide the efficient use of a drug, it is critically important to know its site of interaction with membranes at molecular level [7]. On the other hand, understanding the mechanism of action of the antidepressant drugs is important to shed an insight to resolve the pathogenesis of depression. However, there is limited information in the literature regarding the interaction of agomelatine with the molecular components of cells and biological membranes.

Furthermore, being a melatonergic drug, agomelatine has the potential of being used in therapeutic applications like melatonin as an antioxidant, anti-inflammatory and antiapoptotic drug [8–13]. To the best of our knowledge, the number of studies as to the effect of agomelatine in this sense is limited [9,14–16].

Biological membranes are complex systems containing lipids, proteins and carbohydrates. Therefore, model liposomes prepared from desired membrane components have proven invaluable in membrane research since they mimic biological membranes. The phospholipids used in the liposome formation in the current study have different polar head groups. It is known that, a difference in the phospholipid head group causes different intermolecular interactions, drawing to different packing abilities [17,18]. In our case, the head group of dipalmitoylphosphatidylcholine (DPPC) is zwitterionic

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whereas the head group of dipalmitoylphosphatidylglycerol (DPPG) is negatively charged.

In biomembrane studies, biophysical techniques such as spectroscopy and calorimetry give valuable information on the order–disorder state, phase transition or thermotropic mesomorphism and provide information about molecular motion and molecular moieties. Therefore, this current study aims to investigate the effect of agomelatine on DPPC and DPPG model membranes. DPPC is one of the main lipids in biological membranes. DPPG is chosen as an experimental model lipid since it is the negatively charged counterpart of DPPC and so is used in order to understand the effect of charge status in agomelatine–model membrane interactions as reported in other studies [19–21]. To achieve this aim, different biophysical techniques, namely Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) have been performed. These techniques provide significant and detailed information about changes in the phase transition behavior, mobility and structure of individual molecular moieties [19,22–27].

The action mechanism of agomelatine on the membrane structure has not been clarified yet; for instance, no study has been found in the literature about the interaction of agomelatine with the lipids of biological membranes. Thus, the main objective of the present study is to evaluate the interaction of agomelatine with zwitterionic and anionic membranes DPPC and DPPG at model level, respectively by investigating structural parameters such as phase transition behavior, lipid order, strength of hydrogen bonding and functional parameters such as lipid dynamics. To achieve this, the thermotropic phase transition profile of the liposomes was compared at different drug concentrations by FTIR spectroscopy and DSC techniques. To the best of our knowledge, this is the first study to report the interactions of agomelatine with phospholipid membranes.

2. Results

In the current study, the interaction mechanism of agomelatine at different concentrations (1–18 mol%) on zwitterionic DPPC and charged DPPG liposomes in the gel and fluid phases was analyzed by using FTIR spectroscopy and DSC techniques. For this purpose, FTIR spectra and DSC thermograms were collected for pure and agomelatine containing DPPC and DPPG liposomes as a function of temperature between 20 and 60 °C. Fig. 1A shows the FTIR spectra of DPPC and DPPG liposomes in the whole region. The CH_2 antisymmetric stretching (~2920 cm^{-1}), CH_2 symmetric stretching (~2850 cm^{-1}), C=O stretching (~1740 cm^{-1}) and PO_2^- symmetric stretching (~1080 cm^{-1}) bands were taken into consideration. Fig. 1B and C shows the normalized FTIR spectra of pure and agomelatine containing DPPC and DPPG liposomes, respectively in the C–H stretching region, at a temperature corresponding to the fluid phase of the membrane to illustrate the alterations induced by agomelatine. The figures clearly show that agomelatine induces changes in the frequency and bandwidth values of the spectral bands. Since the normalization process does not reflect the actual variations, the detailed spectral changes were performed from the original subtracted spectra.

The alterations in the frequency values of CH_2 antisymmetric and symmetric stretching vibrations provide information about the membrane phase transition behavior and membrane order [19,22–24,26,27]. Fig. 2A and B shows the temperature dependent frequency changes of CH_2 symmetric stretching bands of DPPC and DPPG liposomes in the absence and presence of agomelatine, respectively. As can be seen, there is an abrupt change in the frequency at ~41 °C for both pure DPPC and DPPG liposomes. These abrupt changes indicate a transition from gel to fluid phase where ~41 °C is the main phase transition temperature (T_m). As it can be inferred from the figures, agomelatine abolishes the pre-transition and decreases the main phase transition temperature of zwitterionic DPPC and charged DPPG liposomes to lower degrees at all agomelatine concentrations. As seen from Fig. 2A, the addition of
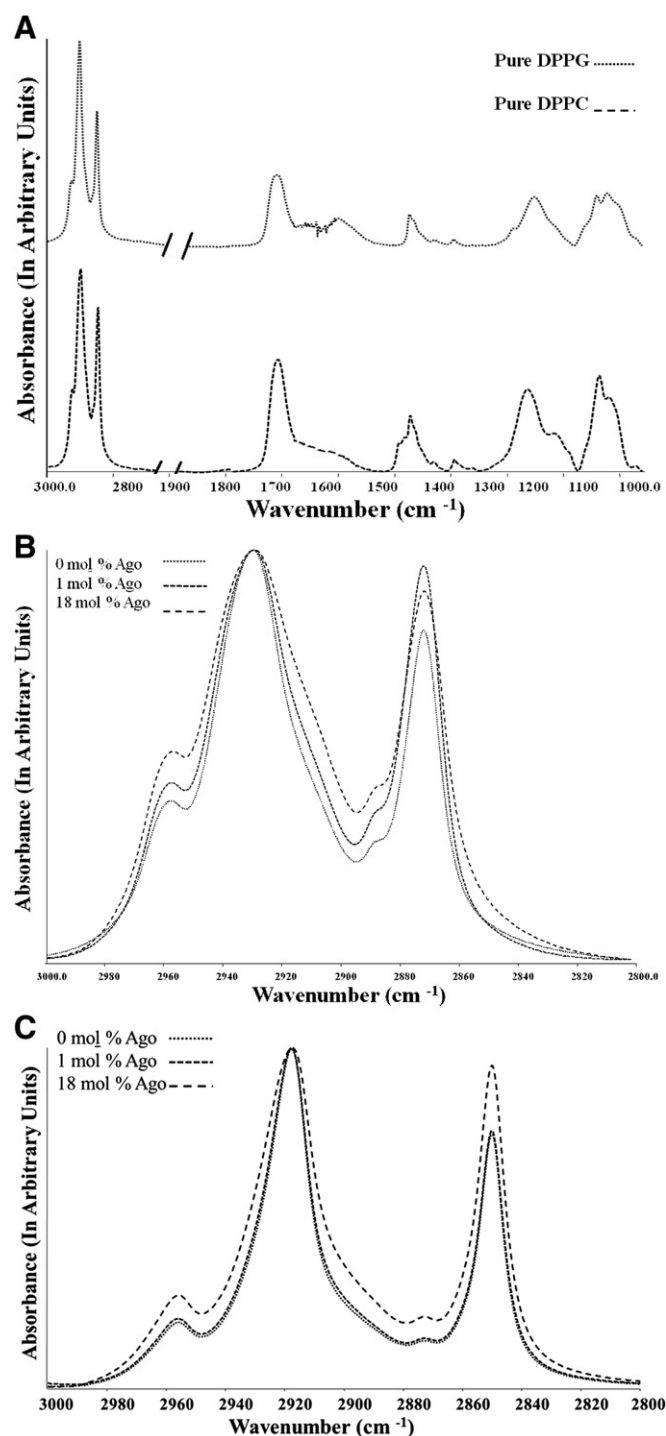

