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Membrane Interactivity Shared by Receptor-Acting Drugs

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Review Article

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ABSTRACT

Background: Although lipids have been regarded as a passive component to constitute biomembranes, they can also play an important role in modulation of the activity or function of membrane-embedded proteins like receptors. Membrane lipids are presumed to be one of additional sites of action for receptor-acting drugs because their broad pharmacological spectra are not necessarily interpretable by the direct action on receptors. In order to obtain novel insights into the drug target and mechanism, we reviewed the membrane interactivity of different classes of drugs to act on representative receptors.
Methods: A search of the scientific articles published between 1979 and 2018 was carried out by using PubMed/MEDLINE, Google Scholar and ACS Publications. The relevant research papers published in recognized international journals and on-line journals in English were preferred, but the review articles of specific importance were also included, although non-English language citations were excluded. Collected articles were reviewed by title, abstract and text for relevance with preference to more recent publications.
Results: Results of the literature search indicate that membrane interactivity is shared by various drugs that act on α- and β-adrenergic, muscarinic and nicotinic acetylcholine, γ-aminobutyric acid type A, *N*-methyl-D-aspartate, opioid and transient receptor potential vanilloid type-1 receptors. These receptor agonists and antagonists not only interact with receptor proteins but also would structure-specifically interact with membrane lipids to affect receptors by modifying the lipid bilayer

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environments surrounding them with the resultant conformational change of receptor proteins.
Conclusion: The structure-specific membrane interaction is pharmacologically contributable to diverse effects of receptor-acting drugs.

Keywords: Membrane interactivity; receptor-acting drug; lipid bilayer membrane; structure-specific.

ABBREVIATIONS

$GABA_A$: γ-aminobutyric acid type A; NMDA: N-methyl-D-aspartate; TRPV1: transient receptor potential vanilloid type-1; DPH: 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH: 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene; PNA: N-phenyl-1-naphthylamine; DSC: differential scanning calorimetry; ESR: electron spin resonance.

1. INTRODUCTION

Receptors have a critical mechanistic contribution to a major class of drugs. In addition to the receptor agonistic and antagonistic activity, receptor-acting drugs exhibit different effects on seemingly unrelated membrane-associated proteins. The pharmacological mechanism(s) underlying them are not necessarily attributable to the direct action on receptors, suggesting the mechanistic interaction independent of receptor proteins. While many of receptor-acting drugs are amphiphilic, such structural characteristics are very likely to allow drug molecules to interact with lipid bilayers [1]. Lipids have been conventionally regarded as a passive component to constitute biomembranes but they can also modulate the activity or function of membrane-embedded proteins: ion channels, enzymes and receptors [2].

A number of drugs target receptors that are responsible for neurotransmission and neuronal functions. Besides the interaction with their relevant receptors, neurotransmitters can diffuse into synaptic membranes to modify their physicochemical properties, thereby shifting the conformational equilibrium of receptor proteins [3]. Considering the membrane effects of neurotransmitters, receptor-acting drugs would biophysically perturb membrane lipid bilayers as neurotransmitters do.

Membrane fluidity, membrane microviscosity (the reciprocal of membrane fluidity), membrane order and membrane elasticity influence the receptors diffusible in lipid bilayer membranes because such membrane properties are associated with the protein conformation optimal for the receptor activity [4]. The formation of ligand and receptor complexes depends on the affinity of ligands to receptors and the accessibility of ligands to receptor-binding sites, both of which are determined by the fluidity of biomembranes. Membrane fluidity, which refers to the relative motional freedom of the lipid components in lipid bilayers, is considered as one of determinants for the functions and dynamics of biomembranes and the conformational equilibria of membrane-embedded proteins [1]. Membrane fluidity can affect the rotation and diffusion of drug molecules in lipid bilayer membranes, thereby modulating the activity and membrane location of drugs. The fluidity of intact membranes is determined by the composition of membrane lipids. Phospholipids with unsaturated acyl chains make membranes more fluid than ones with saturated acyl chains. With regard to characteristic lipids, sphingomyelin stiffens membranes and cholesterol bidirectionally regulates membrane fluidity to decrease or increase depending on experimental conditions.

The interactions of drugs with liposomal and biological membranes are investigated by a variety of spectroscopic and biophysical methodologies including differential scanning calorimetry (DSC), electron spin resonance (ESR), nuclear magnetic resonance, X-ray diffraction and fluorescence anisotropy. Among them, fluorescence polarization has been most frequently used to determine drug-induced changes in membrane fluidity [5]. The polarization of fluorescence emitted by a membrane-incorporated fluorophore reflects its mobility in the surrounding membrane lipid bilayer environments. Fluorescence polarization is measured by excitation performed with monochromatic light that is vertically polarized, and the emission intensity detected through an analyzer oriented parallel or perpendicular to the direction of polarization of the excitation light by using different fluorophores such as 1,6-diphenyl-1,3,5-hexatriene (DPH), 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexa-

triene (TMA-DPH) and *N*-phenyl-1-naphthylamine (PNA). These probes structure- and lipophilicity-dependently penetrate into membranes to align with phospholipid acyl chains and locate in different membrane regions, indicating the fluidity of a membrane region specific to each individual probe. They are subject to the rotational restriction imparted by lipid bilayer rigidity or order. Drugs interact with lipid bilayers to produce more fluid or disordered membranes, which facilitate the probe rotation to emit the absorbed light in all directions, resulting in a decrease of fluorescence polarization. On the contrary, more rigid or ordered membranes produced by drugs disturb the probe rotation to emit the absorbed light in all directions, resulting in an increase of fluorescence polarization.

In order to obtain novel insights into the drug target and mechanism, we reviewed articles and information about the membrane interactivity of different classes of drugs (agonists and antagonists) that act on representative receptors: α- and β-adrenergic, muscarinic and nicotinic acetylcholine, γ-aminobutyric acid type A ($GABA_A$), *N*-methyl-D-aspartate (NMDA), opioid and transient receptor potential vanilloid type-1 (TRPV1) receptors.

2. METHODS

The present review is based on published articles and information retrieved from PubMed/MEDLINE, Google Scholar and ACS Publications. Databases were searched from 1979 to 2018. The papers published earlier than 1979 were exceptionally cited if they are essential to advancing the discussion. The research papers published in recognized international journals and on-line journals in English were preferred, but the review articles of specific importance were also included, although non-English language citations were excluded. Published abstracts were used when their complete articles were not available. The searches were carried out by using the following terms or combinations thereof: “membrane interaction”, “membrane physicochemical property”, “membrane fluidity”, “adrenergic receptor”, “acetylcholine receptor”, “$GABA_A$ receptor”, “NMDA receptor”, “opioid receptor”, “TRPV1 receptor”, “agonist” and “antagonist”. Collected articles were reviewed by title, abstract and text for relevance with preference to more recent publications. Their bibliographies were also searched for additional references.

3. RESULTS AND DISCUSSION

Results of the literature search indicate that different classes of receptor-acting drugs share the property to interact with membranes structure-specifically. Table 1 summarizes the representative membrane interactions of drugs, including relevant receptors and drugs’ effects on membranes.

The membrane interactivity of drugs has been widely investigated using liposomal membranes or unilamellar vesicles. Even if drugs show relatively small changes in such protein-free lipid bilayers at their clinical concentrations, membrane-acting compounds are known to produce greater effects on natural membranes containing protein components.

3.1 Adrenergic Receptor-Acting Drugs

Adrenergic receptors are widely distributed in both the peripheral nervous system and the central nervous system. All of them are G protein-coupled receptors that are primarily classified into four basic subtypes: α_1, α_2, β_1 and β_2. In the autonomic nervous system, α_1-, β_1- and β_2-adrenergic receptors are localized in the postsynaptic terminals of sympathetic postganglionic neurons, whereas α_2-adrenergic receptors are present in the presynaptic terminals of sympathetic postganglionic neurons.

3.1.1 Alpha-adrenergic receptor-acting drugs

While noradrenaline binds to α- and β-adrenergic receptors as a neurotransmitter, it exhibits a relatively strong agonistic effect on α_1- and β_1-adrenergic receptors. Therefore, noradrenaline is clinically usable as an injectable sympathomimetic drug for the treatment of critically low blood pressure. Burgess et al. [6] labelled plasma membranes isolated from rat liver with a fluorescent probe DPH and treated them with noradrenaline at 0.1-5 μM in the presence of 5 μM propranolol to block both β_1- and β_2-adrenergic receptors, followed by measuring DPH fluorescence polarization to determine the membrane interactivity. Noradrenaline interacted with the membranes to induce membrane fluidity-increasing effects, which were inhibited by non-selective α-adrenergic antagonist phentolamine and phenoxybenzamine at 1 μM and 50 μM, respectively.

