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Effects of Dopamine·HCl on Structural Parameters of Bovine Brain Membranes

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Fluorescence probes located in different membrane regions were used to evaluate the effect of dopamine·HCl on the structural parameters (transbilayer lateral mobility, annular lipid fluidity, protein distribution, and thickness of the lipid bilayer) of synaptosomal plasma membrane vesicles (SPMV), which were obtained from the bovine cerebral cortex. An experimental procedure was used based on selective quenching of 1,3-di(1-pyrenyl)propane (Py-3-Py) by trinitrophenyl groups, and radiationless energy transfer from the tryptophan of membrane proteins to Py-3-Py and energy transfer from Py-3-Py monomers to 1-anilinonaphthalene-8-sulfonic acid (ANS) was also utilized. Dopamine·HCl increased both the bulk lateral mobility and annular lipid fluidity, and it had a greater fluidizing effect on the inner monolayer than on the outer monolayer. Furthermore, the drug had a clustering effect on membrane proteins.

Key words: Dopamine·HCl, Transbilayer lateral mobility, Membrane thickness, Annular lipid fluidity, Membrane protein clustering

INTRODUCTION

Kebabian & Calne (1979) first described the two main types of the dopaminergic receptor, which they named D_1 and D_2. Recent advances in genetic cloning have enabled the discovery of 5 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 into 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 through the observation that amphetamines and other stimulant drugs could induce psychosis. 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 (Peroutka & Snyder, 1980; Faedda *et al.*, 1989).

Changes in membrane fluidity are known to be linked to alterations in physiological processes of the cell membrane, such as carrier-mediated transport, activities of membrane bound enzymes, receptor binding, phagocytosis, endocytosis, depolarization dependent exocytosis, cytotoxicity, and cell growth (Spector and Yorek, 1985). Membrane fluidity appears to change during development, aging, and drug therapy of cultured cells (Toplak *et al.*, 1990). Alterations in membrane fluidity may even represent a possible parameter in evaluating malignancy (Shinitzky, 1984).

The ability to regulate their volume belongs to the elementary requirements needed for the survival of cells. The cell volume regulatory mechanisms are an integral part of the intracellular cascade that transmits hormonal

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signals (Lang *et al.,* 1993, 1998). It is important to note that the cell volume is linked to the ion channels and carriers located at the cell membrane as well as being linked to metabolic activity of the cell (Lang *et al.,* 1993, 1998).

Researches on the mechanism of pharmacological action of dopamine have been concentrated on the interaction between dopamine and its receptors. It is not an exaggeration to say that these studies have been entirely about changes of cells that deal with inward and outward inorganic ion transport, and the generation of a second messenger that results from the dopamine-receptor interaction. Because receptors coexist with membrane lipids, we cannot entirely exclude the possibility that changes in lipid fluidity may be accompanied before or after neurotransmitters display interactions with their receptors. It should be kept in mind that the changes of these cells involved in inward and outward inorganic ion transport through membranes are closely related to the fluidity of membrane lipid bilayers.

Therefore, we presume that fluidity of membrane lipid bilayers would be changed before or after the dopamine-receptor interaction. Dopamine·HCl has an increasing effect on the rotational mobility of the bulk bilayer structures and has a greater increasing effect on the mobility of the inner monolayer compared to the outer monolayer of the synaptosomal plasma membrane vesicles (SPMV) from bovine cerebral cortex (Kim *et al.*, 2000). If dopamine·HCl causes an expansion of neuronal membranes, this expansion is probably due to the increased fluidity in the neuronal membrane lipid bilayer. Our questions are what the role of dopamine·HCl (which is believed to have more interactions with protein than other lipids) is and to what degree the neuronal membrane lipid bilayer is expanded by dopamine·HCl. More specifically, our questions are: first, how much of an increase does dopamine·HCl bring to lateral mobility of the neuronal membrane lipid bilayer; second, whether such increasing effects are shown evenly on both lipid bilayers or differently between inner and outer monolayers; third, if the degree of increase is different between the inner and outer monolayers, then which monolayer has been mostly affected; fourth, whether the annular lipid fluidity and protein clustering of the neuronal membrane lipid bilayer are increased or decreased by dopamine·HCl; and fifth, to what degree the neuronal membrane is expanded by dopamine·HCl.