Fig. 1. FTIR spectra of (A) pure DPPC (.......) and DPPG (——) liposomes in the whole region, and (B) DPPC and (C) DPPG liposomes containing agomelatine of 0 mol% (.......), 1 mol% (——), and 12 mol% (- - -), in the CH stretching region at 44 °C (spectra were normalized with respect to the CH_2 asymmetric stretching band).

agomelatine to DPPC liposomes causes a downward shift in the frequency of the CH_2 symmetric band in both phases, which corresponds to the ordering effect (i.e. decrease in acyl chain flexibility) of agomelatine. Contrary, for DPPG liposomes (Fig. 2B), agomelatine causes a slight increase in the frequency values of the CH_2 symmetric stretching band in both phases indicating a disordering effect (i.e. increase in acyl chain flexibility).

Fig. 3A and B gives the temperature dependent bandwidth changes of the CH_2 symmetric stretching bands of DPPC and DPPG liposomes in the absence and presence of agomelatine, respectively. It can be
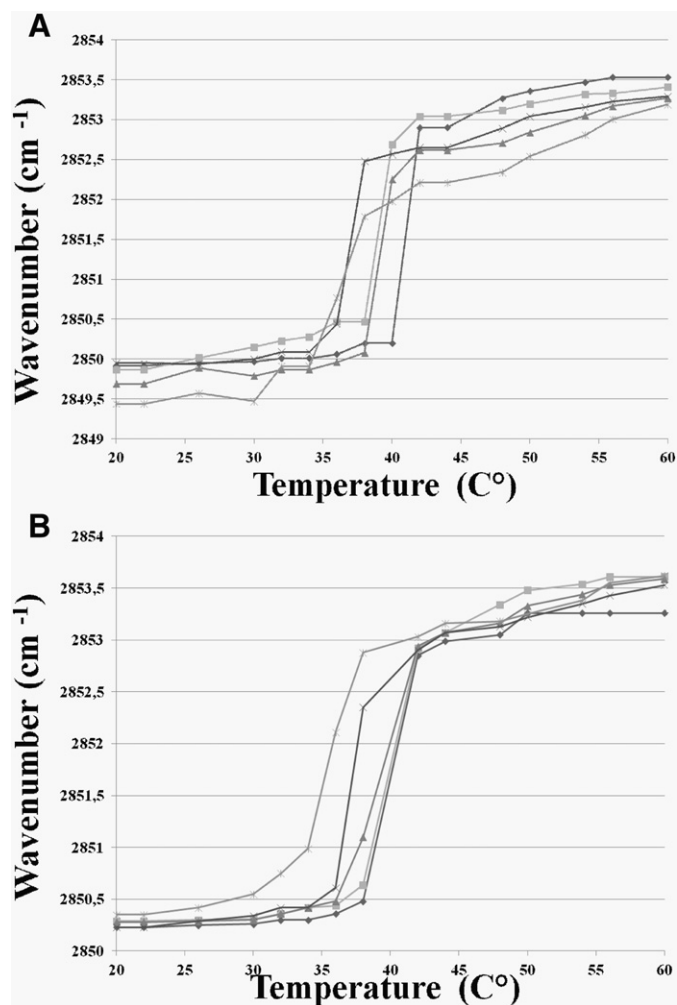
Fig. 2. Temperature dependent frequency changes of the CH$_2$ symmetric stretching mode of (A) DPPC liposomes and (B) DPPG liposomes for 0 mol% (♦), 1 mol% (■), 3 mol% (▲), 12 mol% (X) and 18 mol% (*) agomelatine concentrations.
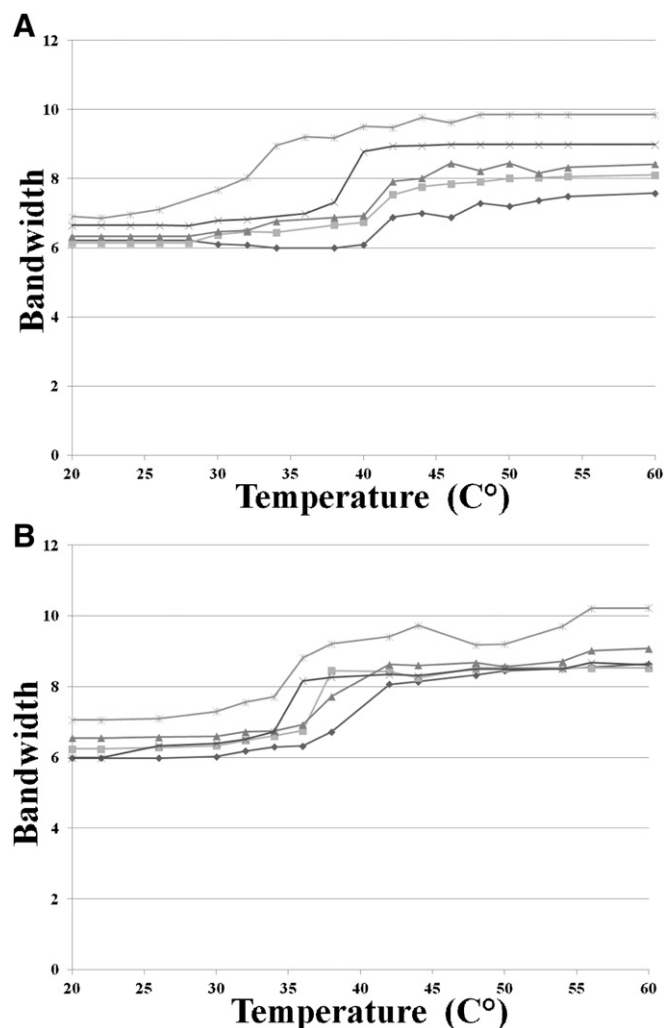

