The effects of melatonin, serotonin, tryptophan and NAS on the biophysical properties of DPPC monolayers

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\textbf{ABSTRACT}

Melatonin is a neurohormone that has been shown to be protective in Alzheimer’s diseases against amyloid-β (Aβ) toxicity, which involves interaction of Aβ with neuronal membrane. Non-specific interactions of melatonin with cell membrane may play a physiological role in this process by preserving membrane fluidity. In the brain, melatonin is derived from the amino acid tryptophan through a pathway that includes serotonin and N-acetylserotonin (NAS). How these molecules affect the membrane properties is not understood. In this work, we studied interactions of melatonin and its metabolic precursors tryptophan, serotonin and NAS with dipalmitoylphosphatidylcholine (DPPC) monolayers at the air-water interface using Langmuir monolayer technique. Analysis of compression isotherms, phase transitions and compressibility moduli indicate that all four molecules alter the DPPC monolayer properties in a structure and concentration dependent manner. This effect was most pronounced for melatonin followed by NAS. Melatonin and NAS both decreased the compressibility modulus and shifted the LE/LC phase transition suggesting an increase in the membrane fluidity. Tryptophan and serotonin caused less pronounced effects on the DPPC isotherm. These differences suggest different interaction mechanisms and may be attributed to the interplay between electrostatic and hydrophobic interactions of these molecules with the zwitterionic DPPC headgroups which correlate with water solubility and oil partition coefficients (LogS and LogP) of each of the four molecules. The results here demonstrate how the physiochemical properties of indoles can affect lipid membranes which may shed light on the functional significance of these important neurochemicals and the neuroprotective mechanisms of melatonin.

1. Introduction

Alzheimer’s disease (AD) is a neurodegenerative disease with no cure, and few viable treatment options, none of which slow disease progression. The primary cause of AD has long-thought to involve over-production, misfolding and aggregation of amyloid-β (Aβ) into neuro-toxic oligomers [1]. The amyloid precursor protein (APP) is cleaved by β- and γ-secretases across the membrane spanning region of APP to produce the neurotoxic Aβ fragment [2]. The neurotoxicity of Aβ is fundamentally linked to interactions with the cell membrane, which provides an interface for misfolding and nucleation of toxic oligomers [3,4]. Aβ has been shown to cause defects in the plasma membrane [4,5], increased membrane permeability [6,7], and the formation of Ca\textsuperscript{2+} ion channel pores in the cell membrane [8,9], all of which disrupt membrane integrity. Furthermore, there is increasing evidence for lipid dysfunction in AD pathogenesis as lipid metabolism genes are amongst the leading risk factors for AD [10], lipidomic studies have identified differences in lipid composition of AD brains compared to age-match controls [11,12], and mechanistic studies reveal how amyloid toxicity is affected by lipid membrane composition and membrane lipid rafts [3,13,14]. Restoring or preventing the changes in lipid membrane properties caused by altered brain lipid composition may be necessary for the successful treatment or prevention of AD.

Plasma membrane lipids serve a key structural role of cells, with the lipid composition – especially cholesterol, affecting the biophysical properties that contribute to the folding, physical conformation, trafficking and activation of membrane proteins in and anchored to the lipid bilayer [15–18]. These important membrane biophysical
properties include membrane potential, curvature and membrane fluidity and are important for normal cell function. These membrane features depend on electrostatic and hydrophobic properties of membrane components, as well as lipid packing density (or ordering parameters). The physical structure of the plasma membrane is important, compounds that can bind or partition into the membrane may exert effects by modifying membrane properties, such as: cell-adhesion, antimicrobial and viral-fusion peptides [19,20], and small molecules, both endogenous ligands [21–23] and pharmaceutical drugs [24–27]. To make predictions about the function of membrane-active small molecules it is important to understand how their chemical structure and properties influence lipid membranes, and their properties.

