**The membrane interaction of drugs as one of mechanisms for their enantioselective effects**

Hironori Tsuchiya [a,*], Maki Mizogami [b]

[a] *Department of Dental Basic Education, Asahi University School of Dentistry, Mizuho, Japan*

[b] *Department of Anesthesiology, Asahi University School of Dentistry, Mizuho, Japan*

Running Title: Enantioselective drug and membrane interaction

* Corresponding author. Hironori Tsuchiya

Address: Department of Dental Basic Education, Building 3, Asahi University School of Dentistry, 1851-1 Hozumi, Mizuho, Gifu 501-0296, Japan.

Tel.: +81 58 329 1266; fax: +81 58 329 1266.

E-mail address: hiro@dent.asahi-u.ac.jp

This study was supported by a Grant-in-Aid for Scientific Research (C) 23593005 from the Japan Society for the Promotion of Science.

## ABSTRACT

The discrimination between different enantiomers of chiral compounds by the biological system is medically important as the pharmacological and toxicological effects of enantiomeric drugs significantly differ depending on their stereostructures. One enantiomer is preferred over its enantiomeric counterpart and a racemic mixture for higher activity or lower toxicity. Such enantioselectivity has been exclusively explained by the stereostructure-specific interactions with receptors, channels and enzymes of drugs including general and local anesthetics, sedatives, hypnotics, anti-inflammatory drugs, analgesics and β-adrenergic antagonists. These drugs can act on not only protein targets but also lipid biomembranes. Almost all of the relevant proteins are embedded in or associated with membrane lipid bilayers. Therefore, we propose one of possible mechanisms that drugs might enantioselectively interact with membrane lipids and induce changes in membrane property like fluidity which are discriminable between enantiomers. If the induced changes are different between enantiomers, enantiomeric drugs would differently influence the membrane lipid environments for receptors, channels and enzymes, resulting in the enantioselectivity of drug effects. The enantioselective membrane interactions of drugs could be mediated by membrane component cholesterol and phospholipids, both of which have chiral centers in structure as well as drug enantiomers. Chiral membrane lipids possibly exhibit the preference for the interactions with drug molecules of either the same chirality or the different chirality, producing the selectivity to one drug enantiomer. The proposed hypothesis may be available to investigate more useful medicines based on the novel concept of drug enantioselectivity.

## INTRODUCTION

More than 50% of currently used drugs are chiral compounds and about 90% of them have been marketed and clinically administered as racemates which contain an equal mixture of two enantiomers [1]. The chirality of drug molecules most commonly arises due to the presence of an asymmetric carbon, chiral center. Enantiomers are a pair of stereoisomers which are mirror images of each other and non-superimposable. They are denoted as *R*- (*rectus*) or *S*- (*sinister*) according to the absolute configuration in which four substituents around the chiral center are assigned priorities based on atomic number. The *R*-configuration is assigned if when looking down the bond from the chiral center to the substituent with the lowest priority, the other substituents are ordered from higher to lower priority in a clockwise direction, and the *S*-configuration is assigned if these substituents are so ordered in a counterclockwise direction. Enantiomers are also termed, but according to the optical activity, as *dextro*-rotatory (+) or *levo*-rotatory (–) by the direction in which they rotate the plane of polarized light clockwise or counterclockwise. The *R*/*S* assignment has no relation to the (+)/(–) assignment, so an *R*-enantiomer is either *dextro*-rotatory or *levo*-rotatory depending on its exact substituents.

The biological system can discriminate different enantiomers of chiral compounds. The medical importance of such discrimination is clear as the pharmacological and toxicological effects are significantly different between enantiomeric drugs. Despite chemically sharing identical molecular formulas, atom-to-atom linkages and binding distances, enantiomers are well known to show the differences in pharmacodynamics, which originate from the stereostructure-specific actions [2]. One enantiomer is preferred over its enantiomeric counterpart and a racemic mixture to increase beneficial

activity and decrease adverse toxicity, indicating the clinical advantage of using a single enantiomer [3]. Such enantiomeric drugs include general and local anesthetics, sedatives, hypnotics, anti-inflammatory drugs, analgesics and β-adrenergic antagonists. In comparative activity and toxicity, the *R*-form is more potent than the *S*-form and the racemate as in bupivacaine, etomidate, salbutamol, methadone, etc., whereas the *S*-form is reversely more potent than the *R*-form and the racemate as in isoflurane, thiopental, ibuprofen, propranolol, ketamine, etc.