Fig. 3. Temperature dependent bandwidth changes of CH$_2$ symmetric stretching mode of (A) DPPC liposomes and (B) DPPG liposomes for 0 mol% (♦), 1 mol% (■), 3 mol% (▲), 12 mol% (X) and 18 mol% (*) agomelatine concentrations.

seen from the figures that, agomelatine causes an increase in the bandwidth implying an increase in the fluidity of both DPPC and DPPG liposomes at all concentrations both in the gel and fluid phases [22–24].

The variations in the frequency of the C=O stretching band monitor the hydration state of glycerol molecules which function as a bridge in between the phosphate head groups and the acyl chains of the phospholipids [19,22,24,26]. The temperature dependent frequency changes of the C=O stretching bands of DPPC and DPPG liposomes in the absence and presence of agomelatine can be seen in Fig. 4A and Fig. 4B, respectively. At a low (1 mol%) agomelatine concentration, the frequency of this band shifts to higher values, indicating a decrease in the hydrogen bonding capacity (i.e. dehydration) of the glycerol backbone of DPPC liposomes (Fig. 4A). In contrast, at higher concentrations of agomelatine, the enhancement of the hydrogen bonding capacity in the DPPC liposomes was noticed, which indicates that new H bonds are formed. In DPPG liposomes, agomelatine decreases the H bonding capacity of the glycerol backbone, i.e. it increases dehydration, in both phases except at 18 mol%, for which an increase in H bonding is observed (Fig. 4B). The changes in the frequency of the PO$_2^-$ symmetric stretching band give information about the hydration profile of head groups of phospholipids. A decrement in the frequency of this band corresponds to either the strengthening of the existing hydrogen bonds or formation of new ones [19,22,26,27]. Fig. 4C and D represents the changes in the PO$_2^-$ symmetric stretching band frequencies of DPPC and DPPG liposomes, respectively as a function of temperature. It can

be seen from Fig. 4C that, agomelatine increases the H bonding of the phosphate head group of DPPC when it is used at high concentrations (>1 mol%). Moreover agomelatine enhances the H bonding capacity of the phosphate head group of DPPG liposomes in the gel phase but lowers it in the fluid phase (Fig. 4D).

