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Different Effects of Dopamine on Differential Rotational Mobility between Inner and Outer Monolayer of Synaptosomal Plasma Membrane Vesicles Isolated from Bovine Brain

Hyun-Gang Kim[1], Chang-Hwa Choi[2], Inn-Se Kim[3], In-Kyo Chung[4], Goon-Jae Cho[5], Hye-Ock Jang[6], and Il Yun[1]

Department of [1]Dental Pharmacology and Biophysics and [4]Oral and Maxillofacial Surgery and Clinical Pharmacology, College of Dentistry and Research Institute for Oral Biotechnology, Pusan National University, Pusan 602−739; Departments of [2]Neurosurgery, [3]Anesthesiology and [5]Internal Medicine, College of Medicine and Research Institute for Oral Biotechnology, Pusan National University, Pusan 602−739; Department of [6]Oriental Pathology and Prescription, College of Oriental Medicine, Dong-Eui University, Pusan 614−054, Korea

Fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) was used to evaluate the effects of dopamine · HCl on the range of the rotatioanl mobility of bulk bilayer structure of the synaptosomal plasma membrane vesicles (SPMV) isolated from whole bovine brain. In a dose-dependent manner, dopamine decreased the anisotropy (γ), limiting anisotropy (γ_∞) and order parameter (*S*) of DPH in the membranes. These indicate that dopamine increased the rotational mobility of the probe in the neuronal membranes. Cationic 1-[4-(trimethylammonio)-phenyl]-6-phenylhexa-1,3,5-hexatriene (TMA-DPH) and anionic 3-[p-(6-phenyl)-1,3,5-hexatrienyl]-phenylpropionic acid (PRO-DPH) were utilized to examine the range of transbilayer asymmetric rotational mobility of the neuronal membranes. Dopamine had a greater increasing effect on the mobility of the inner monolayer as compared to the outer monolayer of the neuronal membranes. It has been proven that dopamine exhibits a selective rather than nonselective fluidizing effect within the transbilayer domains of the SPMV.

Key Words: Dopamine, Neuronal membranes, Transbilayer fluidity, Fluorescent probe technique

INTRODUCTION

Kebabian & Calne (1979) first described the two main types of dopaminergic receptor, which they named D_1 and D_2. Recent advances in genetic cloning have enabled the discovery of 7 dopaminergic receptor subtypes, from D_1 to D_5. When D_1 type receptors are activated by dopamine (or dopamine-agonists), they promote the activation of the enzyme adenylated cyclase, which catalyses the conversion of adenosine triphosphate (ATP) molecules to cyclic adenosine monophosphate (cyclic AMP). Cyclic AMP acts as a second messenger within the cell and can trigger a cascade of intracellular events including the release of other neurotransmitters, the modification of cell wall permeability to chemicals, and the production of proteins. Activation of D_2 receptors either has no effect on cyclic AMP or inhibits the formation of cyclic AMP. The dopamine hypothesis of schizophrenia emerged in the 1960s through the development of antipsychotic drugs such as chlorpromazine and the observation that amphetamines and other stimulant drugs could induce psycosis. The antipsychotic effect of these 'neuroleptic' drugs was found to be due to their ability to block dopaminergic receptors (Cress et al, 1976). Although some neuroleptics (especially the thioxanthenes and some phenothiazines) bind avidly to D_1 sites, those with relatively high affinity for D_1 receptors also bind to and block D_2 receptors (Per-

Corresponding to: Il Yun, Department of Dental Pharmacology and Biophysics, College of Dentistry and Research Institute for Oral Biotechnology, Pusan National University, Pusan 602-739, Korea. (Tel) 051-240-7813, (Fax) 051-254-0576, (E-mail) iyun@hyowon.pusan.ac.kr

outka & Snyder, 1980; Faedda et al, 1989).