Table 1. Membrane interactions of receptor-acting drugs

Relevant receptor	Mode of action	Drug	Interact with	Membrane effect	Reference
Adrenergic	Non-selective α- and β-agonist	Noradrenaline	Plasma membranes isolated from rat liver	Increase membrane fluidity at 0.1-5 μM in the presence of 5 μM non-selective β-antagonist propranolol	[6]
	Non-selective α-antagonist	Phentolamine Phenoxybenzamine		Inhibit 5 μM noradrenaline's membrane effect at 1 and 50 μM	
Adrenergic	Selective α_1-antagonist	Prazosin	Phosphatidylcholine plus cholesterol monolayers	Induce Langmuir monolayer fluidization	[8]
Adrenergic	Selective α_2-agonist	Dexmedetomidine	Neuro-mimetic liposomal membranes	Increase membrane fluidity at 50 μM with the potency being dexmedetomidine > racemic medetomidine > levomedetomidine	[9]
Adrenergic	Non-selective β-agonist	Isoproterenol	Rat reticulocyte membranes	Increase membrane fluidity through phospholipid methylation at 30 μM	[10]
	Non-selective β-antagonist	Propranolol		Inhibit isoproterenol's membrane effect	
Adrenergic	Selective β_2-agonist	Salmeterol	Phospholipid liposomal membranes	Increase membrane fluidity at >1 μM	[11]
Adrenergic	Non-selective β-antagonist	Carvedilol Propranolol	Phosphatidylcholine and phosphatidylglycerol liposomal membranes	Increase membrane fluidity at 50-200 μM	[13]
Adrenergic	Non-selective β-antagonist	Propranolol Alprenolol Oxprenolol	Phospholipids plus cholesterol liposomal membranes	Increase membrane fluidity at 0.2-1 mM with the potency being propranolol > alprenolol > oxyprenolol	[14]
Adrenergic	Non-selective β-antagonist Selective β_1-antagonist	Propranolol Acebutolol	Phosphatidylcholine unilamellar vesicles	Increase membrane fluidity at 10-250 μM with the potency being propranolol > acebutolol	[16]
Acetylcholine	Muscarinic agonist	Oxotremorine Carbamylcholine	Human lymphocyte membranes	Increase membrane fluidity at 10-100 μM	[19]
	Muscarinic antagonist	Atropine		Reverse oxotremorine's membrane effect	
Acetylcholine	Muscarinic agonist	Oxotremorine	Rat splenic lymphocyte membranes and phosphatidylcholine liposomal membranes	Increase membrane fluidity	[20]
	Muscarinic antagonist	Atropine		Antagonize oxotremorine's membrane effect	

Relevant receptor	Mode of action	Drug	Interact with	Membrane effect	Reference
Acetylcholine	Muscarinic agonist	Carbachol	Membrane fragments prepared from rat cerebral cortex	Increase membrane fluidity at nanomolar concentrations	[21]
Acetylcholine	Nicotinic agonist	Pancuronium	Peripheral leukocyte membranes obtained from human subjects	Increase membrane fluidity at 0.01-1 mM	[23]
$GABA_A$	Positive allosteric modulator	Propofol	Phospholipids plus cholesterol liposomal membranes	Structure-specifically increase membrane fluidity at sub-micromolar concentrations	[26]
$GABA_A$	Positive allosteric modulator	Propofol	Phosphatidylcholine liposomal membranes	Fluidize membranes at clinically relevant concentrations	[28]
$GABA_A$	Positive allosteric modulator	Pentobarbital Phenobarbital	Synaptic plasma membranes prepared from mouse brain	Increase membrane fluidity at sub-millimolar concentrations	[33]
$GABA_A$	Positive allosteric modulator	Phenobarbital	Plasma membranes isolated from rat liver	Increase membrane fluidity at 4 mM	[34]
$GABA_A$	Positive allosteric modulator	Thiopental	Peripheral leukocyte membranes obtained from human subjects	Increase membrane fluidity at 0.1-1 mM	[23]
$GABA_A$	Positive allosteric modulator	Diazepam	Crude synaptic membranes isolated from rat hippocampus Rat hippocampal synaptic membranes	Increase membrane fluidity at 0.1 nM to 10 μM Increase membrane fluidity by 10 mg kg^{-1} (i.p.)	[35]
$GABA_A$	Positive allosteric modulator	Chlordiazepoxide Diazepam	Egg-yolk phosphatidylcholine liposomal membranes	Increase membrane fluidity at low nanomolar concentrations	[36]
$GABA_A$	Positive allosteric modulator	Thymol Propofol	Phosphatidylcholine Langmuir films	Destabilize lipid monolayer	[40]
$GABA_A$	Positive allosteric modulator	Thymol Propofol Alkylphenols	Neuro-mimetic phospholipids plus cholesterol liposomal membranes	Structure-specifically increase membrane fluidity at 1-10 μM	[42]
$GABA_A$	Positive allosteric modulator	Menthol	Neuro-mimetic liposomal membranes	Increase membrane fluidity at 50 μM with the potency being (+)-menthol > (–)-menthol	[9]
$GABA_A$	Positive allosteric modulator	Neomenthol	Phosphatidylcholine unilamellar vesicles	Increase membrane fluidity at 0.3-1.8 mM with the potency being (+)-neomenthol > (–)-neomenthol	[63]

Relevant receptor	Mode of action	Drug	Interact with	Membrane effect	Reference
NMDA	Non-competitive antagonist	Ketamine	Synaptic and mitochondrial membranes prepared from rat brain	Increase membrane fluidity at 250 μM	[45,46]
			Rat brain synaptic membranes	Increase membrane fluidity by 50 mg kg^{-1} (i.p.)	
NMDA	Non-competitive antagonist	Ketamine	Pig brain synaptic membranes	Increase membrane fluidity	[47]
NMDA	Non-competitive antagonist	Ketamine	Phospholipids plus cholesterol liposomal membranes	Increase membrane fluidity at 50 μM with the potency being *S*(+)-ketamine > racemic ketamine	[9,48]
Opioid	Agonist	Morphine	Lipid bilayers prepared with crude mitochondrial lipids isolated from rat brain	Increase membrane fluidity by 10-25 mg kg^{-1} (i.p.)	[49]
	Antagonist	Naloxone		Reverse morphine's membrane effects by 1 mg kg^{-1} (i.p.)	
Opioid	Agonist	Morphine	Rat brain lipid preparations	Decrease lipid phase transition temperature at 50 nmol mg^{-1} lipid	[49]
	Antagonist	Naloxone		Reverse morphine's effect at 50 nmol mg^{-1} lipid	
Opioid	Agonist	Levorphanol	Membranes prepared with crude mitochondrial lipids from rat brain	Increase membrane fluidity by 5 mg kg^{-1} (i.p.)	[49]
	Inactive enantiomer	Dextrorphan		Exert no membrane effects when treating rats with 5 mg kg^{-1} (i.p.)	
Opioid	Agonist	Morphine	Membrane preparations from rat hippocampus and caudate	Decrease membrane microviscosity by 25 mg kg^{-1} (i.p.)	[50]
	Antagonist	Naloxone		Increase membrane microviscosity by 2 mg kg^{-1} (i.p.)	
Opioid	Agonist	Morphine	Rat brain membrane preparations	Increase membrane fluidity at 10 nM and 10 μM	[50]
	Antagonist	Naloxone		Reverse 10 nM morphine's membrane effect at 1 nM	
Opioid	Agonist	Codeine *N*-Methylcodeine	Phosphatidylcholine liposomal membranes	Decrease lipid phase transition temperature at 0.1 M	[51]
TRPV1	Agonist	Capsaicin	Rabbit platelet, rat peritoneal mast cell and human erythrocyte membranes	Cell-specifically change membrane fluidity at micromolar concentrations	[54]

Relevant receptor	Mode of action	Drug	Interact with	Membrane effect	Reference
TRPV1	Agonist	Capsaicin	Phosphatidylcholine plus cholesterol liposomal membranes	Increase membrane fluidity at 50 μM and decrease at 100-500 μM	[55]
TRPV1	Agonist	Capsaicin	Phospholipid liposomal membranes	Alter lipid bilayer elasticity at 10-100 μM sufficiently to change the conformational preference of membrane-embedded proteins	[56]
TRPV1	Agonist	Capsaicin	Rat erythrocyte membranes	Increase membrane fluidity and reverse the decreased membrane fluidity of hypercholesterolemic rats by maintaining them on 0.015% capsaicin-containing diets	[60]
TRPV1	Agonist	Capsaicin	Rat intesti nal brush-border membranes	Increase membrane fluidity by maintaining rats on 0.01% capsaicin-containing diets	[61]

Selective α_1-adrenergic antagonists such as prazosin, doxazosin and terazosin are used to treat hypertension, arrhythmia, anxiety and post-traumatic stress disorder. Among them, prazosin has the property to interact with model membranes [7]. Gzyl-Malcher et al. [8] investigated the membrane interaction of prazosin (5 μg ml^{-1}) by using a mixed cholesterol/phosphatidylcholine monolayer at the water/air interface as a simplified cell membrane model. Prazosin affected the film rigidity of lipid Langmuir monolayers to cause membrane fluidization.