Finally, DSC thermograms of pure and agomelatine containing (1–18 mol%) DPPC and DPPG liposomes can be seen in Fig. 5A and Fig. 5B, respectively. Tables 1 and 2 give the corresponding phase transition temperatures (T$_m$), $\Delta T_{1/2}$ and enthalpy changes of DPPC and DPPG liposomes, respectively. The small peaks at ~34 °C and ~33 °C of pure DPPC and DPPG liposomes, respectively, are the pre-transition temperatures. As can be seen, pre-transition disappeared with the addition of agomelatine. On the other hand, the main phase transition of pure DPPC and DPPG liposomes are at ~41 °C and 40 °C with transition enthalpies (ΔH) 40.9 J/g and 42 J/g, respectively which are consistent with the literature values [28–30]. The main phase transition shifted to lower temperatures as the agomelatine concentration is increased. Furthermore, agomelatine causes a broadening in DSC thermograms of both neutral DPPC and charged DPPG liposomes at all drug concentrations which indicates loss in cooperativity in the phase transition profile [23]. Also the system becomes more fluid in the presence of agomelatine [24]. For concentrations of 12 and 18 mol% two peaks are clearly observed on the transition curve, implying the existence
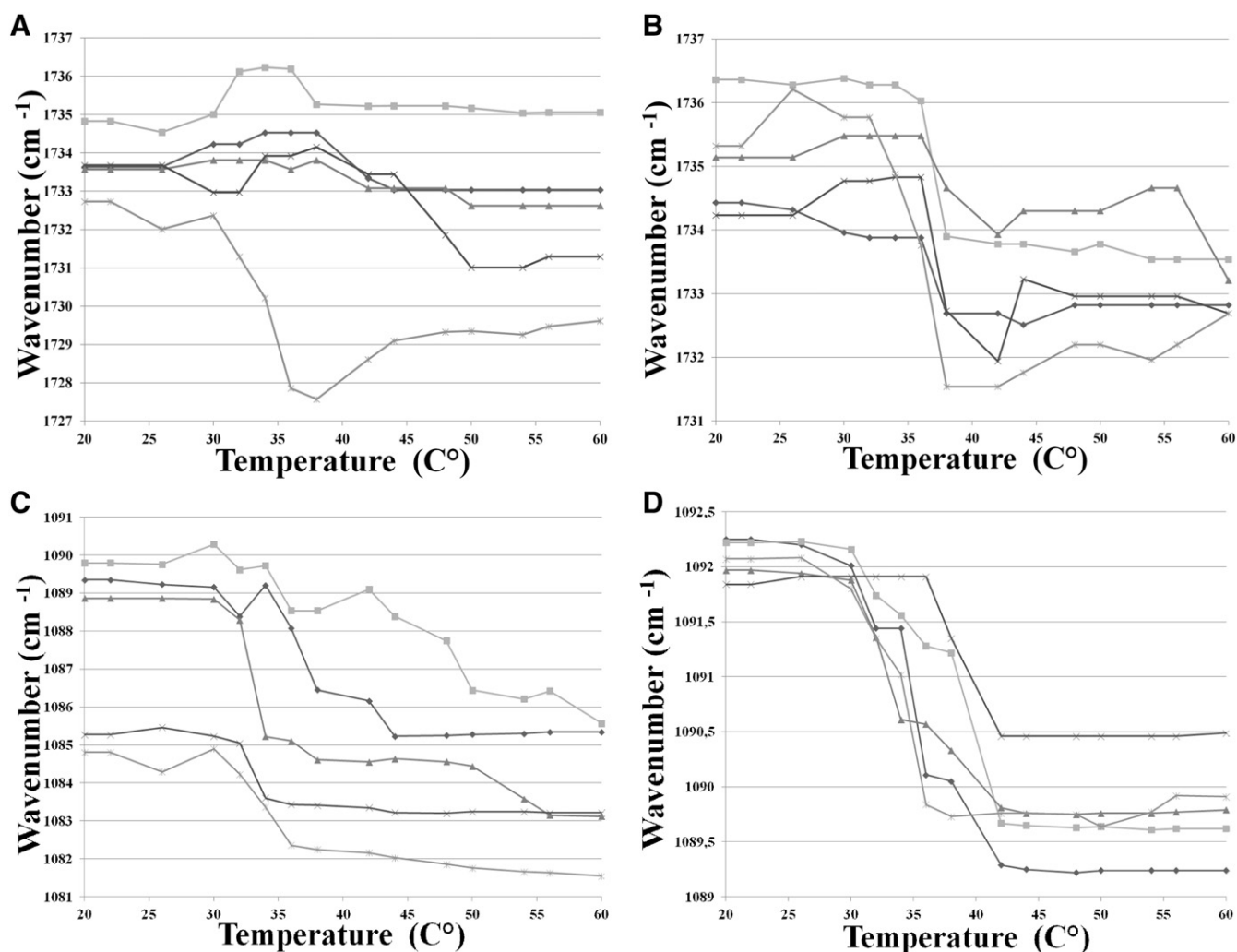
Fig. 4. Temperature dependent frequency changes of (A) C=O stretching band of DPPC liposomes and (B) DPPG liposomes; (C) PO_2^- asymmetric band of DPPC liposomes and (D) DPPG liposomes for 0 mol% (♦), 1 mol% (■), 3 mol% (▲), 12 mol% (X) and 18% mol (*) agomelatine concentrations.

of phase separation (domain formation) in the system. The domain formation can be seen better from Fig. 6A and B which shows the deconvoluted DSC thermograms of DPPC and DPPG liposomes at 18 mol% of agomelatine concentrations, respectively.

3. Discussion

In the current study, interaction of agomelatine with one of the main membrane lipids, namely DPPC, and its negatively charged counterpart DPPG in the form of MLVs was studied. The usage of DPPC in membrane studies is very common [22,24,31,32]. DPPG is also used to understand the charge effect on drug–membrane interactions [19–21]. Furthermore, there are studies that are concerned with the interaction of antidepressants or antipsychotic drugs with DPPG liposomes [20,21,33,34]. Besides, DPPG is one of the model membranes that is used in Alzheimer's disease [35–37] and Parkinson's disease [38,39] studies. Alzheimer's disease is characterized by Aβ aggregation and Parkinson's disease is characterized by α-synuclein aggregation in the brain. These mentioned studies look for the possible role of membrane lipids in Aβ and α-synuclein aggregation and state that both Aβ and α-synuclein bind to negatively charged lipids and use DPPG for this purpose.

Results obtained in the present study extend previous findings which demonstrated that agomelatine interacts around the head group in such a manner that it destabilizes the membrane architecture to a large extent. Thus, it causes alterations in the order, packing and

dynamics of the DPPC and DPPG model membranes. Although agomelatine was thought to exert its action by interacting only with melatonin MT_1 and MT_2, and serotonin $5\text{-}HT_{2C}$ receptors on nerve cells [4], here in the present study, we demonstrated that agomelatine has remarkable effects on T_m, acyl chain order, glycerol backbone and phosphate head groups and on the dynamics of zwitterionic and charged lipids which reveal that agomelatine interacts also with lipids in the membrane structure.

It has been reported that the bioactivity of drugs may alter the physical properties of membranes such as membrane fluidity and formation of lipid rafts [40] as well as membrane protein–protein and protein–lipid interactions [41,42]. Therefore, it is important to evaluate or predict drug–membrane interactions in order to comprehend their action mechanisms.