Melatonin is a lipophilic neuroendocrine hormone produced in the pineal gland that has been of interest as a therapeutic for treating AD [28]. Melatonin is key for regulating sleep-wake cycles and circadian rhythm, and it has been shown that melatonin levels in cerebral spinal fluid decrease with age and are much lower in AD patients than in age-matched controls [29,30]. Melatonin has also been shown to be neuroprotective in several animal models of AD and in vitro against both Aβ, oxidative stress and lipid peroxidation [31,32]. Most curiously, the protective effect of melatonin from Aβ toxicity has shown to be melatonin receptor independent, indicating non-specific interactions are involved [33]. As melatonin has demonstrated potent antioxidant activity it may provide direct protection from lipid peroxidation, preserving the fluidity of the lipid membrane during oxidative stress [31,34,35]. It has also been shown that melatonin increases membrane fluidity directly, absent lipid peroxidation, and has a thinning effect on the membrane that may counteract the rigidity imposed by cholesterol [36,37]. This is important as membrane cholesterol and rigidity potentiates neuronal susceptibility to Aβ and affect Aβ-membrane interactions [5,14]. Unfortunately, the results of melatonin in clinical trials are mixed, with a recent meta-analysis showing no significant improvement in cognitive function, however melatonin appears to be useful in managing AD-related sleep disturbances [38,39]. As of yet, no disease-modifying clinical trials for AD have been successful at changing disease trajectory and it appears preventative trials may be the way forward [40], if so, the use of melatonin as a preventative treatment especially in at-risk and prodromal populations may yet demonstrate promise.

Melatonin is derived from tryptophan, first tryptophan is converted to 5-hydroxytryptophan, then to serotonin, serotonin is then converted into N-acetylserotonin and finally melatonin (Fig. 1). These precursor molecules in the melatonin biosynthesis pathway (Fig. 1) are important for a variety of physiological functions and although interest regarding melatonin's effect on lipid membranes has grown, these similar molecules differing have not been studied. The differences in the structure and physical properties of these molecules (Fig. 1) should affect interactions with membrane lipids and thus how they affect membrane properties. In this report, we studied interactions of melatonin, tryptophan, serotonin and NAS with a DPPC lipid monolayer using the Langmuir monolayer technique to understand and compare how these related molecules incorporate into and affect the DPPC monolayer, used as a model of the lipid membrane.

2. Materials and methods

Dipalmitylphosphocholine (DPPC, ≥99%) was purchased from Avanti Polar Lipids; L-tryptophan (≥99.5%), melatonin (≥98%), HPLC grade chloroform (99%) and methanol (99%) was purchased from Sigma Aldrich; serotonin hydrochloride (≥98%) was purchased from Santa Cruz Biotechnology; and N-acetyl-5-hydroxytryptamine (NAS, ≥99%) was purchased from Cayman Chemicals. Whatman CH1 chromatography paper (10 mm) was used for Wilhelmy Balance.

Pressure-area isotherms of DPPC monolayers were collected on the Langmuir-Blodgett (LB) trough (NIMA-KSV). The LB trough subphase was prepared by dissolving each indole in pure MilliQ water, (resistivity > 18.2 MΩ) at low and high concentration: 0.1 mM and 1.0 mM for melatonin and 1.0 mM to 10.0 mM for tryptophan, serotonin and NAS. Melatonin was tested at lower concentrations as it has a low aqueous solubility that restricts the upper limit at which it can be tested. Pure MilliQ water alone was used as subphase for the DPPC control. The pH of each solution was measured and there was no effect of melatonin or any of its precursors on the pH of the pure water subphase (pH 6.8 ± 0.1). Prior to each experiment, the trough was cleaned thoroughly three times with ethanol then three times with MilliQ water. Once the subphase was filled, the barriers of the trough were fully opened to 145 cm² and the pressure sensor was zeroed. The trough was then closed to 85 cm² and 30 µl DPPC dissolved in HPLC grade chloroform/methanol 4:1 (v/v) at 1.0 mg/ml was deposited using a gas-tight syringe (Hamilton Company, USA) onto the subphase. The volume was determined by the lens method and used for all compressions. The solvent was evaporated for 10 min. The barriers of the trough were then fully opened to an area of 145 cm², the balance was left to come to equilibrium, after 10 min the pressure was checked, minute amount of subphase was vacuum aspirated off to zero any residual pressure (only isotherms with residual pressure no >2 mN/m were used). During data acquisition the barriers were closed to a final area of 25 cm² at a compression rate of 20 cm²/min (2.45 Å²/min × molecule). All experiments were collected in ambient conditions with at least two control isotherms performed between each set of indoles to provide an accurate representation of the variance due to fluctuations in ambient conditions over the course of data acquisition. Pressure versus area isotherms were repeated a minimum of four times, the average isotherm with standard error in the mean (SEM) is shown. The early isotherm region when the monolayer is most expanded was used to correct error in lipid deposition by fitting a slope to each isotherm and shifting the curves to align the slopes.