The different effects between enantiomers are attributable to the enantioselective interactions of drugs with chiral macromolecules. Since two drug enantiomers absolutely differ in spatial configuration, they differently behave in the chiral matrices, but not in the achiral environments such as in an aqueous solution. Almost all of molecules in the body are enantiomers themselves, and proteinous receptors, channels and enzymes are entirely made up of only L-amino acids, not D-amino acids. In the conventional mechanisms, therefore, the enantioselective effects of drugs have been exclusively explained by their stereostructure-specific interactions with receptors, channels and enzymes as reported for general anesthetics, sedatives and hypnotics interacting with $GABA_A$ and NMDA receptors, local anesthetics with sodium and potassium channels, non-steroidal anti-inflammatory drugs with cyclooxygenase, β-adrenergic antagonists with $\beta_1$- or $\beta_2$-receptors and opioid analgesics with opioid receptors.

Contrary to the mechanism reported previously, the effects of enantiomeric local anesthetics on ion channels are not necessarily consistent with their pharmacological comparisons that *R*(+)-enantiomers are more potent than *S*(–)-enantiomers. *S*(–)-Bupivacaine was shown to be more effective in blocking ion channels than racemic

bupivacaine [4]. Non-steroidal anti-inflammatory drugs have been considered to inhibit cyclooxygenase with the different potency between *S*(+)-forms and *R*(–)-forms. However, the effects of ibuprofen and fenoprofen were found to be virtually identical, contradicting the enantioselective enzyme-inhibitory mechanism in which their *S*(+)-enantiomers are more effective than *R*(–)-enantiomers in inhibiting the prostaglandin-synthetic enzyme [5]. The cyclooxygenase-independent action, arising from the partition of drugs into biomembranes, also participates in the effects of non-steroidal anti-inflammatory and analgesic drugs [6]. The adrenoceptor blockade by β-blockers is usually explained by their structure-specific bindings to $\beta_1$- or $\beta_2$-receptors. In addition, another mode of action was suggested for β-adrenergic antagonists [7,8].

Many receptor-, channel- and enzyme-interacting drugs are amphiphiles which also can interact with membrane lipids. Cellular and plasma membranes are structurally the lipid bilayers primarily consisting of phospholipids and steroids. These biomembranes not only function as a matrix holding proteinous drug targets but also may play an important role in the discrimination process for drug molecules. Since phospholipids and cholesterol contain chiral carbon atoms, such membrane lipids potentially mediate the enantioselective actions of drugs. Therefore, we propose one of possible mechanisms that the stereostructure-specific membrane interaction might be, at least in part, responsible for the different effects between drug enantiomers.

## HYPOTHESIS

*Background*

Cellular and plasma membranes are the platform for transmembrane signaling pathways. This platform, in which many receptors, channels and enzymes are embedded,

influences the activities of functional proteins through the physicochemical alterations of lipid bilayers. Receptor-acting neurotransmitters change the synaptic membrane property to cause a shift in receptor conformational equilibrium [9]. Topical capsaicin regulates voltage-dependent sodium channels by altering the lipid bilayer elasticity [10]. Changes in membrane fluidity also modulate the membrane-bound enzyme activity [11].