Researches on mechanism of pharmacological action of dopamine have been all about interaction between dopamine and its receptors. It is not exaggerating to say that these researches have been entirely about changes of inorganic ions transport inward and outward cells, and the generation of second messenger resulting from the dopamine-receptor interaction. Because receptors coexist with membrane lipids, we cannot entirely exclude the possibility that the changes of lipid fluidity may be accompanied before or after neurotransmitters display interactions with their receptors. We think that it should be kept in mind that the changes of inorganic ion transport inward and outward cells through membranes are closely related to the fluidity of membrane lipid bilayers. Therefore, we became to presume that fluidity of membrane lipid bilayers would be changed before or after the dopamine-receptor interaction. If dopamine alters mobility in neuronal membrane lipid bilayer, for example, will it evenly act on both inner and outer monolayers? Sheetz & Singer (1974) proposed that the asymmetry net charge at the surface of the two monolayers of biological membranes could establish an asymmetric transbilayer distribution of charged amphipaths intercalating in the two monolayers. Will the positively charged dopamine in the water solution change mainly the mobility of the negatively charged inner monolayer of neuronal membrane's lipid bilayer?

Exploiting fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH), we examined the effects of dopamine · HCl on rotational mobility of bulk lipid bilayer of synaptosomal plasma membrane vesicles (SPMV). Using sidedness selective fluorescent DPH derivatives, cationic 1-[4-(trimethylammonio)-phenyl]-6-phenylhexa-1,3,5-hexatriene (TMA-DPH) and anionic 3-[p-(6-phenyl)-1,3,5-hexatrienyl]-phenylpropionic acid (PRO-DPH), we evaluated the effects of dopamine · HCl on transbilayer differential rotational mobility of SPMV.

METHODS

Chemicals

The fluorescent probes DPH, PRO-DPH and TMA-DPH were obtained from Molecular probes (Eugene, OR, USA). Dopamine · HCl, and other reagents were obtained from Sigma (St. Louis, MO, USA) and were of analytical grade.

Preparation of SPMV

The SPMV was isolated from a whole bovine brain by the formerly reported method in our laboratory (Yun & Kang, 1990; Yun et al, 1990). The specific activities of Na, K-ATPase, acetylcholinesterase and 5'-nucleotidase in the plasma membrane fraction were approximately 4-, 2.5- and 3-times higher than those in crude homogenates. The electron microscopic examination of the prepared SPMV showed very high purity. The vesicles, which were separated according to size, demonstrated homogeneous distribution and no longer showed the presence of intracellular organelles or leakage. The protein concentration was determined by the method of Lowry et al (1951) using bovine serum albumin (BSA) as a standard.

Fluorescence measurements

The fluorescent probe DPH was dissolved in tetrahydrofuran and a volume of 0.5 ml of tetrahydrofuran per ml of phosphate-buffered saline (PBS) was added directly to the membrane suspension at a concentration of 0.1 μg/100 μg membrane protein. PBS was composed of 8 g/l NaCl, 0.2 g/l KCl, 0.2 g/l KH_2PO_4, 1.15 g/l $Na_2HPO_4 \cdot 7H_2O$, 0.48 g/l Hepes (37°C, pH 7.4). The suspension was incubated in the dark at 37°C for 30 min with frequent vortexing. The excitation wavelength for DPH in SPMV was 362 nm, and the fluorescence emission was monitored at 424 nm.

The polarization (P) was obtained from intensity measurements using $P=(I_{\parallel} - GI_{\perp})/(I_{\parallel} + GI_{\perp})$ where G is a grating correction factor for the monochromator's transmission efficiency for vertically and horizontally polarized light. This polarization (P) value is the ratio of the fluorescence intensities of the vertical to horizontal components when the excited light is polarized in the horizontal direction. This condition yields an equivalent fluorescence intensity (I) entering the monochromator irrespective of the orientation of the observation polarizer. The polarization was expressed as the anisotropy [$\gamma=2P/(3-P)$], the limiting anisotropy (γ_∞) and the order parameter (S). The limiting anisotropy (γ_∞) of membrane-bound DPH, PRO-DPH and TMA-DPH was determined directly from the anisotropy (γ) value using the following relationship (van Blitterwijk et al, 1981)

$\gamma_\infty=(4/3)\gamma - 0.10 \qquad 0.13 < \gamma < 0.28 \qquad (1)$

The limiting anisotropy (γ_∞) reflects restriction to probe motion and can be converted to an order parameter, $S=(\gamma_\infty/\gamma_o)^{1/2}$ (Kawato et al, 1978) where r_o, the anisotropy in the absence of motion, is equal to 0.362 for DPH (Lakowicz et al, 1979).