Here, we present the results of our study on how we solved the aforementioned questions by employing fluorescence techniques, including the fluorescence quenching technique, which was first developed specifically for this study to measure the rate and range of asymmetrical lateral mobility between inner and outer monolayers of the lipid bilayer.

MATERIALS AND METHODS

Materials

The fluorescent probes, 1,3-di(1-pyrenyl)propane (Py-3-Py) and 1-anilinonaphthalene-8-sulfonic acid (ANS), 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 synaptosomes and TNBS labeling

Synaptosomes were prepared as described previously (Yun and Kang, 1990; Yun *et al.,* 1990). To determine the fluorescence parameters of the probe molecules in each of the membrane monolayers, 2,4,6-trinitrobenzenesulfonic acid (TNBS) labeling reactions were performed as described (Yun and Kang, 1990; Yun *et al.,* 1993, 1994; Kang *et al.,* 1996) with a few modifications. The synaptosomal pallet was gently resuspended in 50 mL of 4 mM TNBS in buffer A for 90 min or in buffer A alone. Buffer A consisted of 30 mM NaCl, 120 mM $NaHCO_3$, 11 mM glucose, and 1% bovine serum albumin (BSA). In order to assure complete exposure of all synaptosomal outer monolayers to TNBS, the pellet was passed slowly through an Eberbach tissue grinder (3 up and down strokes). Unless otherwise specified, the treatment was carried out at 4°C. The TNBS labeling reaction was terminated by adding an equal volume of 1% BSA in phosphate-buffered saline (PBS: 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; pH 7.4).

Membrane isolation

SPMV were isolated from synaptosomes by the method that was previously reported (Yun and Kang, 1990; Yun *et al.,* 1990). Their protein concentration was determined through the method of Lowry *et al.* (1951) with BSA as the standard. The purity of SPMV was assessed by enzymatic and morphological criteria. The specific activities of Na^+, K^+-ATPase, acetylcholinesterase, and 5'-nucleotidase were enriched about 4-, 2.5- and 3-fold, respectively, in the plasma membrane fraction with respect to crude homogenates. The transmission electron microscopic examination of the SPMV indicated a level of very high purity. The vesicles, which were separated according to size, were homogeneous in size, and there was no sign of other intracellular organelles or of leakage.

Fluorescence measurements

All fluorescence measurements were made with a Multi Frequency Cross-Correlation Phase and Modulation Fluorometer (Model; ISS K2-003). Cuvette temperature was maintained at 37.0 ± 0.1°C in a circulating water bath (pH 7.4). Bandpass slits were 10 nm on excitation and 5 nm

on emission. Blanks, which were prepared under identical conditions without fluorescent probes, served as controls.

Py-3-Py was incorporated by adding aliquots of a stock solution of 5×10^{-5} M in absolute ethanol to the SPMV, such that the final probe concentration was less than 5×10^{-7} M (Yun *et al.*, 1994; Kang *et al.*, 1996). Mixtures were initially vigorously vortexed for 10 sec at room temperature and then were incubated at 4°C for 18 h with gentle stirring (Yun *et al.*, 1994; Kang *et al.*, 1996).

After probe incorporation the membrane suspension was placed in cuvettes, and the control fluorescence were determined. Concentrated solutions of the dopamine·HCl were prepared in 10 mM Tris-HCl (pH 7.4) and were added to the labeled membrane suspension (or untreated membrane suspension) to give the desired concentration of the agent.