The elastic compressibility modulus (C_{s}^{-1}) was calculated (Origin 2020) from the averaged isotherm using Eq. (1) below, where A is the area of the trough, and P is the surface pressure. Compressibility modulus near the bilayer equivalent pressure was computed at 35 mN/m for each isotherm, then means and standard error in the mean (SEM) were computed.

\[
C_{s}^{-1} = -\frac{\Delta P}{\Delta A}
\]

(1)

One-way ANOVA with Dunnett's multiple comparison was performed to confirm statistical significance of the compressibility modulus near bilayer equivalent pressure with α = 0.05 and p < 0.05 considered significant. Statistical analysis was performed using GraphPad Prism software.

3. Results and discussion

Compression isotherms measure the surface pressure of thin films in response to mechanical compression (reduction in surface area), which provides important information regarding the mechanical and thermodynamic properties of surfactants and lipid monolayers, a schematic is shown in Fig. 2. In this report, the Langmuir-Blodgett trough was used to collect compression isotherms of a self-assembled DPPC monolayer at the water/air interface on a subphase of pure water containing the indoles (tryptophan, serotonin, NAS and melatonin). Once the DPPC monolayer is spread at the air/water interface, the indoles present in the subphase start interacting with and incorporate into the lipid monolayer, thus affecting the molecular area, pressure, compressibility, and phase transitions. These interactions between the lipid and indole depend on the lipid type and the indole molecule, including molecular geometry, structure, charge/polarity, and hydrophobicity. For the control DPPC monolayer in this study the LE/LC phase transition was found to occur near a pressure of 7.9 ± 0.3 mN/m (Fig. 3), which agrees well with other reports that observe the transition to be between 5 and 10 mN/m [36,42]. The large variation in the literature with respect to the pressure of the DPPC phase transition have been
attributed to the differences in temperature, leakage during compression (thus reducing the number of molecules), the presence of buffering salts, and differences in compression rates (affect lipid relaxation times in the membrane and thus affect molecular areas) [43].

In our experiments, melatonin, tryptophan, serotonin, and NAS were dissolved in the water subphase at the following concentrations: 0.1 mM and 1.0 mM for melatonin and 1.0 mM to 10.0 mM for tryptophan, serotonin, and NAS. Melatonin was tested at lower concentrations as it has a low aqueous solubility that restricts the upper limit at which it can be tested. Synaptic vesicles of neurons have serotonin concentrations as high as hundreds of millimolar, and thus may have concentrations at the synaptic cleft reaching tens of millimolar [44]. While systemic melatonin concentrations are on the tens of nanomolar range, local concentration in the pineal gland and circumventricular regions are orders of magnitude higher especially during the dark cycle, with high micromolar to low millimolar range considered to be pharmaceutically relevant [30,45]. These experiments were carried out at a pH of 6.8 ± 0.1, near physiological pH, at which both NAS and melatonin will be uncharged, while it is expected that 99.82% of the serotonin to be cationic (at pH 7) [46], and tryptophan (-NH₃⁺: pKa = 9.39 and -COO⁻: pKa = 2.38) to be in a zwitterionic state.