Dopamine, at the concentrations indicated, was added directly to the membranes resuspended in PBS. The pH of the buffered sample was not changed significantly by addition of dopamine. Measurements commenced usually within 1 min after addition. No effect of longer incubation time was noted.

All fluorescence measurements were obtained with a Multi Frequency Cross-Correlation Phase and Modulation Fluorometer (ISS K2-003, IL, USA) and performed at 37°C (pH 7.4). Before the fluorescence spectra were obtained, all samples were bubbled by dry nitrogen through the solution for at least 5 min in order to eliminate oxygen. Blanks, prepared under the identical conditions without fluorescent probes, served as control for the fluorometric measurements.

Determination of individual monolayer structure in SPMV: preferential distribution of charged DPH derivatives

In order to evaluate transmembrane asymmetry of rotational mobility, plasma membrane sidedness selective fluorescent DPH derivatives, cationic TMA-DPH and anionic PRO-DPH were utilized. Since the negatively charged phospholipids are localized preferentially in the inner monolayer of SPMV, TMA-DPH and PRO-DPH are expected to be located preferentially in the inner monolayer and in the outer monolayer of SPMV, respectively. The excitation wavelength for TMA-DPH and PRO-DPH was 362 nm, and the fluorescence emission was read at 424 nm.

RESULTS

In order to determine the effects of the dopamine on the bulk and asymmetric rotational mobility of monolayers of SPMV, it is first necessary to demonstrate that this drug does not interact directly with DPH, TMA-DPH and PRO-DPH, and thereby quench its fluorescence. Quenching of absorbance-corrected fluorescence intensity by the dopamine is not observed at all of the concentration levels where dopamine was tested. Furthermore, if direct quenching of DPH, TMA-DPH and PRO-DPH by dopamine occurred, fluorescence lifetime would decrease. However, the fluorescene lifetime of DPH is not changed by dopamine in the SPMV. For example, the lifetime of DPH in the SPMV was 9.8±0.01, 9.7±0.2, 9.8±0.3, 9.6±0.1 and 9.7±0.2 ns at 0.1, 0.5, 1.5 and 10 mM dopamine, respectively. There were similar results with TMA-DPH and PRO-DPH. Hence, the possibility of direct quenching of fluorescence of the probes by the drug is ruled out.

Effects of dopamine on the range of rotational mobility of bulk SPMV lipid bilayer

The bulk anisotropy (γ), limiting anisotropy (γ_∞) and order parameter (S) of intact SPMV (dopamine-untreated) were 0.202±0.001, 0.169±0.002 and 0.683±0.003 (Table 1). The effects of increasing concentrations of dopamine on the anisotropy (γ), limiting anisotropy (γ_∞), and order parameter (S) of DPH in bulk SPMV lipid bilayer are shown in Fig.

Table 1. Structural parameters of 1,6-diphenyl-1,3,5-hexatriene (DPH), 1-[4-(trimethylammonio)-phenyl]-6-phenylhexa-1,3,5-hexatriene (TMA-DPH) and anionic 3-[P-(6-phenyl)-1,3,5-hexatrienyl]-phenylpropionic acid (PRO-DPH) in synaptosomal plasma membrane vesicles isolated from bovine brain (SPMV)

Parameters	DPH	TMA-DPH	PRO-DPH
Anisotropy (γ)	0.202±0.001	0.220±0.002	0.186±0.001
Limiting anisotropy (γ_∞)	0.169±0.002	0.193±0.003	0.148±0.002
Order parameter (S)	0.683±0.003	0.730±0.005	0.639±0.004

Fluorescence measurements were performed at 37°C (pH 7.4). Values are represented as the mean±SEM of 5 determinations.