Excitation wavelengths were 280 nm for tryptophan, 330 nm for Py-3-Py, and 380 nm for ANS. Emission wavelengths were 335 nm for tryptophan, 379 nm for Py-3-Py monomer, and 480 nm for Py-3-Py excimer. For Py-3-Py excimer emission, a GG-455 cut-off filter was used. The excimer to monomer fluorescence intensity ratio, I'/I, was calculated from the 480 nm to 379 nm signal ratio.

Dopamine·HCl, at the concentrations indicated, were added directly to the membranes that were resuspended in PBS. The sample (SPMV 2 mL-50 μM/mL) was placed in the thermostated cuvette chamber and was stirred continuously. The pH of the buffered sample was not significantly changed by the addition of dopamine·HCl. Measurements commenced usually within 1 min after the addition. No effect from a longer incubation time was noted.

Effect of dopamine·HCl on individual monolayer structure in SPMV: Selective quenching of Py-3-Py

To determine individual monolayers of SPMV, a new method was devised, which was the selective quenching of Py-3-Py fluorescence by trinitrophenyl groups. This method is based on the assumption that the system is composed of fluorescing compartments that are differentially accessed by TNBS. The excimer to monomer fluorescence intensity ratios, I'/I, of Py-3-Py in bulk (inner plus outer), inner and outer monolayers were calculated using the following equations:

$$(I'/I)_t = I'_t / I_t \quad \text{(equation 1)}$$

$$(I'/I)_i = I'_i / I_i \quad \text{(equation 2)}$$

$$(I'/I)_o = (I'_t - I'_i) / (I_t - I_i) \quad \text{(equation 3)}$$

where $(I'/I)_t$, $(I'/I)_i$, and $(I'/I)_o$ are the excimer to monomer fluorescence intensity ratios of Py-3-Py (I'/I) in bulk, and inner and outer monolayers, respectively. The values of I'_t (excimer fluorescence intensity for the inner plus outer monolayers) and I'_i (excimer fluorescence intensity for the inner monolayer) were determined for Py-3-Py from SPMV that was incubated with buffer A and buffer A plus TNBS at 4°C (pH 8.5) (nonpenetrating conditions), respectively.

Determination of annular lipid fluidity in SPMV

Incorporated Py-3-Py in the SPMV was excited by using radiationless energy transfer (RET) from tryptophan (excitation at 286 nm), and the excimer to monomer fluorescence intensity ratio (I'/I) of Py-3-Py was calculated from the 480 nm to 379 nm signal ratio. Given that the Förster radius (the RET-limiting distance) for the tryptophan-Py-3-Py donor-acceptor pair is 3 nm (Dobretsov *et al.*, 1982), only Py-3-Py located in annular lipids (close to proteins) was excited, and the fluidity of the annular lipids was considered proportional to I'/I (Yun *et al.*, 1993, 1994; Kang *et al.*, 1996).

Determination of protein clustering in the SPMV lipid bilayer

The fluorescence intensity of the endogenous tryptophan in SPMV was determined. Following this measurement, the Py-3-Py probe was incorporated at 10^{-6} M (1 μL of 10^{-3} M in ethanol), and after 10 min, tryptophan emission fluorescence intensity was measured again. The efficiency of RET from tryptophan to Py-3-Py was calculated as:

$$\text{RET} = (I_d - I_{da}) / I_d \quad \text{(equation 4)}$$

where I_d and I_{da} represent the fluorescence intensity of the donor (in this case, endogenous tryptophan) in the absence and presence of the acceptor (in this case, Py-3-Py), respectively. The wavelengths of excitation and emission of tryptophan were 286 and 335 nm, respectively.