The pressure vs area isotherms are plotted (Fig. 3) as a function of the normalized total molecular area ($A_r = A_{DPPC} + A_{subphase}$), with the control ($A_0 = A_{DPPC}$). With each of the isotherms we see a translation in the isotherm along the area axis in a concentration dependent manner, this coincides with a lengthening of the LE phase (Fig. 3). This shift to lower molecular area is due to the reduction of lipids on the surface required to bring the isotherm to the beginning of the LE state at the start of the isotherm. This space in the membrane is then occupied by the subphase molecule during the compression isotherm. This can be measured as the change in molecule area of isotherm which has been plotted as a function of subphase concentration in Fig. 4A. We observe a
correlation between the change in molecular area (ΔA) and the hydrophobicity, as measured by the LogP values for melatonin and its precursors (Fig. 1). Melatonin is the most hydrophobic (LogP 1.42) and at 1.0 mM increased the area per molecule by ΔA = 15.8 ± 2.6%, which is about four-fold higher than NAS at that same concentration (ΔA = 4.2 ± 2.8%, 1.0 mM) and was not significantly different than NAS at 10.0 mM (ΔA = 16.8 ± 1.9%), despite an order of magnitude lower concentration. The LogP of NAS is 0.98, indicating a nearly 10-fold preference for the hydrophobic phase over the hydrophilic phase. Tryptophan (LogP -1.1) also caused an increase in the molecular area of

Fig. 3. Pressure-Area Isotherms. DPPC monolayers prepared with subphases containing various indoles at low (light) and high (dark) concentration, the area is expressed in terms of the normalized total molecular area as defined in the text, average curves ± SEM are presented.

Fig. 4. Change in area (ΔA) due to incorporation of the isotherm subphase vs concentration (A) shows a dose-dependent relationship between hydrophobicity and the increased change in area. The change in pressure at the LE/LC phase transition vs concentration (B).
M. Robinson, et al.

BBA - Biomembranes 1862 (2020) 183363

Serotonin had no effect on the monolayer area at any concentration despite a higher LogP value (0.58) than tryptophan, the difference here may be explained by the lower water solubility of tryptophan (1.36 mg/ml vs 2.5 mg/ml). This suggests that melatonin and then NAS are most effective at incorporating into the monolayer, to a lesser extent tryptophan can also penetrate the monolayer whereas serotonin cannot, the trend is proportional with the inverse of LogS, rather than LogP.

We also see a similar trend for the change in the pressure at which the LE/LC transition occurs with one interesting difference, we observe that serotonin, but not tryptophan causes an increase in the pressure. The effects of NAS and melatonin on the DPPC monolayer were similar, with a stronger effect for melatonin than NAS at 1.0 mM, this is likely explained by the difference in hydrophobicity between melatonin and NAS, compare LogP value of 1.42 to 0.98, respectively. NAS at 1.0 mM caused a small effect on the isotherm, increasing the pressure at which the LC/LE phase transition occurred by 1.1 ± 0.4 mN/m compared to control (Fig. 4B). This suggests that NAS can incorporate into the monolayer and take up space in the monolayer increasing the molecular area and surface pressure at low concentration. On the other hand, melatonin at 1.0 mM was much more dramatic and pronounced than NAS at 1.0 mM increasing the pressure by 2.9 ± 0.3 mN/m. Melatonin has a lower water solubility than NAS, restricting the upper limit of concentrations for these experiments. Serotonin had an interesting effect on the DPPC monolayer at high concentration (10.0 mM) where it caused an increase in the pressure (II) at the LE/LC phase transition of the isotherm (Fig. 4, right). The pressure increase at the LE/LC phase transition was 1.7 ± 0.4 mN/m compared to control, to 9.6 ± 0.4 mN/m (Fig. 4), which was significantly greater than the effect of tryptophan. However, since serotonin does not change area per lipid of the monolayer even at high concentration (Fig. 4), this suggests serotonin may be binding directly to the PC headgroup of the DPPC molecules, without incorporating into the monolayer. Overall, the effect of the indoles on the LE/LC phase transition pressure correlates with LogP, and not with the LogS, in contrast to the molecular area, ΔA.