The broadening of the phase transition curves imply that agomelatine enters into the hydrophobic part of the multilamellar bilayers and disturb strong van der Waals interactions between the hydrophobic acyl chains which causes each phospholipid acyl chain to melt slightly at a different temperature [23,24]. This shows loss in cooperativity. The broadening and decrement of T_m suggest that agomelatine is partially buried in the hydrocarbon core of the bilayer, interacting primarily with the C2–C8 methylene region of the hydrocarbon chains [43]. This may lead to the enhancement of interactions between lipid head groups and/or drug and lipid head groups resulting with the disturbances of the packing of the system [44,45]. The order–
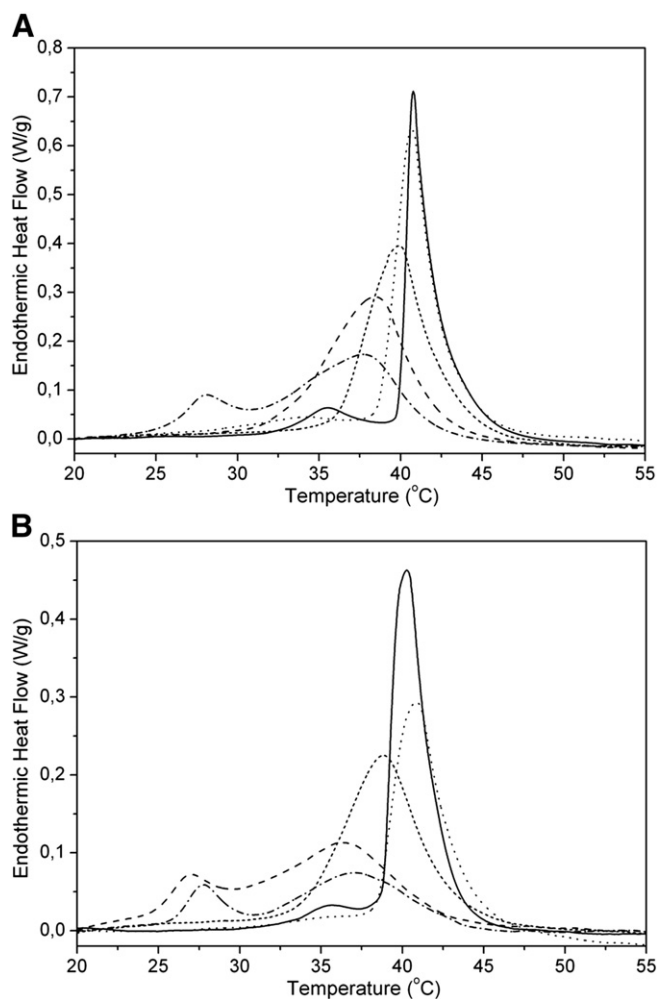
Fig. 5. DSC thermograms of (A) DPPC liposomes with and without different concentrations of agomelatine and (B) DPPG liposomes with and without different concentrations of agomelatine (0 mol% (point), 1 mol% (short dash), 3 mol% (dash), 12 mol% (long dash) and 18% mol (dash & dot)).

Table 2
Main phase transition temperatures (T_m), full width at half maximum ($\Delta T_{1/2}$) and enthalpies of the main phase transitions (ΔH) for DPPG liposomes.

Sample	T_m (°C)	$\Delta T_{1/2}$ (°C)	ΔH (J/g)
DPPG	40.27	2.2	42
DPPG + 1 mol% ago	40.9	3.1	31
DPPG + 3 mol% ago	38.9	4.9	38
DPPG + 12 mol% ago	36.3	–	–
DPPG + 18 mol% ago	36.9	–	–

optimally adapted trans-membrane segments in biological membranes [42]. Those disturbances in turn, may lead to impairments in membrane fusion and trafficking [45]. Also, controlled membrane fluidity is essential for the proper functioning of transmembrane receptors such as G coupled receptors [46].

DSC thermograms (in Fig. 5A and B) clearly showed that the thermograms broaden as the concentration of agomelatine is increased for both DPPC and DPPG liposomes. Starting from 12 mol% concentration of agomelatine for DPPG and at 18 mol% concentration of agomelatine for DPPC, two peaks are observed in the thermograms. The deconvolution of DSC thermograms at an 18 mol% concentration of agomelatine for DPPC and DPPG liposomes gave these peaks in resolved form (Fig. 6A and B). These indicate that there is lateral phase separation into drug-rich and drug-poor domains as observed in other studies [22,24,47,48]. Domain formation following drug addition may result in loosely packed lipid bilayers [24,49], which causes alterations in membrane permeability [50]. Therefore, the loose packing of lipid bilayers may innervate the intermolecular interactions [51]. The H bonding differences in the DPPG head groups among the gel and fluid phases may be due to the differences in the permeability of membranes at these two phases. In particular, gel phase bilayers are characterized by tight lipid packing and low permeability, whereas fluid phase analogs are loosely packed and have relatively high permeability. The highest permeation rate may happen around T_m where both gel and fluid phases co-exist. Therefore a mismatch can be suggested in lipid packing between the two phases that produces defects in which permeable molecules can pass [52].

In addition to temperature changes, gel to fluid phase transition can also be induced by changes in hydration. Observed progressive decreases in T_m with increasing hydration indicates that the adsorption of water molecules or H atoms decreases the strength of interactions between neighboring molecules in the lipid bilayer causing a disturbance at the polar head group [28]. Alterations at the phosphate head group may affect acyl chains [44], and the glycerol backbone region modifies most of the polar/non-polar interfacial parts of the bilayer where the chemical structure of the interfacial region can influence the overall conformation of the lipid molecule [28].