Selective α_2-adrenergic agonist clonidine and dexmedetomidine are used as a therapeutic agent for hypertension and as an analgesic and sedative agent in anesthetic practice, respectively. Tsuchiya and Mizogami [9] treated phospholipids plus cholesterol liposomal membranes with dexmedetomidine at 50 μM. Their DPH fluorescence polarization measurements indicated that dexmedetomidine interacts with neuro-mimetic membranes to increase their fluidity.

3.1.2 Beta-adrenergic receptor-acting drugs

Non-selective β-adrenergic agonist isoproterenol is one of drugs used for bradycardia and atrioventricular block. Hirata et al. [10] determined the physicochemical changes of rat reticulocyte membranes after treating with β-adrenergic drugs. Isoproterenol was found to enhance the membrane fluidity through an increase of phospholipid methylation with an EC_{50} of about 30 μM. Its induced membrane fluidization was inhibited by non-selective β-adrenergic antagonist propranolol, but not by non-selective α-adrenergic antagonist phentolamine. It was also suggested that the enhancement of membrane fluidity by isoproterenol may promote the lateral movement of β-adrenergic receptors in the membranes.

Selective β_2-adrenergic agonists such as indacaterol and salmeterol are effective in treating chronic obstructive pulmonary disease and persistent asthma. DPH fluorescence anisotropic experiments of Lombardi et al. [11] showed that salmeterol interacts with phospholipid liposomal membranes to increase their fluidity at concentrations above 1 μM.

Selective β_1-adrenergic antagonists (acebutolol, atenolol, metoprolol, etc.) and non-selective β-adrenergic antagonists (propranolol, alprenolol, carvedilol, oxyprenolol, timolol, pindolol, etc.) are usable as general sympatholytics to treat or prevent hypertension, arrhythmia, angina pectoris and myocardial infarction. Short-acting selective β_1-blocker landiolol and esmolol are perioperatively used to reduce the risk of heart events of tachycardia, hypertension, myocardial ischemia and infarction. Many studies [12-16] support that β-adrenergic antagonists interact with model and biological membranes to increase their fluidity with the relative potencies correlating to those of cardio-protective effects. Butler et al. [13] determined the effects of different β-adrenergic antagonists on liposomal membranes prepared with dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol by DSC. Carvedilol most strongly perturbed the membranes at 50-200 μM, followed by propranolol. In DPH fluorescence polarization experiments of Mizogami et al. [14], β-blockers interacted with biomimetic membranes consisting of different phospholipids and cholesterol to increase their fluidity with the potency being propranolol > alprenolol > oxprenolol at 0.2-1 mM. By measuring fluorescence anisotropy of phosphatidylcholine unilamellar vesicles with DPH and TMA-DPH, Pereira-Leite et al. [16] revealed that propranolol induces larger increases of membrane fluidity than acebutolol at 10-250 μM. These comparative results indicate that non-selective β-blockers possess greater membrane interactivity than selective β_1-blockers.

3.1.3 Beta-adrenergic receptor and membrane property

The activity of cardiac β-adrenergic receptors is determined by membrane physicochemical properties. In in vivo model experiments of Ma et al. [17], cardiomyocyte plasma membranes prepared from cirrhotic rats showed a significant reduction of membrane fluidity, which was associated with a functional decrease of β-adrenergic receptors. Their following DPH fluorescence polarization study indicated that β-adrenergic receptor signaling is affected by the fluidity of cardiac plasma membranes [18].

3.2 Acetylcholine Receptor-Acting Drugs

Acetylcholine receptors are classified into muscarinic receptors (G protein-coupled) and nicotinic receptors (ionotropic). The former is peripherally localized in the postsynaptic terminals of parasympathetic postganglionic

neurons, and the latter, in autonomic ganglia, neuromuscular junctions and adrenal medullae.

3.2.1 Muscarinic acetylcholine receptor-acting drugs

Muscarinic acetylcholine receptors are one of pharmacological targets for neurological diseases. Among muscarinic agonists, carbachol and pilocarpine are useful for glaucoma treatment, while oxotremorine experimentally induces tremor, ataxia and spasticity. Masturzo et al. [19] treated human lymphocytes, in which muscarinic acetylcholine receptors are possibly present, with oxotremorine or carbachol at 10 and 100 μM, and then measured fluorescence polarization with DPH. Consequently, both muscarinic agonists were found to decrease the membrane microviscosity. Such membrane fluidization induced by 100 μM oxotremorine was reversed by muscarinic antagonist atropine pretreated at 10 μM. Tang et al. [20] demonstrated that oxotremorine concentration-dependently increases the membrane fluidity of both rat splenic lymphocytes and dimyristoylphosphatidylcholine liposomes, and its membrane-fluidizing effects are antagonized by atropine. Manevich et al. [21] reported the binding experiment of specific ligands to muscarinic receptors, in which carbachol increased the fluidity of membrane fragments prepared from rat cerebral cortex at nanomolar concentrations.

3.2.2 Muscarinic acetylcholine receptor and membrane property

Muscarinic acetylcholine receptor binding is influenced by the fluidity change of rat frontal cortex membranes [22].

3.2.3 Nicotinic acetylcholine receptor-acting drugs

Since pancuronium, vecuronium and atracurium competitively inhibit nicotinic acetylcholine receptors at the neuromuscular junction, these nicotinic antagonists are used as a muscle relaxant in general anesthesia and an aid to intubation. Aloui et al. [23] incubated peripheral leukocytes obtained from human subjects with pancuronium for 30 min, and then measured fluorescence polarization after labelling the leukocyte membranes with TMA-DPH. Pancuronium increased the membrane fluidity at 0.01-1 mM in allergic patients and control subjects, while its membrane-fluidizing effects were more pronounced in the allergic groups.

3.2.4 Nicotinic acetylcholine receptor and membrane property

Studies to reconstitute the purified *Torpedo californica* receptors into defined lipid environments suggested that the functions of nicotinic acetylcholine receptors require the optimal fluidity of bulk membranes in addition to the membrane composition of cholesterol and negatively charged phospholipids [24,25].

3.3 $GABA_A$ Receptor-Acting Drugs

$GABA_A$ receptors are ligand-gated Cl^- channels that are expressed in the central nervous system such as cortex, hippocampus and cerebellum. Inhibitory neurotransmitter GABA binds to $GABA_A$ receptors to allow the influx of Cl^- into postsynaptic neurons, resulting in inhibition of neuronal excitability. Heteromeric $GABA_A$ receptors, which are composed of α and β subunits arranged around a central pore, have a specific binding site for GABA and different allosteric binding sites (distinct from the GABA recognition site) for general anesthetics and anesthetic adjuvants (propofol, barbiturates, benzodiazepines, volatile anesthetics, etc.) and also for plant components (thymol, menthol, other related terpenoids, etc.). These drugs and phytochemicals act as a positive allosteric modulator of $GABA_A$ receptors to induce anesthesia, sedation, anxiolysis and convulsion cessation.

3.3.1 Propofol

Given its high lipophilicity, propofol is very likely to penetrate into lipid bilayers and modify the fluidity of liposomal membranes [26-29] and biological membranes [30]. Tsuchiya [26] compared the effects of propofol and its structurally-related compounds on dipalmitoylphosphatidylcholine liposomal membranes and biomimetic phospholipids plus cholesterol membranes by measuring fluorescence polarization with PNA, DPH and TMA-DPH. Of alkylphenolic derivatives, propofol most potently interacted with the membranes to increase their fluidity at 0.125-1.0 μM, which correspond to free propofol concentrations in blood during anesthesia. The structure-specific membrane interactivity of propofol is also evident when comparing between constitutional isomers of diisopropylphenol [29]. Propofol is able to

interact with biomimetic membranes significantly at clinically relevant concentrations [26,28].