Determination of thickness of SPMV lipid bilayer

The effect of dopamine·HCl on the thickness of SPMV lipid bilayer was determined using the energy transfer between the surface fluorescent probe ANS and the hydrophobic fluorescent probe Py-3-Py. Py-3-Py was excited at 330 nm, and the monomer fluorescence intensity was determined to be at 379 nm. Next, 1 min after 30 μM ANS (60 μL of 10^{-3} M solution in water) was added, Py-3-Py monomer fluorescence was measured again. ANS was located on the membrane surface and this concentration was chosen because it produced 50% quenching of Py-3-Py monomer fluorescence in SPMV. Efficiency of Py-3-Py quenching by ANS was calculated using equation 4, considering Py-3-Py monomer as the donor and ANS as the acceptor. Membrane thickness was considered to be proportional to the distance (D) between the donor and the acceptor and can be calculated as follows:

$$D = [(\pi\Phi/6)(\langle E\rangle^{-1} 1)]^{1/3} \quad \text{(equation 5)}$$

where Φ is the number of acceptor/volume, and $\langle E\rangle$ is

the average efficiency of RET (Eisinger and Flores, 1982). In order to calculate D, we used a modified equation:

$$D = [(F'/F_{max})(<E>^{-1} - 1)]^{1/3} \quad \text{(equation 6)}$$

where F' is the fluorescence intensity of the acceptor concentration that was utilized in the RET experiments, and F_{max} is the maximal fluorescence of the acceptor in the membrane (both values were obtained by ANS binding studies in the absence of Py-3-Py). The ratio F'/F_{max} provides a measure of binding of ANS to the membrane surface and provides a correction for the effect of dopamine·HCl on this binding.

ANS (2 μL of 10^{-2} M solution) was added repeatedly to the sample in 15 sec intervals, and measurements were made as soon as the probe was added. In each case, at least 14 concentrations of the probe were used. Dissociation constants and F_{max} values for ANS-membrane complex were calculated from double-reciprocal plots by linear regression analysis. Correlation coefficients were >0.99.

RESULTS AND DISCUSSION

In order to determine the effect of the dopamine·HCl on the bulk and asymmetric lateral mobility of the monolayers of SPMV, on annular lipid fluidity and clustering proteins in the SPMV, and on thickness of the SPMV lipid bilayer, it is, first, necessary to demonstrate that this drug does not interact directly with Py-3-Py and thereby quench its fluorescence. Quenching of absorbance-corrected fluorescence intensity by the dopamine·HCl was not observed with all tested concentrations. Furthermore, if direct quenching of Py-3-Py by dopamine·HCl occurred, fluorescence lifetime would decrease. However, the lifetime of Py-3-Py in the SPMV was 15.1±0.02, 15.2±0.01, 15.1±0.02, 15.0±0.05, and 15.3±0.01 ns at 0.1, 0.5, 1, 5, and 10 mM dopamine·HCl concentrations, respectively. Hence, the possibility of the drug directly quenching the fluorescence of the probe was ruled out.

Changes in the membrane structure, which includes fluidity, cause changes in membrane properties and functions. As this study shows, neuronal membrane fluidity and structure are changed by dopamine·HCl. Therefore, it can easily be presumed that properties and functions of the neuronal membranes will be changed by the drug.

Our data presented herein have shown that dopamine·HCl (even at 2×10^{-8} M) increased the rate and range of lateral mobility in the SPMV, which indicates that dopamine·HCl has an increasing effect of lateral mobility on the bulk lipid bilayer. Dopamine·HCl increased the annular lipid fluidity in the SPMV but dercreased the thickness of the SPMV lipid bilayer. Furthermore, the drug had a clustering effect on proteins in the SPMV.