Agomelatine, having a 1H donor and a 2H acceptor site causes alterations in the H bonding capacity of all types of liposomes that were used in the current study. These H bonds might have been formed with glycerol backbones of adjacent phospholipids and/or with agomelatine [22, 26]. Furthermore, the interaction of agomelatine with the charged phospholipid DPPG is different from that of zwitterionic DPPC when the H bonding of the glycerol backbone and the state of order of acyl chains are considered. One possible reason for this observation could be that the cationic side chains of the drug may favor electrostatic interactions with the anionic lipid head group of DPPG, rather than interacting through hydrophobic interactions with the lipid acyl chain region or the glycerol backbone.

The main phase transition enthalpy of DPPC liposomes were found to be increased whereas DPPG liposomes were found to be decreased from the DSC results (Tables 1 and 2). The different inclinations in the main phase transition enthalpies of agomelatine–DPPC liposomes and agomelatine–DPPG liposomes that arose with respect to their pure

disorder state of lipids can be inferred from the shifts in the frequency of the CH_2 symmetric stretching band; a decrease in the frequency corresponds to an increase in the lipid order (decrease in acyl chain flexibility) of the system [22,24]. The alterations in the bandwidth of the C–H stretching bands reflect the changes in the dynamics and hence fluidity of the system [7,22]. The increase of bandwidth indicates an increase in the fluidity of the system or vice versa. According to the results of this study, agomelatine enhances the membrane fluidity in both the gel and fluid phases for all liposomes studied (Fig. 3A and B). Besides, agomelatine has an ordering effect on zwitterionic DPPC liposomes and disordering effect on charged DPPG liposomes in both phases (Fig. 2A and B). These changes reveal that agomelatine has induced alterations in membrane thickness and structure [28,41]. Those changes may cause disturbances in the selection of certain proteins with

Table 1
Main phase transition temperatures (T_m), full width at half maximum ($\Delta T_{1/2}$) and enthalpies of the main phase transitions (ΔH) for DPPC liposomes.

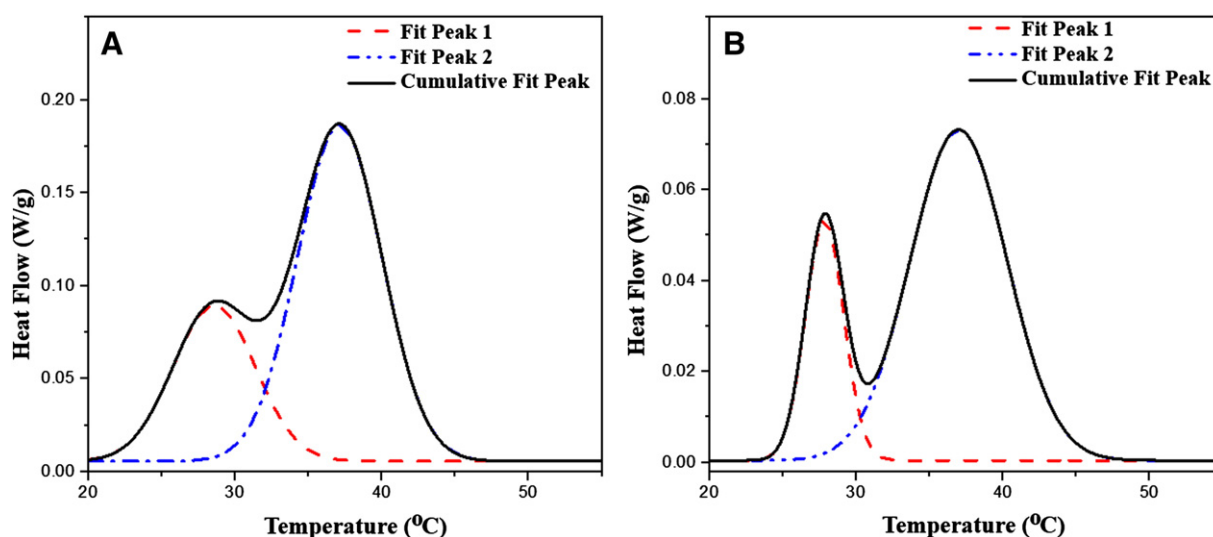| Sample	T_m (°C)	$\Delta T_{1/2}$ (°C)	ΔH (J/g)
DPPC	40.8	1.5	40.9
DPPC + 1 mol% ago	40.8	2.3	49.7
DPPC + 3 mol% ago	39.9	4.0	53.6
DPPC + 12 mol% ago	38.4	5.4	56.1
DPPC + 18 mol% ago	37.9	–	–

Fig. 6. Deconvoluted DSC thermograms of (A) DPPC and (B) DPPG liposomes at 18 mol% of agomelatine concentrations.

DPPC and DPPG forms are due to the differences in the head group charges of liposomes.

The main phase transition of liposomes is affected mostly from the van der Waals and *trans/gauche* rotameric energy [31,53]. In pure liposomes, an increment in the *gauche* conformations takes place which causes the liposomes to gain a rotational mobility during the main phase transition [54]. However, FTIR spectroscopic results showed that agomelatine interaction with DPPC liposomes caused the lipids to go to an ordered state, i.e. less *trans/gauche* isomerization of fatty acyl chains and so a reduction in the rotameric energy [31]. This in turn suggests that, the increment in the enthalpy is not due to the rotational excitations of fatty acyl chains. Furthermore, as stated, the broadening of the DSC curve implies that agomelatine interacts with the hydrophobic part of the membranes, i.e. fatty acyl chains. Thus, the increase in enthalpy can be due to the changes in the van der Waals energy as a result of agomelatine interaction with the hydrophobic part of DPPC liposomes [31,55–59].