The elastic compressibility modulus (C_{50}^{-1}) has been extracted from the isotherm (according to Eq. (1)) and presented in Fig. 5 as a function of the pressure. The C_{50}^{-1} is a parameter that correlates with the bending and bulk modulus of the monolayer that is proportional to the stiffness of the membrane [26,47]. C_{50}^{-1} depends on the thermodynamic properties of the lipid membrane, for instance it is proportional to the compression rate and inversely proportional to the temperature, i.e., higher compression rates increase membrane stiffness, while higher temperature increases membrane fluidity.

Monolayer-bilayer correspondence theory can be used to analyze the isotherm near bilayer equivalent pressure, which has been obtained both experimentally and theoretically, with values around 35 mN/m [48,49]. We find that at low concentration (1.0 mM), tryptophan slightly increased the compressibility of the monolayer across the whole isotherm and at the bilayer equivalent pressure from 170 ± 24 mN/m to 203 ± 8 mN/m (P < 0.01**, Fig. 6). There was no significant effect on compressibility modulus at the bilayer equivalent pressure at high concentration (Fig. 5). This indicates that tryptophan may have an ordering effect on the membrane at high concentration and low surface pressure but is to some extent squeezed out of the membrane at higher pressure. This may suggest multiple possible binding sites or interaction mechanisms between tryptophan and DPPC monolayer, at low concentration and toward the end of the isotherm, at high pressure, tryptophan may participate in electrostatic interactions with the headgroup, but at high concentration and low pressure hydrophobic effects may drive the equilibrium position of tryptophan deeper into the headgroup region.

Serotonin was observed to reduce the compressibility modulus of the DPPC monolayer of pressures between 9 mN/m (just before the LE/LC phase transition) and 30 mN/m (near the bilayer equivalent pressure). This is suggestive of a reduction in fluidity; however serotonin is not likely taking up space in the membrane, due to negligible ΔA. This may indicate that the serotonin interaction with the PC headgroup may be interfering with PC-PC headgroup interactions. Moreover, with increasing pressure (>30 mN/m) the influence of serotonin not significantly different from control suggesting that increasing DPPC-DPPC interactions may reduce serotonin-DPPC interactions, this could be evidence of a competitive process. Serotonin did not affect collapse pressure.

Melatonin and NAS both caused similar trends in compressibility modulus versus pressure functions. Both significantly decreased compressibility over much of the isotherm at high concentration indicating a fluidizing effect. NAS (10.0 mM) caused a decrease in compressibility modulus across most of the isotherm including at the bilayer equivalent pressure of 35 mN/m, where it reduced the compressibility from control, 170 ± 24 mN/m to 119 ± 6 mN/m (**p < 0.001)(Fig. 6). NAS had no effect at 1.0 mM, however melatonin did, it caused a decrease to 141 ± 11 mN/m (*p < 0.05) at the bilayer equivalent pressure (Fig. 6).

The effects of NAS and melatonin were similar, though the effect of melatonin was observed at 10-fold lower concentration. One difference in the compressibility between NAS and Melatonin is the end behavior, near collapse, it appears as though melatonin at both concentrations stabilized the collapse process, increasing collapse pressure whereas NAS at had no effect or reduced pressure at collapse (Figs. 3 and 5).

Molecular dynamics (MD) simulations provide evidence that serotonin and tryptophan primarily interact with phospholipid headgroups via electrostatics: hydrogen bonds, salt-bridges and cation-π interactions [50]. These MD simulations suggest that the intensity of the salt-bridge interaction is strongest for protonated serotonin which when taken together with our report would suggest a direct interaction with the PC headgroup of DPPC [23,50]. MD simulations also reported that tryptophan has a weaker interaction with lipids compared to serotonin due to the carboxylic acid group of tryptophan interacting with water and destabilizing the tryptophan-lipid interaction [50]. Despite greater predicted electrostatic interactions of serotonin for phospholipids than tryptophan (a local phenomena), our results suggest that electrostatics are secondary to the hydrophobic effects of indoles, at least in terms of how much each subphase can incorporate into the membrane and in terms of the bulk mechanical properties of the membrane, as measured by the compressibility modulus. To our knowledge no theoretical or MD studies of NAS with lipids have been done. Previously, our group has performed MD simulations that show melatonin sits near the headgroup region partitioned into the membrane with the long axis of the molecule parallel to the monolayer plane [36,37]. This is partially supported by NMR and FTIR spectroscopy experiment, where melatonin was found to reside near the headgroup region [51], although the orientation of the molecule has not been experimentally verified. These MD simulations by our group predict that melatonin has a disordering effect on the acyl chains of the phospholipids, our study here suggests that NAS also has a disordering effect (as indicated by the increase in fluidity), albeit to a lesser degree. Future MD simulations may explain whether the magnitude of the disordering effect between NAS and melatonin can be attributed to the partition coefficient, size, the position and/or orientation within the membrane.