Effect of dopamine·HCl on the rate and range of lateral mobility of bulk bilayer SPMV

To determine the rate and range of lateral mobility in the SPMV, Py-3-Py was used, which is a pyrene derivative that has been used to quantify lateral mobility within native and model membranes (Zachariasse *et al.*, 1982; Schachter, 1984; Yun *et al.*, 1993, 1994; Kang *et al.*, 1996). With this probe, emission of both the monomer (*I*) and the excimer (*I'*) components can be monitored in such a way that a ratio can be derived and used as a measure for lateral mobility (Zachariasse *et al.*, 1982; Schachter, 1984; Yun *et al.*, 1993, 1994; Kang *et al.*, 1996). As the probe mobility increases, emission from the excimer predominates since the formation of the intramolecular excimer is dependent upon lateral movement of its two components. Therefore, an increase in the *I'/I* ratio indicates an increased lateral mobility of the probe within the membranes. The excimer fluorescence technique using Py-3-Py has the advantage over its counterpart based on intermolecular excimerization in which very low probe concentrations can be used (<10^{-7} M), and perturbation of the SPMV by the probe molecule is minimized.

The *I'/I* value in intact SPMV (dopamine·HCl untreated) was 0.412 ± 0.005 (at 37°C, pH 7.4). Incubation with dopamine·HCl increased the rate and range of lateral mobility of bulk (inner + outer monolayer) SPMV at concentrations as low as 2×10^{-8} M (n = 5, $P<0.05$), as demonstrated in Fig. 1. The *I'/I* value of Py-3-Py in bulk SPMV incubated with 10^{-7} M dopamine·HCl was 0.441 ± 0.012** (n = 5, $P<0.01$), and the change in *I'/I* value before and after adding the dopamine·HCl was 0.029. The *I'/I* values of Py-3-Py in the bilayer were 0.412 ± 0.005 (n = 5) and 0.356 ± 0.006 (n = 5) at 37 and 25°C (pH 7.4), respectively. Hence, the effect of 10^{-7} M dopamine·HCl was equivalent to that produced by a temperature increase of approximate 6.2°C.

Effect of dopamine·HCl on the rate and range of transbilayer lateral mobility of SPMV monolayers

The covalently linked trinitrophenyl group has a broad absorbance range with a maximum near 420 nm. This peak has a large overlap with the fluorescence emission of Py-3-Py. This overlap is responsible, in part, for the high transfer (quenching) efficiency of the probe. Approximately half of the Py-3-Py fluorescence was quenched in the trinitrophenylated SPMV. When TNBS labeling was conducted under penetrating conditions (37°C), nearly all of the Py-3-Py fluorescence was quenched. The *I'/I* of Py-3-Py in the outer monolayer was 0.066, which was greater than the value calculated for the inner monolayer. This means that the rate and range of lateral mobility of the outer monolayer is greater than that of the inner monolayer.

The effect of increasing concentrations of dopamine·

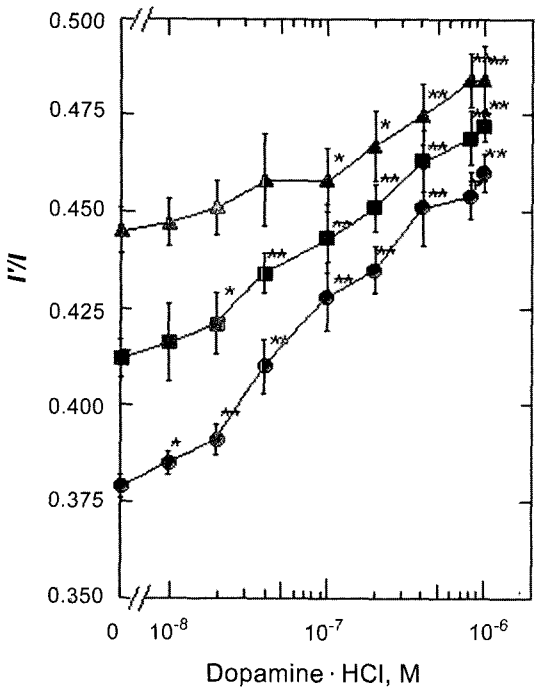

Fig. 1. Effects of dopamine·HCl on excimer to monomer fluorescence intensity ratio (*I'/I*) of Py-3-Py in SPMV. The excitation wavelength of Py-3-Py was 330 nm, and the *I'/I* values were calculated from the 480 nm to 379 nm signal ratio. SPMV was treated ± 4mM TNBS, pH 8.5, at 4°C for 90 min. Py-3-Py was incorporated into SPMV, and fluorescence measurements were performed at 37°C (pH 7.4). Untreated (inner and outer monolayers, ■); TNBS treated (inner monolayer, ●); calculated for outer monolayer (▲) by eq. 3 as described in Materials and 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 the control by Student's *t*-test.