3.3.2 Barbiturates

Harris et al. [31-33] performed a series of studies to determine the effects of barbiturates on synaptic plasma membranes prepared from mouse brain by measuring fluorescence polarization. Pentobarbital and phenobarbital were confirmed to increase the membrane fluidity at sub-millimolar concentrations [33]. When comparing between DPH and TMA-DPH polarization changes, pentobarbital was more effective in fluidizing the membrane core than the membrane surface [32]. An ESR spectroscopic experiment of Houslay et al. [34] showed that phenobarbital increases the fluidity of liver plasma membranes isolated from rats at 4 mM by interacting with the external half of lipid bilayers. In TMA-DPH fluorescence polarization measurements of Aloui et al. [23], thiopental increased the membrane fluidity of peripheral leukocytes obtained from human subjects at 0.1-1 mM and its membrane effects were greater in allergic patients than in non-allergic subjects.

3.3.3 Benzodiazepines

Mennini et al. [35] treated crude synaptic membranes isolated from rat hippocampus with diazepam and measured fluorescence polarization with DPH. They revealed that diazepam increases the membrane fluidity at concentrations ranging from 0.1 nM to 10 μM. Based on the in vitro results, they intraperitoneally injected rats with diazepam (10 mg kg^{-1}), and then prepared brain synaptosomes 15 min after injection. Diazepam was found to increase the fluidity of hippocampal synaptic membranes, although such in vivo membrane-fluidizing effects were not significantly influenced by selective benzodiazepine receptor antagonist flumazenil (30 mg kg^{-1}, p.o.) given 1 min after diazepam injection. In DPH fluorescence polarization experiments of Kurishingal et al. [36], chlordiazepoxide and diazepam interacted with egg-yolk phosphatidylcholine liposomal membranes to increase their fluidity at nanomolar concentrations.

3.3.4 Phytochemicals

Thymol from thyme (*Thymus vulgaris*, Lamiaceae) and menthol from peppermint (*Mentha piperita*, Lamiaceae) or spearmint (*Mentha spicata*, Lamiaceae) positively allosterically modulate $GABA_A$ receptors [37-39]. Several studies showed that thymol and menthol interact with lipid membranes to modify their physicochemical properties [9,40-42]. Reiner et al. [40] compared the effects of GABAergic phenols on dipalmitoylphosphatidylcholine Langmuir films. Thymol, propofol and other structural analogs destabilized the lipid monolayers depending on lipophilicity. By ^{1}H-nuclear magnetic resonance spectroscopy, they revealed that thymol and propofol insert into phosphatidylcholine unilamellar vesicles and locate in the region between the choline polar group, the glycerol and the acyl chain first atom. In DPH fluorescence polarization experiments of Tsuchiya and Mizogami [42], thymol, propofol and their related alkylphenols structure-specifically increased the fluidity of neuro-mimetic membranes prepared with phospholipids and cholesterol at 1-10 μM. Menthol also has the property to fluidize the similar neuro-mimetic membranes at 50 μM [9].

3.3.5 $GABA_A$ receptor and membrane property

Sooksawate and Simmonds [43] investigated the influence of membrane cholesterol on $GABA_A$ receptors in rat hippocampal neurons by a whole-cell patch clamp technique. Consequently, GABA effects were decreased by cholesterol enrichment that decreases the membrane fluidity and impedes the receptor protein conformational change, whereas increased by cholesterol depletion. By ligand binding assays with HEK293 and CHO cells expressing human $GABA_A$ $\alpha_1\beta_2\gamma_{2S}$ or $\alpha_5\beta_2\gamma_{2S}$, Søgaard et al. [44] revealed that [^{3}H]-muscimol binding to $GABA_A$ receptors is promoted by the depletion of membrane cholesterol to decrease lipid bilayer stiffness.

3.4 NMDA Receptor-acting Drugs

NMDA receptors are specific-type ionotropic receptors that are activated by neurotransmitter glutamate and glycine to open the ion channels nonselective to positively charged cations, resulting in neuronal excitation. Ketamine and its related drugs antagonize or inhibit NMDA receptor functions to produce dissociative anesthesia characterized by catalepsy, amnesia and analgesia. Ketamine is frequently used as an analgesic, sedative and anesthesia-maintaining agent because this non-competitive antagonist binds to the allosteric sites of NMDA receptors.

3.4.1 Ketamine

Mazzanti et al. [45] treated synaptic and mitochondrial membranes prepared from rat brains with 0-5 mM ketamine to determine its membrane effects by ESR spectroscopy using spin label 5-doxylstearate and 16-doxylstearate. Ketamine increased the membrane fluidity at concentrations as low as 0.25 mM. Based on the in vitro results, they intraperitoneally injected rats with ketamine (50 mg kg^{-1}) to prepare brain synaptosomes, followed by ESR spectroscopic analysis with 5-doxylstearate. Ketamine showed in vivo effects to increase the fluidity of synaptic membranes after 30 min of anesthesia [46]. Lenaz et al. [47] carried out ESR spectroscopic experiments for different anesthetics and found the ability of ketamine to increase the fluidity of synaptic membranes from pig brains. DPH fluorescence polarization measurements of Tsuchiya and Mizogami [48] indicated that ketamine interacts with neuro-mimetic phospholipids plus cholesterol membranes to increase their fluidity at 50 μM.

3.5 Opioid Receptor-Acting Drugs

Morphine and its related drugs act on opioid receptors that are inhibitory G protein-coupled receptors with multiple subtypes of μ, κ and δ expressed in the neuronal circuits responsible for nociception. Their analgesic effects are induced through opioid receptor activation. In addition to opioid receptor proteins, membrane lipids have been suggested as one of possible acting sites for opioid analgesics and their antagonists.

3.5.1 Opiates

Hosein et al. [49] reported the in vivo effects of opioid receptor-acting drugs by injecting rats intraperitoneally with morphine (5, 10, 15 and 25 mg kg^{-1}) or its antagonist naloxone (1 mg kg^{-1}) 15 min after morphine injection. They isolated crude brain mitochondrial lipids from drug-treated rats and dispersed the lipids in aqueous media to prepare lipid bilayers, followed by DSC analysis. Consequently, morphine was found to decrease the phase transition temperature of lipids, suggesting that it increases membrane fluidity. Naloxone completely reversed such membrane-fluidizing effects of morphine. By using brain lipid preparations from control rats (not treated with drugs), they demonstrated that morphine (50 nmol mg^{-1} lipid) exerts in vitro effects to decrease the phase transition temperature of lipids, which were reversed by naloxone (50 nmol mg^{-1} lipid). Heron et al. [50] verified the membrane interactivity of morphine by in vivo and in vitro experiments. They intraperitoneally injected rats with morphine (25 mg kg^{-1}) or naloxone (2 mg kg^{-1}), and then measured DPH fluorescence polarization of the membranes prepared from different brain regions 10-30 min after injection. Morphine induced a significant decrease in microviscosity of hippocampus and caudate membrane preparations, whereas naloxone increased the membrane microviscosity of both brain regions. Morphine also showed in vitro effects to increase the fluidity of rat brain membrane preparations at 0.01 and 10 μM, but the membrane-fluidizing effect of 10 nM morphine was reversed by 1 nM naloxone. Budai et al. [51] revealed that different morphine derivatives interact with dipalmitoyl-phosphatidylcholine liposomal membranes by DSC and electron paramagnetic resonance spectroscopic experiments. Of particular, codeine and *N*-methylcodeine significantly decreased the phase transition temperature of liposomes at 0.1 M.

3.5.2 Opioid receptor and membrane property

By [^{3}H]D-Ala-enkephalinamide binding and DPH fluorescence polarization studies, Heron et al. [52] showed that the ligand binding to opioid receptors is reduced by increasing the fluidity of crude mitochondrial membranes isolated from mouse forebrains, whereas the ligand accessibility to opioid receptors is elevated by increasing the microviscosity of the membranes.