While the most important targets for anesthetics, sedatives and hypnotics have been referred to as a $GABA_A$ receptor and ion channels, the effects of these drugs are mediated by interacting with not only the relevant proteins but also membrane lipids [12]. The effects of $GABA_A$ potentiators such as benzodiazepines and barbiturates are modulated by changes in membrane fluidity [13]. Anesthetic enantiomers were shown to be equally effective in disturbing membrane lipid bilayers and affecting membrane lipid phase transition [14]. To compare them, however, liposomal membranes were prepared only with dipalmitoylphosphatidylcholine, not with heterogeneous components as in biological membranes. Other comparative studies also used only a few phospholipid compositions for model membranes despite the fact that stereoisomers exert the structure-specific effects on high cholesterol-containing biomembranes [15]. The previous comparison between *S*(+)- and *R*(–)-isoflurane showed no differences in their effects on membranes consisting of phospholipids plus 40 mol% cholesterol [16]. However, this study determined only the partition coefficients of enantiomers into lipid bilayers, not their influences on membrane physicochemical property. While ketamine is an NMDA receptor antagonist, it also has the ability to fluidize synaptic and mitochondrial membranes [17]. The generation of ionic currents affected by anesthetic drugs requires the interplay of ion channels and membrane lipids [18]. Membrane

disordering modifies the functions of ion channels, which is mechanistically related to local anesthetic effects [19]. Cyclooxygenase inhibition is the primary mode of action of non-steroidal anti-inflammatory drugs. The cyclooxygenase-mediated catalysis to produce prostaglandins takes place in the membrane lipid environments [20]. The therapeutic and toxic effects of anti-inflammatory drugs are influenced by their affinity to lipid bilayers and such drugs commonly have the ability to change membrane fluidity [21]. There is a relationship between the change of membrane fluidity and the activation of phospholipase $A_2$ as a target of anti-inflammatory drugs [22]. The β-adrenergic receptor signaling is associated with the altered fluidity of cardiomyocyte membranes [23]. While morphine and synthetic opioid methadone bind to opioid receptors to cause remarkable analgesia, such analgesic agonists also increase the brain mitochondrial membrane fluidity and this membrane-fluidizing effect is reversible by an antagonist naloxone [24].

*Enantioselective membrane interactions of drugs*

Drugs may enantioselectively interact with lipid membranes and induce membrane fluidity changes discriminable between enantiomers. Such a membrane interaction is mechanistically applicable to a wide range of enantiomeric drugs which have been generally considered to stereostructure-specifically act on receptors, channels and enzymes. Membrane phospholipids and cholesterol are chiral molecules, so they should display the enantioselective interactions with chiral drugs.

Odorant carvone enantiomers were reported to differently interact with phosphatidylcholine [25]. Monoterpenes also enantioselectively fluidized liposomal membranes of 1,2-dipalmitoylphosphatidylcholine with the relative potency being (+)-

> (–)-neomenthol, (+)- > (–)-carvone, (+)- > (–)-α-pinene, and (–)- > (+)-β-pinene [26]. The antifungal activity of amphotericin B is attributed to its association with membrane ergosterol to form a transmembrane channel which leaks monovalent ions to cause the death of fungal cells. A derivative of amphotericin B showed the differential interaction with enantiomeric membranes formed from 1-palmitoyl-2-oleoxylphosphatidylcholine *R*- and *S*-enantiomers [27]. While green tea catechins show optimal isomerism, they acted on lipid bilayers to modify the membrane fluidity stereostructure-specifically [28]. *Vibrio cholera* cytolysin is a bacterial pathogen which binds to cell membranes and forms a transmembrane pore. By interacting with membrane lipids, not membrane proteins, this toxin permeabilized the membranes consisting of phospholipids and cholesterol with the potency depending on enantiomeric membrane components [29].

The enantioselective mode of action of these membrane-interacting compounds is applicable to receptor-, channel- and enzyme-interacting drugs. Our mechanistic concept is supported by the enantioselective membrane interactions of drugs which have been considered to act on proteinous targets. Opioid receptor-acting morphine was found to stereostructure-specifically bind to membrane phospholipids from different brain regions [30]. While $\Delta^1$-tetrahydrocannabinol exerts several effects by binding to the cannabinoid receptor, it also fluidized lipid bilayer membranes of phosphatidylcholine and cholesterol with the relative potency being (–)- > (+)-enantiomer [31].