HCl on the *I'/I* values in the individual SPMV monolayers is shown in Fig. 1. Dopamine·HCl illustrates a greater fluidizing effect on the rate and range of lateral mobility of the inner monolayer (Fig. 1, filled circles) than on the rate and range of lateral mobility of the outer monolayer (Fig. 1, filled triangles). Since the changes observed in the *I'/I* values were derived primarily from the effect on the inner monolayer, we studied the selective effects of dopamine·HCl on the rate and range of the mobility of the probe. To the best of our knowledge, the results presented here are the first to demonstrate that the Sheetz-Singer hypothesis (1974) is valid in neuronal membranes.

Although many researchers have reported that the inner and outer monolayers of native and model membranes differ in fluidity, all previous studies of asymmetric bilayer fluidity have examined the rotational range but not the rate and range of lateral mobility. In this study, using the selective quenching of Py-3-Py fluorescence by trinitrophenyl groups, transbilayer asymmetric lateral mobility was examined.

The TNBS labeling reaction must be carefully monitored in order to ensure that the reagent does not penetrate into the synaptosomes cause labeling of both sides of the plasma membrane. For this purpose, three control procedures are routinely used. First, as an "internal control", mitochondria and microsomes are isolated from the synaptosomes from which the trinitrophenylated plasma membranes are isolated. If any significant degree of penetration of TNBS into the synaptosome occurs, these intracellular organelles also become trinitrophenylated. Only 1.8±0.2% and 2.1±0.4% of microsomal and mitochondrial phosphatidylethanolamine were trinitrophenylated through our treatment, respectively. In contrast, when the TNBS treatment is performed under penetrating conditions (37°C), 60-80% of the phosphatidylethanolamine in microsomes or mitochondria is trinitrophenylated (Yun and Kang, 1990). Second, approximately half of the Py-3-Py fluorescence was quenched in the trinitrophenylated SPMV. Third, the trinitrophenylation of the synaptosomes may alter the membrane enzyme activities. Unlike the results obtained under penetrating conditions (37°C), the activity of neither Na^+,K^+-ATPase nor 5'-nucleotidase was significantly altered by the TNBS reaction under non-penetrating conditions (Yun and Kang, 1990).

It is important to note that the term "membrane fluidity" is often misused. It arose from a combination of spectroscopic studies, which specifically are the realization that membranes can be regarded as two-dimensional fluids and the desire to obtain a simple single physical parameter that would describe their properties. The difficulty with the membrane fluidity concept is that any physical parameter chosen will be a function of the spectroscopic method that is employed, specifically its particular time window and the properties of the probe (shape, charge, location etc) (Stubbs and Williams, 1992). The membrane fluidity concept also depends on the assumption that the hydrophobic region of cell membranes is structurally and dynamically homogeneous, an assumption that is now under serious challenge. Thus, while it may be true to say that the bulk or average spectroscopic properties of cell membranes may not be useful in building a hypothesis for the pharmacological action(s) of drug(s), local properties pertaining to domains or the immediate environment of a membrane protein may be very relevant.

As it has been already pointed out, membrane bilayer mobility is one of the more important factors that control membrane microviscosity or fluidity. Membrane bilayer mobility includes lateral mobility, rotational mobility, and flip-flop, and it is well known that the most important of these is lateral mobility. We are pleased to have been able to develop and describe, for the first time, a fluorescence quenching technique that can measure the membrane transbilayer lateral mobility. Therefore, this study can make

a contribution to the study of drug-membrane interactions.