3.6 TRPV1 Receptor-Acting Drugs

Capsaicin acts on vanilloid or TRPV1 receptors that are expressed in primary afferent sensory neurons of the pain pathway [53]. Since TRPV1 receptors are the non-selective cation channels to modulate nociceptive and pain transmission, capsaicin concentration-dependently exerts analgesic and algesic effects. Capsaicin also opens TRPV1 channels to give Na^+ channel blockers like local anesthetics the access to cell interiors, promoting their transport to nociceptors.

3.6.1 Capsaicinoids

Meddings et al. [54] treated rabbit platelets, rat peritoneal mast cells and human erythrocytes with capsaicin at 40-320 μM, followed by measuring fluorescence polarization with DPH and TMA-DPH. Their results indicated that capsaicin specifically increases the fluidity of platelet membranes at all the tested

concentrations, although it biphasically acts on mast cells and erythrocytes to rigidify their membranes at 320 μM but fluidize at lower concentrations. Tsuchiya [55] investigated the effects of capsaicin on biomimetic phosphatidylcholine plus cholesterol liposomal membranes by determining its induced changes in PNA, DPH and TMA-DPH fluorescence polarization. Capsaicin interacted with platelet-mimetic and bacterial cell-mimetic membranes to show concentration-dependent biphasic effects to increase the membrane fluidity at 50 μM but decrease at 100-500 μM. Lundbæk et al. [56] reported that capsaicin alters phospholipid bilayer elasticity at 10-100 μM sufficiently to change the conformational preference of membrane-embedded proteins. As a background for such membrane interaction, Aranda et al. [57] speculated that capsaicin penetrates into phospholipid membranes with an alkyl chain aligned along the acyl chains and with hydroxyl and amide groups located closer to the lipid/water interface. By spectroscopically analyzing intrinsic fluorescence of capsaicin, Swain and Mishra [58] indicated the location of capsaicin in membrane phospholipid bilayers that a phenolic group is present near the head group region, while a hydrophobic tail, inside the core region.

Jensen et al. [59] compared the biophysical effects of structurally different amphiphiles on membrane lipid bilayers. Among them, capsaicin decreased the lipid bilayer stiffness. Kempaiah and Srinivasan [60] maintained hypercholesterolemic rats on diets containing 0.015% capsaicin for eight weeks, and then isolated erythrocytes for ESR spectroscopic and DPH fluorescence anisotropic measurements. They found that capsaicin increases the fluidity of erythrocyte membranes and also reverses the decreased membrane fluidity of hypercholesterolemic rats. Prakash and Srinivasan [61] reported in vivo membrane effects of capsaicin by the similar experiment, in which rats were maintained on 0.01% capsaicin-containing diets for eight weeks. Their DPH fluorescence polarization data indicated that capsaicin increases the fluidity of intestinal brush-border membranes.

3.7 Stereostructure-Specific Drug and Membrane Interaction

Unlike the interaction with functional proteins, the membrane interaction has a problem for the mechanism of drug action: whether drugs stereostructure-specifically interact with lipid bilayers to exert different effects between enantiomers. One enantiomer exhibits higher activity or toxicity than its enantiomeric counterpart and a racemic mixture. Enantioselectivity of receptor-acting drugs has been exclusively explained by their stereospecific affinity or binding to receptor proteins. Since two enantiomers absolutely differ in spatial configuration, they should differently behave in chiral matrices. Proteins are entirely made up of only L-amino acids, allowing drug stereoisomers to interact with receptor proteins enantioselectively. By contrast, the drug and membrane interaction has been conventionally recognized to equally affect membrane lipid bilayers. However, as Goldstein [62] described that the effects of drugs on membrane fluidity do not exclude some specificity, membrane lipids potentially contribute to the stereostructure-specific interaction with drugs.

Alpha$_2$-adrenergic agonist medetomidine interacts with neuro-mimetic membrane consisting of phospholipids and cholesterol with the potency being dexmedetomidine (D-medetomidine) > racemic medetomidine > levomedetomidine (L-medetomidine) at 50 μM for each [9]. Of β-adrenergic antagonists, *R*(+)-propranolol most potently increases the fluidity of cholesterol-containing membranes at 50 μM, followed by racemic propranolol and *S*(–)-propranolol in the decreasing order of membrane interactivity [9]. Menthol, a positive allosteric modulator of $GABA_A$ receptors, interacts with neuro-mimetic membranes containing cholesterol to increase their fluidity with the potency being (+)-menthol > (–)-menthol at 50 μM for each [9]. Zunino et al. [63] compared the membrane interactivity of neomenthol stereoisomers, which have the $GABA_A$ receptor-modulatory activity to enhance the currents induced by low concentration GABA [64]. Their DPH fluorescence anisotropic experiments revealed that (+)-neomenthol increases the membrane fluidity of dipalmitoylphosphatidylcholine unilamellar vesicles at 0.3-1.8 mM more significantly than (–)-neomenthol. NMDA receptor-acting ketamine interacts with neuro-mimetic phospholipids plus cholesterol membranes to increase the membrane fluidity with the potency being S(+)-ketamine > racemic ketamine at 50 μM for each [9,48]. Hosein et al. [49] intraperitoneally injected rats with levorphanol (5 mg kg^{-1}) or dextrorphan (5 mg kg^{-1}). In their following DSC study, levorphanol increased the fluidity of membrane

preparations from crude rat brain mitochondrial lipids as well as morphine (15 mg kg^{-1}, i.p.), whereas its pharmacologically inactive enantiomer dextrorphan showed no significant membrane effects.

Biological membranes play an important role not only as the matrix to hold receptor proteins but also in the process to discriminate drug molecules. Cellular and plasma membranes structurally consist of lipid bilayers of phospholipids and cholesterol. Such lipid components potentially mediate the enantioselective action of drugs because they have chiral centers. Phospholipids interact preferentially with molecules of the same chirality, producing higher selectivity for one enantiomer than its enantiomeric counterpart [65]. Cholesterol with more chiral carbons than phospholipids is more likely to impart chirality to lipid bilayers and its absolute configuration would influence the membrane physicochemical property [66]. Drugs are considered to interact with chiral lipid membranes to induce membrane fluidity changes that are discriminable between enantiomers.

The composition of membrane lipids, which modulate the function and location of proteins in biomembranes, varies according to cell and tissue types [67,68]. Such variations would make the drug and membrane interaction characteristic to neuronal, cardiovascular and other cells. The effects of membrane-acting drugs remarkably differ by membrane lipid components and their compositions [5], possibly enhancing the membrane interaction specificity for cells.

3.8 Receptors and Lipid Raft Microdomains

The conventional concept “membranes consisting of uniformly distributed lipids” has been substantially modified by the recent theory that a microdomain biophysically differing from bulk membranes is present in biological membranes. The most intensively studied membrane microdomain is lipid rafts, the highly ordered membrane compartments enriched in cholesterol, sphingolipids and characteristic proteins. Lipid rafts float in the liquid-disordered lipid bilayers to play a role of the platform for functional proteins. Since lipid rafts influence the fluidity of membrane compartments and regulate the neurotransmission and receptor trafficking, they are responsible for a variety of cell membrane-related physiological process and pathogenesis. Another type of membrane microdomain is caveolae that are regarded as a special group of rafts to contain peculiar protein caveolins. The localization in lipid raft microdomains and the functional regulation by lipid rafts are known for α_1-adrenergic receptor [69], β_2-adrenergic receptor [70], muscarinic acetylcholine receptor [71], nicotinic acetylcholine receptor [72], $GABA_A$ receptor [73], NMDA receptor [74], opioid receptor [75] and TRPV1 receptor [76]. Lipid rafts potentially modify the receptor affinity to ligands and the integrity of lipid rafts modulates the effects of agonists on their relevant receptors [77,78].

Several drugs and compounds act on receptor-localizing lipid rafts to cause their physicochemical modification [79]. The drugs to induce a significant increase in membrane fluidity are likely to affect the ordered membrane compartments more effectively than membrane-inactive ones. Membrane fluidity-modifiers also show raft-making or raft-breaking effects depending on their located region in lipid bilayers [80]. The interactivity with lipid bilayers may be associated with the selectivity of drugs for receptor subtypes that are activated by the same ligands but are differently localized in lipid rafts. For example, β_2-adrenergic receptors are concentrated in lipid rafts, while controversy remains for β_1-adrenergic receptors [81]. The localization in caveolae/lipid rafts is prerequisite to β_2-adrenergic receptors in cardiac myocytes for physiological signaling, but not to β_1-adrenergic receptors [70]. Non-selective β-adrenergic antagonists interact with lipid raft model membranes to change their fluidity, although selective β_1-adrenergic antagonists are not membrane-active [14-16]. Therefore, non-selective β-blockers would inhibit the activity of β_2-receptors by interacting with lipid rafts together with acting on β_1-receptor proteins antagonistically, whereas selective β_1-blockers could affect the activity of β_1-receptors through the specific action on β_1-receptor proteins but not the activity of β_2-receptors through the interaction with lipid rafts.