As for the DPPG liposomes, it was found that agomelatine interaction caused the disordering of liposomes, meaning that the number of *gauche* conformers increased (acyl chain flexibility) and this should result in an increase in the phase transition enthalpy. Thus, agomelatine might be causing a weakening in the van der Waals interactions due to strong dipole–dipole interactions and/or changes in the hydration profile of phosphate head groups that may affect fatty acyl chains as mentioned above [60]. Furthermore, the reduction in the main phase transition enthalpy due to agomelatine interaction with DPPG might also be due to the inhibition of some of the DPPG liposomes to undergo phase transition [61–64].

It has been reported that agomelatine binds to melatonin receptors, suppresses cyclic adenosine monophosphate (cAMP) formation and mimics the actions of melatonin in a dose dependent manner, inhibiting the firing rate of suprachiasmatic nucleus neurons [4]. These observations were later substantiated when it was shown that agomelatine also potently activates cloned human MT_1 and MT_2 receptors and mimics melatonin [65]. Regarding the previous studies held in our laboratory, agomelatine exerts the same effects on DPPC liposomes when compared with melatonin [22]. Both drugs increase the number of *trans* conformers and the dynamics of the membrane in the DPPC liposome. However for DPPG liposomes, there are some differences between the effects of these drugs. For example, melatonin induces opposite effects on lipid order at high and low concentrations. While melatonin causes an increase in the order of membranes both in the gel and fluid phases at low concentrations, it increases the number of *gauche* conformers, which indicates a decrease in the order of the bilayer at high concentrations [66]. Nonetheless, agomelatine decreases the lipid order of the DPPG liposome at all phases. Furthermore, in DPPG liposomes, melatonin slightly increases the membrane dynamics both in the gel and fluid phases at high concentrations, but in low concentrations, it decreases the dynamics. However, agomelatine enhances the membrane fluidity among all types of liposomes studied at all concentrations. Finally, both melatonin and agomelatine increase the strength of H bonding around the phosphate head group for DPPC liposomes. It is obvious that agomelatine does not show the same effect with melatonin on every liposome type. The reason for this dissimilarity may be the differences between their chemical structures. Melatonin has 2H-bond donors and 2H-bond acceptors whereas agomelatine has a 1H-bond donor and 2H-bond acceptors. Besides, these two drugs might have some unknown different action mechanisms, which may be another reason for this difference.

Rodrigues et al. (2002) [67] stated that the perturbation of the cell membrane structure represents an immediate component of the apoptotic pathway in cells, which results in a rapid disruption of membrane lipid polarity and fluidity, altered protein order, and increased oxidative injury, which precede metabolic and morphologic manifestations of apoptosis. Functionally, the increase in plasma membrane fluidity was found to be associated with the apoptosis of nerve cells [67]. Regarding our findings of agomelatine-induced increase in the membrane fluidity of DPPC and DPPG liposomes, agomelatine may also play a role in the activation of apoptotic pathways in nerve cells.

The molecular organization of the membrane landscape plays an extremely important role in a great variety of processes associated with the membrane [68]. Phospholipids were mainly recognized as second messengers and their effect on membrane dynamics and structure was correlated with their role as a host to signaling molecules [69–71]. Except for protein–protein contacts, the membrane-spanning segments of integral membrane proteins are surrounded by a shell of adjacent boundary lipids that mediates the coupling between the mostly hydrophobic intra-membranous residues of the protein and the lipid bilayer. In addition to electrostatic interactions, specific lipid–protein interactions have also been reported. The alterations in the structure of hydrophobic regions of the membrane may also influence both the structure as well as the function of a number of integral membrane proteins [46,72]. Since we have observed structural changes in model membranes of all of the phospholipids investigated in this study, these alterations in lipid structure may also affect the protein–lipid interactions in biological membranes, therefore it can be concluded that agomelatine

may have an effect on the action mechanisms of integral membrane-spanning proteins in biological membranes. Membrane fluidity is an important concept for membrane fusion and it is required for membrane trafficking, regeneration of various sub-cellular compartments after cell division, and cell growth. Both proteins and lipids have a role in the regulation of membrane fluidity [45] and a controlled membrane fluidity is essential for the proper functioning of transmembrane receptors, such as G coupled receptors [73]. Also membrane fusion is a process that is regulated by both lipids and proteins [74]. According to the results of this model lipid membrane study, agomelatine enhances the membrane fluidity among all types of liposomes studied; therefore, the membrane fusion mechanism may be affected following the administration of agomelatine in biological membranes.

In conclusion, we shed valuable insights into the molecular mechanisms of the interaction of agomelatine with DPPC and DPPG MLVs. Our results revealed that agomelatine causes alterations in the order, packing and dynamics of the DPPC and DPPG model membranes. Changes in the order–disorder state of liposomes were monitored from the CH_2 symmetric stretching bands. It was found that agomelatine causes the ordering of DPPC liposomes, whereas it causes the disordering of DPPG liposomes in both gel and fluid phases. The effect of agomelatine on the fluidity of liposomes was found from the changes in the bandwidth of the CH_2 symmetric stretching bands and an increase in the fluidity of both lipids in both phases was found. The hydration states of glycerol molecules and head groups of phospholipids were found by monitoring the frequency of the $C{=}O$ stretching and the PO_2^- symmetric stretching bands, respectively which revealed that, for DPPC liposomes, the H bonding capacity of the glycerol backbone of both liposomes decreases (dehydration) at low concentration (1 mol%) and increases at higher concentrations in both phases. However for DPPG liposomes, agomelatine generally dehydrates the system in the fluid phases. In the gel phase, it induces dehydration around the glycerol backbone, while the H bonding capacity increases around the head group. DSC studies clearly showed the agomelatine-induced domain formation for both lipids. Although it has been stated that agomelatine only interacts with MT_1, MT_2 and $5\text{-}HT_{2C}$ receptors in the cell membrane, here in this study, we have demonstrated that it strongly interacts with membrane phospholipids.