Considering that all drug molecules cannot interact with receptors, channels and enzymes, and a number of drugs are transported through lipid bilayers to the proteinous targets, membrane lipids would play an important role in recognition and discrimination processes of drugs. Drug and membrane interactions to induce membrane perturbation

or fluidity change are discriminated by chiral membrane lipids and the enantiomeric discrimination occurs in lipid membranes by the direct interactions of chiral centers [32]. Chiral membrane lipids possibly exhibit the preference for the interactions with drug molecules of either the same chirality or the different chirality. Phospholipids interact preferentially with molecules of the same chirality, producing higher selectivity to one enantiomer than its enantiomeric counterpart [33]. Membrane steroids with several chiral centers are more likely to contribute to the enantioselective interaction compared with phospholipids having one chiral center in the head group. Lipid membranes consisting of cholesterol enantiomers showed the chirality depending on an increase of cholesterol composition and the absolute configuration of cholesterol influenced the membrane physicochemical property [34]. Local anesthetic enantiomers were recently reported to differently interact with biomimetic lipid membranes to increase their fluidity with the relative potencies which were consistent with those of anesthetic and cardiotoxic effects [35]. In such interactions, membrane cholesterol was closely associated with the discrimination between *R*(+)- and *S*(–)-drugs.

## CONCLUSION

We have hypothesized a membrane-interacting mechanism for the enantioselective effects of drugs. Because of the conventional belief that drug enantiomers produce different effects by stereostructure-specifically acting on receptors, channels and enzymes, little effort has gone into studying the discriminative interactions of enantiomeric drugs with cellular and plasma membranes. Our hypothetical mechanism may be available to investigate medicines with higher activity or lower toxicity from the novel viewpoint of drug enantioselectivity.

## Conflict of interest

All authors declare that they do not have any conflicts of interest.

## Acknowledgement

This study was supported by a Grant-in-Aid for Scientific Research (C) 23593005 from the Japan Society for the Promotion of Science.

**References**

[1] Rentsch KM. The importance of stereoselective determination of drugs in the clinical laboratory. J Biochem Biophys Meth 2002;54:1–9.

[2] Bernards CM, Ulma GA, Kopacz DJ. The meningeal permeability of *R*- and *S*-bupivacaine are not different: evidence that pharmacodynamic differences between the enantiomers are not the result of differences in bioavailability. Anesthesiology 2000;93:896–7.

[3] Smith SW. Chiral toxicology: it's the same thing…only different. Toxicol Sci 2009;110:4–30.

[4] González T, Arias C, Caballero R, et al. Effects of levobupivacaine, ropivacaine and bupivacaine on HERG channels: stereoselective bupivacaine block. Br J Pharmacol 2002;137:1269–79.

[5] Evans AM. Enantioselective pharmacodynamics and pharmacokinetics of chiral non-steroidal anti-inflammatory drugs. Eur J Clin Pharmacol 1992;42:237–56.

[6] Villanueva M, Heckenberger R, Palmér M, Schrör K. Stereospecific and non-stereospecific effects of ibuprofen on human platelet and polymorphonuclear leukocyte functions. Agents Actions Suppl 1992;37:162–70.

[7] Lombardi D, Cuenoud B, Krämer SD. Lipid membrane interactions of indacaterol and salmeterol: do they induce their pharmacological properties? Eur J Pharm Sci 2009;38:533–47.

[8] Mizogami M, Takakura K, Tsuchiya H. The interactivities with lipid membranes differentially characterize selective and nonselective $\beta_1$-blockers. Eur J Anaesthesiol 2010;27:829–34.

[9] Cantor RS. Receptor desensitization by neurotransmitters in membranes: are

neurotransmitters the endogenous anesthetics? Biochemistry 2003;42:11891–7.

[10] Lundbæk JA, Birn P, Tape SE, et al. Capsaicin regulates voltage-dependent sodium channels by altering lipid bilayer elasticity. Mol Pharmacol 2005;68:680–9.

[11] Kopeikina LT, Kamper EF, Siafaka I, Stavridis J. Modulation of synaptosomal plasma membrane-bound enzyme activity through the perturbation of plasma membrane lipid structure by bupivacaine. Anesth Analg 1997;85:1337–43.

[12] Cantor RS. The lateral pressure profile in membranes: a physical mechanism of general anesthesia. Toxicol Lett 1998;100–101:451–8.