4. CONCLUSION

Lipids are no longer only a structural component to constitute biomembranes but a critical factor to modulate the location and activity of membrane proteins as well as define membrane microdomains. Since integral membrane proteins are not rigid entities, the modification of membrane fluidity would influence the functions

of proteins embedded in membrane lipid bilayers. In addition to acting on receptors directly, drugs are considered to interact with membranes to affect their relevant receptors by changing the lipid bilayer environments surrounding them. The structure-specific membrane interaction is pharmacologically contributable to diverse effects of receptor-acting drugs. Not all receptor-acting drugs interact with membrane lipids and not all membrane-interacting drugs act on receptor proteins. However, the membrane interactivity may be useful as a tool for screening lead compounds in drug discovery, while biophysical and biochemical studies on the interactions of drugs with lipid bilayers were conventionally of pharmacokinetic interest for drug design.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Kopeć W, Telenius J, Khandelia H. Molecular dynamics simulations of the interactions of medicinal plant extracts and drugs with lipid bilayer membranes. FEBS J. 2013;280(12):2785-2805.
DOI: 10.1111/febs.12286
[PMID: 23590201]
2. Burger K, Gimpl G, Fahrenholz F. Regulation of receptor function by cholesterol. Cell Mol Life Sci. 2000; 57(11):1577-1592.
DOI:10.1007/PL00000643
[PMID: 11092453]
3. Cantor RS. Receptor desensitization by neurotransmitters in membranes: Are neurotransmitters the endogenous anesthetics? Biochemistry. 2003;42(41): 11891-11897.
DOI: 10.1021/bi034534z
[PMID: 14556619]
4. Hershkowitz M, Heron D, Csamuel D, Shinitzky M. The modulation of protein phosphorylation and receptor binding in synaptic membranes by changes in lipid fluidity: Implications for ageing. Prog Brain Res. 1982;56:419-434.
DOI:10.1016/S0079-6123(08)63788-7;
[PMID: 6298878]
5. Tsuchiya H, Mizogami M. Interaction of local anesthetics with biomembranes consisting of phospholipids and cholesterol: Mechanistic and clinical implications for anesthetic and cardiotoxic effects. Anesthesiol Res Pract. 2013; 297141.
DOI: 10.1155/2013/297141
[PMID: 24174934]
6. Burgess GM, Giraud F, Poggioli J, Claret M. α-Adrenergically mediated changes in membrane lipid fluidity and Ca^{2+} binding in isolated rat liver plasma membranes. Biochim Biophys Acta. 1983;731(3):387-396.
DOI:10.1016/0005-2736(83)90033-0;
[PMID: 6305417]
7. Gzyl-Malcher B, Handzlik J, Klekowska E. Interaction of prazosin with model membranes – a Langmuir monolayer study. Bioelectrochemistry. 2012;87:96-103.
DOI:10.1016/j.bioelechem.2011.2012 12.005
[PMID: 22260980]
8. Gzyl-Malcher B, Handzlik J, Klekowska E. Temperature dependence of the interaction of prazosin with lipid langmuir monolayers. Colloids Surf B Biointerfaces. 2013;112:171-176.
DOI: 10.1016/j.colsurfb.2013.07.030
[PMID: 23973675]
9. Tsuchiya H, Mizogami M. Discrimination of stereoisomers by their enantioselective interactions with chiral cholesterol-containing membranes. Molecules. 2018; 23(1):E49.
DOI: 10.3390/molecules23010049;
[PMID: 29295605]
10. Hirata F, Strittmatter WJ, Axelrod J. β-adrenergic receptor agonists increase phospholipid methylation, membrane fluidity, and β-adrenergic receptor–adenylate cyclase coupling. Proc Natl Acad Sci USA. 1979;76(1):368-372.
DOI: 10.1073/pnas.76.1.368;
[PMID: 34151]

11. Lombardi D, Cuenoud B, Krämer SD. Lipid membrane interactions of indacaterol and salmeterol: Do they influence their pharmacological properties? Eur J Pharm Sci. 2009;38(5):533-547.
DOI: 10.1016/j.ejps.2009.10.001
PMID: 19819331
12. Herbette L, Katz AM, Sturtevant JM. Comparisons of the interactions of propranolol and timolol with model and biological membranes. Mol Pharmacol. 1983;24(2):259-269.
[PMID: 6888369]
13. Butler S, Wang R, Wunder SL, Cheng HY, Randall CS. Perturbing effects of carvedilol on a model membrane system: Role of lipophilicity and chemical structure. Biophys Chem. 2006;119(3):307-315.
DOI: 10.1016/j.bpc.2005.09.004
[PMID: 16243429]
14. Mizogami M, Takakura K, Tsuchiya H. The interactivities with lipid membranes differentially characterize selective and nonselective β_1-blockers. Eur J Anaesthesiol. 2010;27(9):829-834.
DOI:10.1097/EJA.0b013e32833bf5e4
[PMID: 20601889]
15. Tsuchiya H, Mizogami M. Characteristic interactivity of landiolol, an ultra-short-acting highly selective β_1-blocker, with biomimetic membranes: Comparisons with β_1-selective esmolol and non-selective propranolol and alprenolol. Front Pharmacol. 2013;4:150.
DOI: 10.3389/fphar.2013.00150
[PMID: 24339816]
16. Pereira-Leite C, Carneiro C, Soares JX, Afonso C, Nunes C, Lúcio M, et al. Biophysical characterization of the drug-membrane interactions: The case of propranolol and acebutolol. Eur J Pharm Biopharm. 2013;84(1):183-191.
DOI: 10.1016/j.ejpb.2012.12.005
[PMID: 23291047]
17. Ma Z, Meddings JB, Lee SS. Membrane physical properties determine cardiac β-adrenergic receptor function in cirrhotic rats. Am J Physiol. 1994;267(1 Pt 1):G87-G93.
DOI: 10.1152/ajpgi.1994.267.1.G87
[PMID: 8048535]
18. Ma Z, Lee SS, Meddings JB. Effects of altered cardiac membrane fluidity on β-adrenergic receptor signalling in rats with cirrhotic cardiomyopathy. J Hepatol. 1997; 26(4):904-912.
DOI:10.1016/S0168-8278(97)80259-0
[PMID: 9126806]
19. Masturzo P, Salmona M, Nordstrom O, Consolo S, Ladinsky H. Intact human lymphocyte membranes respond to muscarinic receptor stimulation by oxotremorine with marked changes in microviscosity and an increase in cyclic GMP. FEBS Lett. 1985;192(2):194-198.
DOI:10.1016/0014-5793(85)80106-X
[PMID: 2998866]
20. Tang C, Castoldi AF, Costa LG. Effects of the muscarinic agonist oxotremorine on membrane fluidity in rat lymphocytes. Biochem Mol Biol Int. 1993;29(6):1047-1054.
[PMID: 8330013]
21. Manevich EM, Köiv A, Järv J, Molotkovsky JG, Bergelson LD. Binding of specific ligands to muscarinic receptors alters the fluidity of membrane fragments from rat brain. A fluorescence polarization study with lipid-specific probes. FEBS Lett. 1988; 236(1):43-46.
DOI:10.1016/0014-5793(88)80282-5
[PMID: 3402616]
22. Ghosh C, Dick RM, Ali SF. Iron/ascorbate-induced lipid peroxidation changes membrane fluidity and muscarinic cholinergic receptor binding in rat frontal cortex. Neurochem Int. 1993;23(5):479-484.
DOI:10.1016/0197-0186(93)90133-P
[PMID: 8251930]
23. Aloui R, Gallet H, Biot N, Perrin-Fayolle M, Lagarde M, Pacheco Y. Behaviour of leukocyte membrane fluidity in presence of anesthetic drugs. Comparison between allergic patients and control subjects. Gen Pharmacol. 1993;24(2):419-422.
DOI:10.1016/0306-3623(93)90326-S
[PMID: 8482526]
24. Fong TM, McNamee MG. Correlation between acetylcholine receptor function and structural properties of membranes. Biochemistry. 1986;25(4):830-840.
DOI: 10.1021/bi00352a015
[PMID: 3008814]
25. Sunshine C, McNamee MG. Lipid modulation of nicotinic acetylcholine receptor function: The role of membrane lipid composition and fluidity. Biochim Biophys Acta. 1994;1191(1):59-64.
DOI:10.1016/0005-2736(94)90233-X
[PMID:7512384]