It is known that, lipid structure and dynamics may have an influence on the structure of membrane bound proteins. Therefore ion conductivity and cell signaling may be affected following the perturbation of membrane bound proteins. All those results highlight the fact that agomelatine interact with the head group in such a manner that it destabilizes the membrane architecture to a large extent. The overall DSC and FTIR spectroscopic data indicate that agomelatine induces changes in the structure and psychico-chemical characteristics of the liposomes. This means that agomelatine is able to change the neuroplasticity of neuronal networks and this statement may contribute to its clinical effects. Although this idea was supported by a recent study which demonstrated that agomelatine modulates the expression of cytoskeletal microtubular proteins, synaptic markers and brain derived neurotrophic factor (BDNF) in the rat hippocampus, amygdala and prefrontal cortex [75], further studies should be conducted to specifically address the effect of agomelatine on lipid–protein interactions and neuroplasticity.

It is important to understand the action mechanism of a drug to understand its pharmacologic effect. In the current study, we studied the general effects of agomelatine on the structure and dynamics of model membranes obtained from two different lipids in order to see the charge effect. Since there is no published study yet on this topic, the information that is derived from this study would be very valuable as a control study of future studies on the interaction of agomelatine with other lipid model membranes which, for example, mimic the brain membranes, and real biological membranes such as liver microsomal membranes and brain membranes. However in order to better understand the antidepressant activity and/or other therapeutic effects of agomelatine in addition to its overall pharmacological effects, agomelatine-treated healthy and diseased animal membrane and tissue studies should be performed for which the results of model membrane studies would be essential.

4. Materials and methods

4.1. Chemicals

Agomelatine, (N-[2-(7-metoksinaftalen-1-yl)etil]acetamid), dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidyl-glycerol (DPPG) and phosphate buffered saline (PBS) tablets were purchased from Sigma (St. Louis, MO, USA). All chemicals were obtained from commercial sources at the highest grade of purity available.

4.2. FTIR studies

For FTIR spectroscopic studies, multilamellar vesicles (MLVs) were prepared in the absence and the presence of 1, 3, 12 and 18 mol% agomelatine according to the procedure reported in [22,49]. Briefly, 5 mg of DPPC and DPPG were separately dissolved in 150 μl chloroform in tubes and the solution was subjected to a stream of nitrogen to remove excess chloroform followed by vacuum drying for 2 h. Subsequently, a dry film was obtained. Thin films of lipids were hydrated by adding 25 μl of PBS buffer solution, pH 7.4. MLVs were formed by vortexing the mixture for 20 min at a temperature of 20 °C above the main transition temperature of lipids. To prepare agomelatine containing MLVs, the appropriate amount of agomelatine from stock solution (2.5 mg/ml) was initially placed inside the sample tube. Excess ethanol was removed by a stream of nitrogen, then the phospholipid was added and MLVs were prepared as described above.

The PBS solution that we used during the experiments was obtained by dissolving the PBS tablets in deionized water. This buffer was also used in other MLV studies [76–78]. This results in a solution of 0.01 M phosphate buffer with 0.0027 M potassium chloride and 0.137 M sodium chloride with a pH level of 7.4. The resultant ionic strength of the solution is 162.7 mM. This results in a Debye length, i.e. the screening length of charges that are in solutions with salts, of 0.76 nm at 300 K [29,79]. Such a Debye length has the same order of magnitude as the lipid–lipid spacing of MLVs [80].

For FTIR spectroscopic data collection, 20 μl of liposomes was placed between CaF_2 windows with a 12 μl spacer to obtain consistent sample thickness. Spectra were recorded using a Perkin Elmer Spectrum 100 FTIR spectrometer (Perkin Elmer, Inc., Norwalk, CT, USA) equipped with a deuterated triglycine sulfate (DTGS) detector, in the temperature range of 20–60 °C. Temperature was controlled digitally by a Graseby Specac controller unit. Samples were incubated for 5 min at each temperature before the acquisition of a spectrum. Interferograms were averaged for 100 scans at 2 cm^{-1} resolution. The spectrum of the air was recorded as a background spectrum and subtracted automatically from the spectra of samples by using the Perkin Elmer Spectrum One software, which was also used for data analyses.

Since the OH stretching bands due to the buffer appear in the regions of 3400–3200 cm^{-1} and 1800–1500 cm^{-1}, these bands overlap with the bands of interest. Therefore, the spectrum of the buffer was taken at different temperatures and was subtracted from the spectrum of liposomes at corresponding temperatures. The subtraction process was performed till the bulk water region located around 2100 cm^{-1} was flattened using the Perkin Elmer software program.

For the determination of variations in peak positions and bandwidths, each original spectrum was analyzed by using the same software. The band positions and bandwidths were measured from the center of weight (0.80× peak height position), respectively. The detailed analyses were performed from the subtracted native spectra.

However, for visual demonstration of the spectral differences in the spectra, the spectra were normalized with respect to the specific bands.

4.3. DSC studies

For calorimetric studies, MLVs were prepared in the absence and the presence of 1, 3, 12, and 18 mol% agomelatine. For the preparation of MLVs, thin films containing 2 mg lipid were hydrated by adding 50 μl of PBS buffer solution, pH 7.4, and the procedure mentioned above was followed. 50 μl MLV suspensions were encapsulated in hermetically sealed standard aluminum DSC pans. An indium containing pan was used as reference during the analysis.

Investigation was performed with a Universal TA DSC Q100 v 6.21 instrument. The scans were collected at 1 °C/min. Only heating curves are presented. Cooling curves were essentially identical. The enthalpy ($\Delta H°$ cal) values were calculated by calculating the area under main transition.

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