In comparing the effects of melatonin and its precursors on the DPPC compression isotherm we observe that melatonin had the greatest effect on monolayer properties followed by NAS, tryptophan then serotonin. Melatonin and NAS caused large increases in monolayer fluidity whereas tryptophan and serotonin did not, in fact, though the effect was small, low concentration tryptophan marginally increased membrane rigidity. The effects of serotonin were less pronounced, binding to the DPPC headgroups early in the isotherm but being completely excluded as the surface pressure increased, suggesting serotonin can only bind at low molecular area when the headgroup is perpendicular to the tails and are otherwise excluded from the headgroup region at bilayer equivalent pressures. Therefore the electrostatic properties of tryptophan and serotonin are likely keeping them near the headgroup/water

35 mN/m (near the bilayer equivalent pressure).
region, unlike NAS and melatonin which appear to penetrate more deeply into the headgroup and perhaps acyl chain region \[50\]. The increase in molecular area correlates with the relative hydrophobicity of the molecules and in general is true of the increase in LE/LC phase pressure transition, with the exception of serotonin which is likely explained by the strong electrostatic interactions of serotonin for zwitterionic PC headgroups. Melatonin and NAS are both uncharged at near physiological pH used, and their structures differ in one side chain (–OH or –OCH₃) at the 5th position of the indole ring (Fig. 1). This difference causes an increase in hydrophobicity of melatonin compared to NAS (logP 1.42 vs 0.98) and slightly larger which may, in part, explain its increased potency at affecting the DPPC monolayer. It is important to note that DPPC is a zwitterionic lipid with fully saturated tails, and that interactions of these different indoles with lipid membranes would be lipid composition dependent \[22,52\], such that charged phospholipids, sphingolipids, fatty acids or sterols in the

Fig. 5. Compressibility Modulus-Pressure curves computed from the pressure-area isotherms shown in Fig. 3. Because the compressibility modulus is the first derivative of the pressure vs area isotherm, the LE/LC phase transition can be clearly identified on these plots where the function goes to its first local minimum. These curves were used to identify the pressure of the phase transition reported in Fig. 4B.

Fig. 6. Compressibility modulus at the Bilayer Equivalent Pressure of 35 mN/m for (A) low and (B) high concentration.
of indoles. It has been shown that sterols and sphingolipids can have evidence that headgroup interactions with indoles are possible, a phenotype more effective than melatonin at preventing lipid peroxidation in membranes. This is also true of serotonin which has been found to prevent radicals in the medium from attacking lipids in the group region rather than deeper into the acyl tail region of the membrane. Thus would function more effectively upstream, perhaps in the headgroup region rather than downstream consequences for physiology such as their antioxidant activities, subsequent receptor signaling systems and in various diseases. The ability of melatonin and other indoles to interact with the membrane may localize them near the membrane and protection from oxidative stress and lipid peroxidation. Melatonin may be preferentially selected over NAS as a hormone because lower concentrations may penetrate the hydrophobic core and cross the membrane more efficiently. As a model system these indoles, varying in electrostatic properties and relative hydrophobicity, but all having a similar indole ring backbone demonstrate that increasing hydrophobicity is the primary factor of how well a small molecule will partition into the hydrophobic core of a lipid membrane, be it cellular membranes or liposomes, but that charged molecules (like serotonin) can still interact with lipid membranes through headgroup interactions. That being said to better measure the effects of molecular charge on indole DPPC interactions, and address which specific form of the subphase molecules interact most strongly with DPPC, controlled pH dependent experiments, likely involving spectroscopic techniques (such as Raman or FTIR) and molecular dynamics simulations are required.