26. Tsuchiya H. Structure-specific membrane-fluidizing effect of propofol. Clin Exp Pharmacol Physiol. 2001;28(4):292-299.
DOI: 10.1016/j.1440-1681.2001.03441.x;
[PMID: 11251643]
27. Bahri MA, Heyne BJ, Hans P, Seret AE, Mouithys-Mickalad AA, Hoebeke MD. Quantification of lipid bilayer effective microviscosity and fluidity effect induced by propofol. Biophys Chem. 2005;114(1):53-61.
DOI:10.1016/j.bpc.2004.11.006
[PMID: 15792861]
28. Bahri MA, Seret A, Hans P, Piette J, Deby-Dupont G, Hoebeke M. Does propofol alter membrane fluidity at clinically relevant concentrations? An ESR spin label study. Biophys Chem. 2007;129(1):82-91.
DOI:10.1016/j.bpc.2007.05.011
[PMID: 17574724]
29. Tsuchiya H, Ueno T, Tanaka T, Matsuura N, Mizogami M. Comparative study on determination of antioxidant and membrane activities of propofol and its related compounds. Eur J Pharm Sci. 2010;39(1-3):97-102.
DOI: 10.1016/j.ejps.2009.11.001
[PMID: 19897032]
30. Tsuchiya M, Asada A, Kasahara E, Sato EF, Shindo M, Inoue M. Antioxidant protection of propofol and its recycling in erythrocyte membranes. Am J Respir Crit Care Med. 2002;165(1):54-60.
DOI: 10.1164/ajrccm.165.1.2010134
[PMID: 11779730]
31. Harris RA, Schroeder F. Effects of barbiturates and ethanol on the physical properties of brain membranes. J Pharmacol Exp Ther. 1982;223(2):424-431.
[PMID: 7131297]
32. Harris RA, Bruno P. Membrane disordering by anesthetic drugs: Relationship to synaptosomal sodium and calcium fluxes. J Neurochem. 1985;44(4):1274-1281.
DOI: 10.1111/j.1471-4159.1985.tb08754.x
[PMID: 2579208]
33. Mitchell MA, Peris J, Harris RA. Barbiturate tolerance and dependence: Effects on synaptosomal sodium transport and membrane fluidity. Pharmacol Biochem Behav. 1985;22(6):955-960.
DOI: 10.1016/0091-3057(85)90302-8
[PMID: 4040639]
34. Houslay MD, Dipple I, Gordon LM. Phenobarbital selectively modulates the glucagon-stimulated activity of adenylate cyclase by depressing the lipid phase separation occurring in the outer half of the bilayer of liver plasma membranes. Biochem J. 1981;197(3):675-681.
DOI: 10.1042/bj1970675
[PMID: 7325977]
35. Mennini T, Ceci A, Caccia S, Garattini S, Masturzo P, Salmona M. Diazepam increases membrane fluidity of rat hippocampus synaptosomes. FEBS Lett. 1984;173(1):255-258.
DOI:10.1016/0014-5793(84)81058-3
[PMID: 6086398]
36. Kurishingal H, Brain PF, Restall CJ. Benzodiazepine-induced changes in biomembrane fluidity. Biochem Soc Trans. 1992;20(2):157S.
DOI: 10.1042/bst020157s
[PMID: 1397547]
37. Priestley CM, Williamson EM, Wafford KA, Sattelle DB. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human $GABA_A$ receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. Br J Pharmacol. 2003;140(8):1363-1372.
DOI: 10.1038/sj.bjp.0705542
[PMID: 14623762]
38. García DA, Bujons J, Vale C, Suñol C. Allosteric positive interaction of thymol with the $GABA_A$ receptor in primary cultures of mouse cortical neurons. Neuropharmacology. 2006;50(1):25-35.
DOI:10.1016/j.neuropharm.2005.07.009
[PMID: 16185724]
39. Watt EE, Betts BA, Kotey FO, Humbert DJ, Griffith TN, Kelly EW, et al. Menthol shares general anesthetic activity and sites of action on the $GABA_A$ receptor with the intravenous agent, propofol. Eur J Pharmacol. 2008;590(1-3):120-126.
DOI: 10.1016/j.ejphar.2008.06.003
[PMID: 18593637]
40. Reiner GN, Perillo MA, García DA. Effects of propofol and other GABAergic phenols on membrane molecular organization. Colloids Surf B Biointerfaces. 2013;101:61-67.
DOI: 10.1016/j.colsurfb.2012.06.004
[PMID: 22796773]
41. Reiner GN, Fraceto LF, de Paula E, Perillo MA, García DA. Effects of gabaergic phenols on phospholipid bilayers as evaluated by ^{1}H-NMR. J Biomater Nanobiotechnol. 2013;4(3A):28-34.
DOI: 10.4236/jbnb.2013.43A004

42. Tsuchiya H, Mizogami M. Comparative interactions of anesthetic alkylphenols with lipid membranes. Open J Anesthesiol. 2014;4(12):308-317.
DOI: 10.4236/ojanes.2014.412044
43. Sooksawate T, Simmonds MA. Effects of membrane cholesterol on the sensitivity of the $GABA_A$ receptor to GABA in acutely dissociated rat hippocampal neurones. Neuropharmacology. 2001;40(2):178-184.
DOI: 10.1016/S0028-3908(00)00159-3
[PMID: 11114396]
44. Søgaard R, Werge TM, Bertelsen C, Lundbye C, Madsen KL, Nielsen CH, et al. $GABA_A$ receptor function is regulated by lipid bilayer elasticity. Biochemistry. 2006;45(43):13118-13129.
DOI: 10.1021/bi060734+
[PMID: 17059229]
45. Mazzanti L, Pastuszko A, Lenaz G. Effects of ketamine anesthesia on rat-brain membranes: fluidity changes and kinetics of acetylcholinesterase. Biochim Biophys Acta. 1986;861(1):105-110.
DOI: 10.1016/0005-2736(86)90408-6
[PMID: 3756149]
46. Mazzanti L, Lenaz G, Marinelli F, Cinti S. Structural and functional modifications induced by ketamine on synaptosomes in the rat. Neuropharmacology. 1991; 30(12A):1343-1349.
DOI: 10.1016/0028-3908(91)90032-7
[PMID: 1664918]
47. Lenaz G, Curatola G, Mazzanti L, Bertoli E, Pastuszko A. Spin label studies on the effect of anesthetics in synaptic membranes. J Neurochem. 1979;32(6): 1689-1695.
DOI: 10.1111/j.1471-4159.1979.tb02280.x
[PMID: 221616]
48. Tsuchiya H, Mizogami M. Analgesic agents share the membrane interactivity possibly associated with the diversity of their pharmacological properties. Br J Pharm Res. 2015;7(2):110-121.
DOI: 10.9734/BJPR/2015/18269
49. Hosein EA, Lapalme M, Vadas EB. Monitoring the stereospecificity of morphine action *in vivo* and *in vitro* through brain membrane lipid fluidity. Biochem Biophys Res Commun. 1977;78(1):194-201.
DOI: 10.1016/0006-291X(77)91239-6
[PMID: 907670]
50. Heron DS, Shinitzky M, Zamir N, Samuel D. Adaptive modulations of brain membrane lipid fluidity in drug addiction and denervation supersensitivity. Biochem Pharmacol. 1982;31(14):2435-2438.
DOI:10.1016/0006-2952(82)90543-3
[PMID: 6889866]
51. Budai M, Szabó Z, Szógyi M, Gróf P. Molecular interactions between DPPC and morphine derivatives: A DSC and EPR study. Int J Pharm. 2003;250(1):239-250.
DOI: 10.1016/S0378-5173(02)00560-4
[PMID: 12480289]
52. Heron D, Israeli M, Hershkowitz M, Samuel D, Shinitzky M. Lipid-induced modulation of opiate receptors in mouse brain membranes. Eur J Pharmacol. 1981;72(4): 361-364.
DOI:10.1016/0014-2999(81)90576-8
[PMID: 6268424]
53. Szallasi A, Cortright DN, Blum CA, Eid SR. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. Nat Rev Drug Discov. 2007;6(5):357-372.
DOI: 10.1038/nrd2280
[PMID: 17464295]
54. Meddings JB, Hogaboam CM, Tran K, Reynolds JD, Wallace JL. Capsaicin effects on non-neuronal plasma membranes. Biochim Biophys Acta. 1991; 1070(1):43-50.
DOI: 10.1016/0005-2736(91)90144-W
[PMID: 1751537]
55. Tsuchiya H. Biphasic membrane effects of capsaicin, an active component in *Capsicum* species. J Ethnopharmacol. 2001;75(2-3):295-299.
DOI:10.1016/S0378-8741(01)00200-8
PMID: 11297867
56. Lundbæk JA, Birn P, Tape SE, Toombes GE, Søgaard R, Koeppe 2nd RE, et al. Capsaicin regulates voltage-dependent sodium channels by altering lipid bilayer elasticity. Mol Pharmacol. 2005;68(3):680-689.
DOI: 10.1124/mol.105.013573
[PMID: 15967874]
57. Aranda FJ, Villalaín J, Gómez-Fernández JC. Capsaicin affects the structure and phase organization of phospholipid membranes. Biochim Biophys Acta. 1995; 1234(2):225-234.
DOI: 10.1016/0005-2736(94)00293-X
[PMID: 7696298]
58. Swain J, Mishra AK. Location, partitioning behavior, and interaction of capsaicin with lipid bilayer membrane: Study using its intrinsic fluorescence. J Phys Chem B. 2015;119(36):12086-12093.