1~3. In the SPMV, dopamine decreased the anisotropy (γ), limiting anisotropy (γ_∞) and order parameter (S) of DPH (increased rotational mobility) in a concentration-dependent manner. The significant decreases in the anisotropy (γ) by dopamine were observed even at 40×10^{-9}, 10×10^{-8}, 20×10^{-8}, 40×10^{-8}, 80×10^{-8} and 10×10^{-7} M (Fig. 1). The difference in the anisotropy (γ) of DPH found in the bulk SPMV lipid bilayer before and after adding 10×10^{-7} M dopamine was 0.027. This can be illustrated by comparing the effect of temperature on this parameter. The anisotropy (γ) values of DPH in the bilayer are 0.202 ± 0.001 (n=5), 0.257 ± 0.002 (n=5) at 37 and 25°C (pH 7.4), respectively. Thus, the difference in the anisotropy (γ) of DPH in the lipid bilayer was 0.027, which was as large as that produced by the temperature increase of approximately 15.7°C.

Effects of dopamine on the range of transbilayer rotational mobility of SPMV lipid bilayer

The anisotropy (γ) value of TMA-DPH in the inner monolayer was 0.034 greater than the value of PRO-DPH in the outer monolayer of the SPMV. This means that the range of rotational mobility of the outer monolayer is greater than that of the inner monolayer.

The effects of increasing concentrations of dopamine on the anisotropy (γ), limiting anisotropy (γ_∞) and order parameter (S) of TMA-DPH and PRO-DPH in the SPMV individual monolayers are shown in Fig.

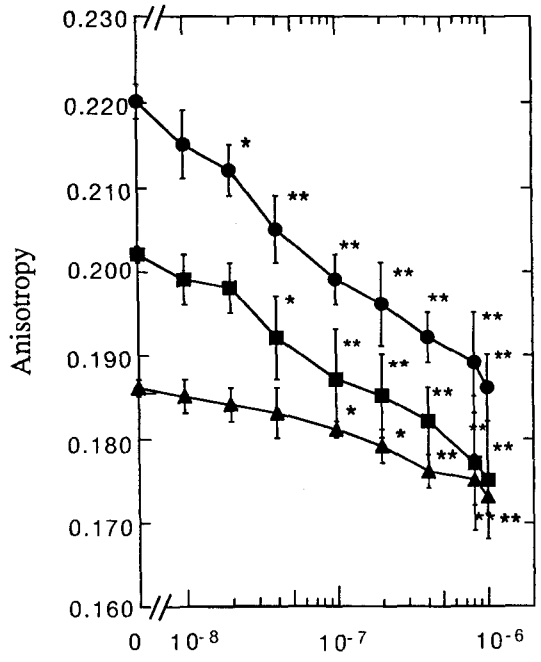

Fig. 1. Effects of dopamine · HCl on the anisotropy (γ) values of DPH, TMA-DPH and PRO-DPH in the SPMV isolated from bovine brain. The excitation and emission wavelengths of the probes were 362 nm and 424 nm, respectively. The probes were incorporated into SPMV and fluorescence measurements were performed at 37°C (pH 7.4). Inner plus outer monolayer (DPH, ■); inner monolayer (TMA-DPH, ●); outer monolayer (PRO-DPH, ▲) as described in Methods. Each point represents the mean ± SEM of 5 determinations. An asterisk and double asterisk signify $P<0.05$ and $P<0.01$, respectively, compared to control by Student's t-test.