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 had been widely known that different lipids could affect the physical properties of the membrane. Membrane cholesterol is one of the major lipids of plasma membranes and is asymmetrically distributed in the outer and inner monolayers of membranes (Kier *et al.*, 1986; Wood *et al.*, 1990; Schroeder *et al.*, 1991a,b). Interest in cholesterol is derived from the fact that cholesterol has a rigidifying effect on the membrane above the phase transition temperature of the membrane lipid. In erythrocytes, differences in fluidity between the two monolayers have not been consistently observed. Some studies have reported that the outer monolayer was less fluid (Seigneuret *et al.*, 1984; Chabanel *et al.*, 1985), whereas other studies have found that the fluidity of the outer monolayer was the exact opposite: more fluid than the inner monolayer (Cogan and Schachter, 1981; Schachter *et al.*, 1983). The discovery that the inner monolayer of the synaptic plasma membrane, which was isolated from rat brain (SPM), was less fluid than outer monolayer was consistent with data showing that the SPM inner monolayer contains approximately 7-times as much cholesterol compared to the outer monolayer (Wood *et al.*, 1990). Thus, a possible explanation for the range of asymmetric lateral mobility between the outer and inner monolayers of SPMV in this study is that the amount of cholesterol may differ in the outer and inner monolayers. Although these differences have been ascribed to cholesterol (which is asymmetrically distributed between the inner and outer monolayers of the neuronal membrane), it does not seem to be the sole responsible agent for such differences. This is because the differences in asymmetrical lateral diffusion between the inner and outer monolayers of the model membrane lipid bilayer prepared from total lipids that were isolated from SPMV were 0.452 ±0.008 and 0.500±0.013, respectively (I'/I values from bispyrenyl propane, Min, 1997). The differences in asymmetrical lateral diffusion between the inner and outer monolayers of the model membrane lipid bilayer made with phospholipids that were separated from SPMV were also 0.540±0.013 and 0.572±0.016, respectively (I'/I values from bispyrenyl propane, Lee, 1999). It is presumed that the asymmetrical mobility between the inner and outer monolayers of the model membranes formed with total phospholipids, where cholesterol and protein are not present, can be attributed to the type of phospholipids (which are likely distributed asymmetrically between inner and outer monolayers) and to the composition of each phospholipid's unsaturated or saturated fatty acids. We can also presume that the law of physics may dictate asymmetrical movements for the stability of the lipid bilayer.

Effect of dopamine·HCl on the annular lipid fluidity in the SPMV lipid bilayer

I'/I values showed that the annular lipid fluidity of SPMV (intact membrane) was 0.156±0.006 (37°C, pH 7.4) and that this increased in response to concentrations of 10^{-8} M dopamine·HCl and higher (Fig. 2).

The clear action mechanism of the drug on the increasing effects of annular lipid fluidity of the SPMV is unknown. However, the mechanism through which dopamine·HCl increases the annular lipid fluidity of the SPMV lipid bilayer can be assumed as follows.

Annular lipids are known to surround proteins with or without any physical association with them. Dopamine·HCl may alter the stereo structure or dynamics of these proteins by combining with lipids, especially with annular lipids, which increases their mobility and indirectly affects the dynamic behavior of the proteins. Biological membranes have a highly complex composition, and it has not been feasible to monitor changes in the local lipid environment and to determine their effect on membrane protein function. Nevertheless, it is likely that the observed effects are not only due to the influence of dopamine·HCl on lipids but are magnified by the interactions between lipids and proteins.