DOI: 10.1021/acs.jpcb.5b05351
[PMID: 26302022]

59. Jensen LD, Hansen AJ, Lundbæk JA. Regulation of endothelial cell migration by amphiphiles – are changes in cell membrane physical properties involved? angiogenesis. 2007;10(1):13-20.
DOI: 10.1007/s10456-006-9060-y
[PMID: 17265099]
60. Kempaiah RK, Srinivasan K. Influence of dietary spices on the fluidity of erythrocytes in hypercholesterolaemic rats. Br J Nutr. 2005;93(1):81-91.
DOI: 10.1079/BJN20041317
[PMID: 15705229]
61. Prakash UN, Srinivasan K. Beneficial influence of dietary spices on the ultrastructure and fluidity of the intestinal brush border in rats. Br J Nutr. 2010; 104(1):31-39.
DOI: 10.1017/S0007114510000334
[PMID: 20178671]
62. Goldstein DB. The effects of drugs on membrane fluidity. Ann Rev Pharmacol Toxicol. 1984;24:43-64.
DOI:10.1146/annurev.pa.24.040184.00035 5
[PMID: 6329077]
63. Zunino MP, Turina AV, Zygadlo JA, Perillo MA. Stereoselective effects of monoterpenes on the microviscosity and curvature of model membranes assessed by DPH steady-state fluorescence anisotropy and light scattering analysis. Chirality. 2011;23(10):867-877.
DOI: 10.1002/chir.20998
[PMID: 21932211]
64. Zhang XB, Jiang P, Gong N, Hu XL, Fei D, Xiong ZQ, et al. A-type GABA receptor as a central target of TRPM8 agonist menthol. PLoS One. 2018;3(10):e3386.
DOI: 10.1371/journal.pone.0003386
[PMID: 18852885]
65. Nandi N, Vollhardt D. Chiral discrimination and recognition in Langmuir monolayers. Curr Opin Colloid Interface Sci. 2008;13(12):40-46.
DOI: 10.1016/j.cocis.2007.07.016
66. Lalitha S, Kumar AS, Stine KJ, Covey DF. Chirality in membranes: first evidence that enantioselective interactions between cholesterol and cell membrane lipids can be a determinant of membrane physical properties. J Supramol Chem. 2001; 1(2):53-61.
DOI: 10.1016/S1472-7862(01)00013-2
67. Spector AA, Yorek MA. Membrane lipid composition and cellular function. J Lipid Res. 1985;26(9):1015-1035.
[PMID: 3906008]
68. Ingólfsson HI, Carpenter TS, Bhatia H, Bremer PT, Marrink SJ, Lightstone FC. Computational lipidomics of the neuronal plasma membrane. Biophys J. 2017; 113(10):2271-2280.
DOI: 10.1016/j.bpj.2017.10.017
[PMID: 29113676]
69. Morris DP, Lei B, Wu YX, Michelotti GA, Schwinn DA. The α_{1a}-adrenergic receptor occupies membrane rafts with its G protein effectors but internalizes via clathrin-coated pits. J Biol Chem. 2008;283(3): 2973-2985.
DOI: 10.1074/jbc.M705795200
[PMID: 18048357]
70. Xiang Y, Rybin VO, Steinberg SF, Kobilka B. Caveolar localization dictates physiologic signaling of β_2-adrenoceptors in neonatal cardiac myocytes. J Biol Chem. 2002;277(37):34280-34286.
DOI:10.1074/jbc.M201644200
[PMID:12097322]
71. Lai HH, Boone TB, Yang G, Smith CP, Kiss S, Thompson TC, et al. Loss of caveolin-1 expression is associated with disruption of muscarinic cholinergic activities in the urinary bladder. Neurochem Int. 2004;45(8):1185-1193.
DOI: 10.1016/j.neuint.2004.06.016
[PMID: 15380628]
72. Zhu D, Xiong WC, Mei L. Lipid rafts serve as a signaling platform for nicotinic acetylcholine receptor clustering. J Neurosci. 2006;26(18):4841-4851.
DOI: 10.1523/JNEUROSCI.2807-05.2006
[PMID: 16672658]
73. Dalskov SM, Immerdal L, Niels-Christiansen LL, Hansen GH, Schousboe A, Danielsen EM. Lipid raft localization of $GABA_A$ receptor and Na^+, K^+-ATPase in discrete microdomain clusters in rat cerebellar granule cells. Neurochem Int. 2005;46(6):489-499.
DOI: 10.1016/j.neuint.2004.11.010
[PMID: 15769551]
74. Swanwick CC, Shapiro ME, Yi Z, Chang K, Wenthold RJ. NMDA receptors interact with flotillin-1 and -2, lipid raft-associated proteins. FEBS Lett. 2009;583(8):1226-1230.
DOI: 10.1016/j.febslet.2009.03.017
[PMID: 19298817]

75. Ge X, Qiu Y, Loh HH, Law PY. GRIN1 regulates μ-opioid receptor activities by tethering the receptor and G protein in the lipid raft. J Biol Chem. 2009;284(52): 36521-36534.
DOI: 10.1074/jbc.M109.024109
[PMID: 19861419]
76. Szőke É, Börzsei R, Tóth DM, Lengl O, Helyes Z, Sándor Z, et al. Effect of lipid raft disruption on TRPV1 receptor activation of trigeminal sensory neurons and transfected cell line. Eur J Pharmacol. 2010;628(1-3):67-74.
DOI: 10.1016/j.ejphar.2009.11.052
[PMID: 19958765]
77. Allen JA, Halverson-Tamboli RA, Rasenick MM. Lipid raft microdomains and neurotransmitter signaling. Nat Rev Neurosci. 2007;8(2):128-140.
DOI: 10.108/nrn2059
[PMID: 17195035]
78. Nothdurfter C, Tanasic S, Di Benedetto B, Uhr M, Wagner EM, Gilling KE, et al. Lipid raft integrity affects $GABA_A$ receptor, but not NMDA receptor modulation by psychopharmacological compounds. Int J Neuropsychopharmacol. 2013;16(6):1361-1371.
DOI: 10.1017/S146114571200140X
[PMID: 23217923]
79. Patra SK, Rizzi F, Silva A, Rugina DO, Bettuzzi S. Molecular targets of (–)-epigallocatechin-3-gallate (EGCG): Specificity and interaction with membrane lipid rafts. J Physiol Pharmacol. 2008; 59(Suppl 9):217-235.
[PMID: 19261982]
80. Tarahovsky YS, Muzafarov EN, Kim YA. Rafts making and rafts braking: How plant flavonoids may control membrane heterogeneity. Mol Cell Biochem. 2008; 314(1-2):65-71.
DOI: 10.1007/s11010-008-9766-9
[PMID: 18414995]
81. Chini B, Parenti M. G-protein coupled receptors in lipid rafts and caveolae: How, when and why do they go there? J Mol Endocrinol. 2004;32(2):325-338.
DOI: 10.1677/jme.0.0320325
[PMID: 15072542]

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