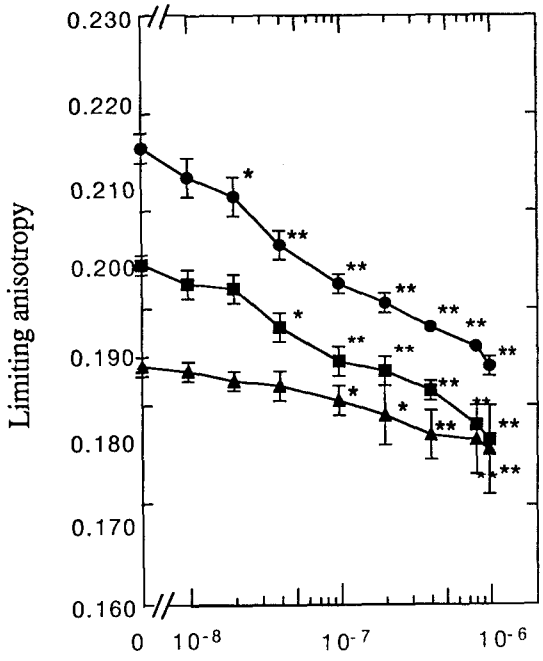

Fig. 2. Effects of dopamine · HCl on the limiting anisotropy (γ_∞) values of DPH, TMA-DPH and PRO-DPH in the SPMV isolated from bovine brain. The excitation and emission wavelengths of the probes were 362 nm and 424 nm, respectively. The probes were incorporated into SPMV and fluorescence measurements were performed at 37°C (pH 7.4). Inner plus outer monolayer (DPH, ■); inner monolayer (TMA-DPH, ●); outer monolayer (PRO-DPH, ▲) as described in Methods. Each point represents the mean ± SEM of 5 determinations. An asterisk and double asterisk signify $P<0.05$ and $P<0.01$, respectively, compared to control by Student's t-test.

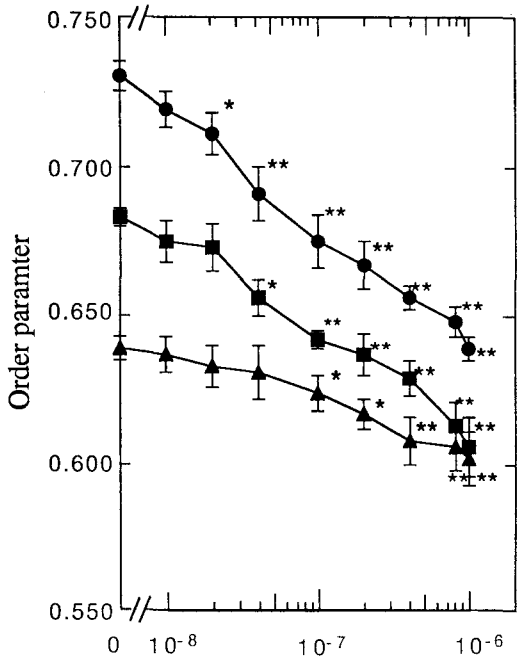

Fig. 3. Effects of dopamine · HCl on the order parameter (S) of DPH, TMA-DPH and PRO-DPH in the SPMV isolated from bovine brain. The excitation and emission wavelengths of the probes were 362 nm and 424 nm, respectively. The probes were incorporated into SPMV and fluorescence measurements were performed at 37°C (pH 7.4). Inner plus outer monolayer (DPH, ■); inner monolayer (TMA-DPH, ●); outer monolayer (PRO-DPH, ▲) as described in Methods. Each point represents the mean ± SEM of 5 determinations. An asterisk and double asterisk signify $P < 0.05$ and $P < 0.01$, respectively, compared to control by Student's t-test.

1 ~ 3, respectively. Dopamine showed a greater increasing effect on the range of rotational mobility of inner monolayer (Fig. 1 ~ 3, filled circles) compared with the outer monolayer (Fig. 1 ~ 3, filled triangles). There was a little, if any, effect on the outer monolayer. Since changes observed in the anisotropy (γ), limiting anisotropy (γ_∞) and order parameter (S) of TMA-DPH and PRO-DPH were derived primarily from changes in the inner monolayer, we studied the selective effects of the drug on the component of the range of mobility of the probes. To the best of our knowledge, the results presented herein are the first to demonstrate that the Sheetz-Singer hypothesis (1974) is valid in neuronal membranes.