The effects of membrane-active small molecules like melatonin on lipid membrane properties may have downstream consequences for physiology such as their antioxidant activities, subsequent receptor signaling systems and in various diseases. The ability of melatonin and other indoles to interact with the membrane may localize them near where they can prevent membrane lipid peroxidation, however melatonin is not an effective chain breaking antioxidant and thus would function more effectively upstream, perhaps in the headgroup region rather than deeper into the acyl tail region of the membrane, preventing radicals in the medium from attacking lipids in the membrane. This is also true of serotonin which has been found to protect red blood cells from lipid peroxidation and has been shown to be more effective than melatonin at preventing lipid peroxidation in aqueous liposome solution. Our work here provides experimental evidence that headgroup interactions with indoles are possible, a phenomenon that may be involved in explaining the antioxidant properties of indoles. It has been shown that sterols and sphingolipids can have functional effects on serotonin (5-HT1A) receptor signaling which was attributed to an increase in membrane order induced by these lipids. The effect of melatonin on the membrane is relevant for AD at multiple levels: the production of Aβ, the direct interaction of Aβ with the membrane and protection from oxidative stress and lipid peroxidation. Several studies have shown that Aβ production is membrane-lipid raft dependent and that membrane cholesterol levels correlate with trafficking of β-secretase and amyloid precursor protein (APP) to co-localize within rafts. Our previous report showed the melatonin can interact with cholesterol within membranes, and thus may affect cholesterol binding sites within membrane proteins such as APP or 5-HT1A receptors, reducing APP production. In addition, Aβ has been shown to interact preferentially with ordered, rigid membrane domains, so called “lipid rafts”. Therefore, melatonin and NAS may have direct protective effects against Aβ by increasing membrane fluidity which may reduce Aβ-membrane interactions and prevent Aβ binding.

4. Conclusions

In this report we used Langmuir compression isotherms to measure some biophysical properties of DPPC monolayers in the presence of four biologically-related indoles (tryptophan, serotonin, NAS and melatonin) to gain insight into how the chemical structure of indoles affect their interaction with lipid membranes and discuss the possible physiological relevance. This report shows that tryptophan, serotonin, NAS and melatonin can alter the properties of the DPPC monolayer in a structure dependent fashion that can be correlated with their physicochemical properties (such as LogP and water solubility). The interaction of melatonin with the DPPC monolayer was most pronounced of the four followed by NAS which followed a similar interaction. Serotonin and NAS both incorporated into the membrane and caused significant decreases in compressibility modulus near the bilayer equivalent pressure and shifted the LE/LC phase transition to lower molecular areas and higher surface pressures suggesting a strong effect to increase membrane fluidity. Tryptophan (zwitterionic) and serotonin (cationic) caused less pronounced effects on the DPPC isotherm and in markedly different ways that suggest contributions from different interaction mechanisms, such as electrostatics. Tryptophan was able to insert into the membrane at high concentration, but had no effect on compressibility, however at low concentration mildly increased the compressibility modulus across most of the isotherm. Serotonin did not significantly affect compressibility at the bilayer equivalent pressure of the isotherm, though it did at lower pressures, and at high concentration serotonin increased the pressure corresponding to phase transition without affecting the molecular area, which may indicate a direct electrostatic interaction with the PC headgroups. In general, hydrophobic interactions appear to be the dominant factor that affects fluidity and membrane phase transitions in lipid membranes. The data presented here adds to the understanding of how neurologically important molecules interact non-specifically with lipid membranes which has many implications for understanding the membrane-dependent effect of indoles and small molecules more generally.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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M. Robinson, et al.

BBA - Biomembranes 1862 (2020) 183863

8