[13] Sooksawate T, Simmonds MA. Influence of membrane cholesterol and modulation of the $GABA_A$ receptor by neuroactive steroids and other potentiators. Br J Pharmacol 2001;134:1303–11.

[14] Tomlin SL, Jenkins A, Lieb WR, Franks NP. Stereoselective effects of etomidate optical isomers on gamma-aminobutyric acid type A receptors and animals. Anesthesiology 1998;88:708–17.

[15] Goldstein DB. The effects of drugs on membrane fluidity. Ann Rev Pharmacol Toxicol 1984;24:43–64.

[16] Dickinson R, Franks NP, Lieb WR. Can the stereoselective effects of the anesthetic isoflurane be accounted for by lipid solubility? Biophys J 1994;66:2019–23.

[17] Mazzanti L, Lenaz G, Marinelli F, Cinti S. Structural and functional modifications induced by ketamine on synaptosomes in the rat. Neuropharmacology 1991;30:1343–9.

[18] Friederich P. Basic concepts of ion channel physiology and anaesthetic drug effects. Eur J Anaesthesiol 2003;20:343–53.

[19] Harris RA, Bruno P. Membrane disordering by anesthetic drugs: relationship to synaptosomal sodium and calcium fluxes. J Neurochem 1985;44:1274–81.

[20] Rand Doyen J, Yucer N, Lichtenberger LM, Kulmacz RJ. Phospholipid actions on PGHS-1 and -2 cyclooxygenase kinetics. Prostaglandins Other Lipid Mediat 2008;85:134–43.

[21] Lúcio M, Ferreira H, Lima JLFC, Matos C, de Castro B, Reis S. Influence of some anti-inflammatory drugs in membrane fluidity studied by fluorescence anisotropy measurements. Phys Chem Chem Phys 2004;6:1493–8.

[22] Baciulis V, Luthy C, Hofer G, Toplak H, Wiesmann UN, Oetliker OH. Specific and non specific stimulation of prostaglandin release by human skin fibroblasts in culture. Are changes of membrane fluidity involved? Prostaglandins 1992;43:293–304.

[23] 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;26:904–12.

[24] 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;78:194–201.

[25] Nandi N. Molecular origin of the recognition of chiral odorant by chiral lipid: interaction of dipalmitoyl phosphatidyl choline and carvone. J Phys Chem A 2003;107:4588–91.

[26] 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. Chrality 2011;23:867–77.

[27] Jeon OY, Carreira EM. Differential interaction of an AmB analogue and ergosterol in enantiomeric membranes. Org Lett 2010;12:1772–5.

[28] Tsuchiya H. Stereospecificity in membrane effects of catechins. Chem Biol Interact 2001;134:41–54.

[29] Zitzer A, Westover EJ, Covey DF, Palmer M. Differential interaction of the two cholesterol-dependent, membrane-damaging toxins, streptolysin O and *Vibrio cholerae* cytolysin, with enantiomeric cholesterol. FEBS Lett 2003;553:229–31.

[30] Abood LG, Hoss W. Stereospecific morphine adsorption to phosphatidyl serine and other membranous components of brain. Eur J Pharmacol 1975;32:66–75.

[31] Makriyannis A, Banijamali A, Van der Schyf C, Jarrell H. Interactions of cannabinoids with membranes. The role of cannabinoid stereochemistry and absolute configuration and the orientation of delta-9-THC in the membrane bilayer. NIDA Res Monogr 1987;79:123–33.

[32] Arnett EM, Gold JM, Harvey N, Johnson EA, Whitesell LG. Stereoselective recognition in phospholipid monolayers. Adv Exp Med Biol 1988;238:21–36.

[33] Nandi N, Vollhardt D. Chiral discrimination and recognition in Langmuir monolayers. Curr Opin Colloid Interface Sci 2008;13:40–6.

[34] 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;1:53–61.

[35] Tsuchiya H, Ueno T, Mizogami M. Stereostructure-based differences in the interactions of cardiotoxic local anesthetics with cholesterol-containing biomimetic membranes. Bioorg Med Chem 2011;19:3410–5.