DISCUSSION

DPH is a rod-shaped molecule that orients with high affinity in hydrophobic regions (core) of the bilayer structures. The fluorescence polarization mainly reflects the rotational mobility of lipid fluorophores (Schachter, 1984; Molitoris & Hoilien 1987; Yun et al, 1993a, b). The results of fluorescence polarization determination are conveniently expressed as the fluorescence anisotropy (γ). The limiting anisotropy (γ_∞) reflects the hindrance to full 90° rotation of a fluorophore in a particular microenvironment. For example, the rod-like hydrocarbon DPH is free to rotate a full 90° in certain organic solvents, and the γ_∞ value is zero. In native and model membranes, the γ_∞ values of the DPH are high and largely determine γ. In biological experiments, both dynamic (rotational relaxation time of fluorophores) and structural (γ_∞) or static components may be significant, and it seems reasonable to use "fluidity" to designate both. The structural organization of the lipid environment in the bilayers limits the rotational extent or the range of DPH, and γ_∞ can be used to define order parameter (S).

Plasma membranes consist of two monolayers that are asymmetric in lipid distribution, electrical charge, fluidity, protein distribution and function, and do not appear to be coupled. It has been widely known that different lipids could affect the physical properties of the membrane. Several different domains have been described, e.g., hydrophilic, hydrophobic, lateral, outer and inner monolayers (Chin & Goldstein, 1981; Seigneuret et al, 1984; Chabanel et al, 1985; Hitzemann et al, 1986; Treistman & Wilson, 1987). The surface of the membrane is more hydrophilic as compared to the interior that is more hydrophobic. Lateral domains are lipid patches that extend laterally along the horizontal plane of the membrane and are thought to differ in their fluidity and lipid composition (Treistman & Wilson, 1987). Two other domains to be considered are the transbilayer or vertical domains of the membrane (i.e., the outer and inner monolayers). Our data explicitly show that the SPMV consists of vertical domains or monolayers that differ in rotational mobility. Hence, the bulk lipid fluidity change obtained will represent an average of the affected and unaffected portions of the membrane core, and may underestimate the effect on specific domains. Very little attention has been given to the selective effects of dopamine on vertical domains.

The investigation of the binding site of drug at a cellular level provides important basic materials for the research of pharmacological actions of drug. This is because the binding site of drug at a cellular level coincides with the site of drug action, even though which is not necessarily the case at all times. From results of this study, the reactions between the membrane lipids and the drugs can be presumed as follows. The unusually hydrophobic ion channel macromolecule has an anomalously high detergent-binding capacity, due in part to more than a dozen long-chain fatty acids associated with each channel molecule (Butterworth & Strichartz, 1990). Bound to the protein by covalent or noncovalent bonds, these acyl chains may anchor and orient the channel in the membrane, stabilizing the channel's three-dimensional structure. Long-chain fatty acids also may participate in binding of lipophilic drugs such as dopamine. The drug binding site may exist in the channel's pore at the membrane-protein interface, or within the protein subunits of the channel. The clear mechanism of action of the drug in the disordering effects on the lipid bilayer of the neuronal membrane is unknown. However, the mechanism through which dopamine increases the rotational mobility of the SPMV lipid bilayers of the neuronal membranes can be assumed as follows.

The phospholipid molecules in the bilayer of the SPMV, the dopamine binds weakly to the phosphate moiety and effectively establish formation of hydrogen bonds with the carbonyl moiety. Present results show that the dopamine may interact with the phosphate and carbonyl moieties of phospholipids in the bilayer. The interaction of the dopamine with the bilayer's hydrocarbon region will generate rearrangements of the intermolecular hydrogen-bonded network among phospholipid molecules and/or protein molecules that are associated with the liberation of hydrated water molecules on the monolayer of the membranes. The interaction will also change the orientation of the P-N dipole of phospholipid molecules. These changes should cause disordering of the hydrocarbon interior of the bilayer.

According to the results of this study, we can presume the mechanism of pharmacological action of dopamine as follows. First, the increasing effects on the rotational mobility of the neuronal membrane lipid bilayers by dopamine provide proper environment for the generation of the dopamine-receptor interaction. Second, due to the increased rotational mobility of the lipid bilayers resulting from the dopamine-receptor interaction, intracellular influx of inorganic ions is facilitated, thereby leading to depolarization of postjunctional membrane.

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