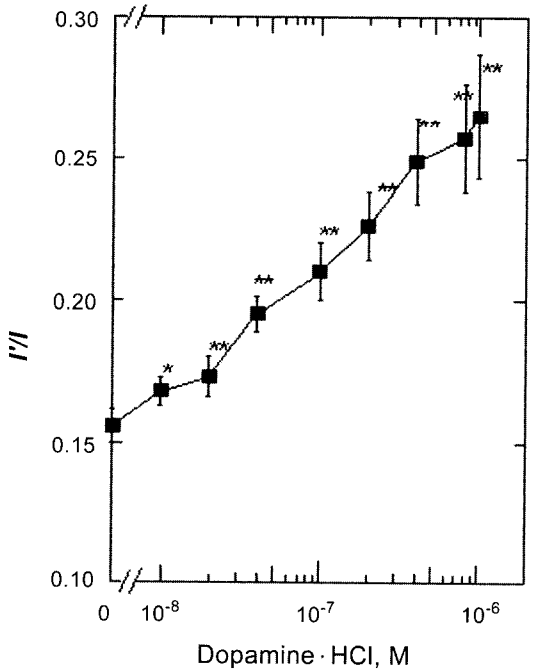

Fig. 2. Effects of dopamine·HCl on annular lipid fluidity in SPMV. Py-3-Py was excited through RET from tryptophan (excitation wavelength, 286 nm), and the excimer to monomer fluorescence intensity ratio (I'/I) was calculated from the 480 nm to 379 nm signal ratio. Fluorescence measurements were performed at 37°C (pH 7.4). 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 the control by Student's *t*-test.

Effect of dopamine·HCl on protein clustering of SPMV

Protein distribution was evaluated by RET from tryptophan to Py-3-Py. The RET value of untreated SPMV was 0.295 ±0.003 (37°C pH 7.4), and it was lowered by concentrations of 2×10^{-8} M dopamine·HCl or higher (Fig. 3).

Protein clustering is probably caused by the interaction between phospholipids, in particular annular lipids, whose movement is increased by dopamine·HCl and the proteins around them.

Effect of dopamine·HCl on the thickness (D) of SPMV lipid bilayer

Within the cellular machinery, cell volume is an integral element in regulating cellular performance. The role of the cell volume in cell functions involves erythrocyte volume and shape, epithelial transport, regulation of metabolism, receptor recycling, hormone and transmitter release, excitability and contraction, migration, pathogen host interactions, cell proliferation, cell death, and others (Lang *et al.*, 1998).

It is known that the increase or decrease of molecular movement eventually increases or decreases the molecular volume. However, the amount of molecular movement required for the increase or decrease of the molecular volume is not known. In this study, the amount of membrane thickness (D) that was increased (or decrease) by dopamine·HCl was measured, using the advantage of the fluorescence quenching technique.

Membrane thickness (D) was measured by the energy transfer from Py-3-Py to ANS in SPMV. F'/F_{max}, and E values are listed in Table I. The intact membrane thick-

Table I. F'/F_{max} and E values

dopamine·HCl Conc. (M)	F'/F_{max}	E
0	0.724 ± 0.016	0.389 ± 0.003
10×10^{-9}	0.721 ± 0.015	0.386 ± 0.002
20×10^{-9}	0.717 ± 0.016	0.383 ± 0.004
40×10^{-9}	0.715 ± 0.009	0.378 ± 0.003
10×10^{-8}	0.710 ± 0.012	0.368 ± 0.002
20×10^{-8}	0.704 ± 0.018	0.363 ± 0.004
40×10^{-8}	0.699 ± 0.013	0.357 ± 0.002
80×10^{-8}	0.693 ± 0.009	0.350 ± 0.002
10×10^{-7}	0.689 ± 0.007	0.346 ± 0.004

F′ is the fluorescence intensity of 5 μM 1-anilinonaphthalene-8-sulfonic acid (ANS) and F_{max} is the maximal fluorescence of ANS in synaptosomal plasma membrane vesicles isolated from bovine cerebral cortex. Both values were obtained by ANS binding studies in the absence of 1,3-di(1-prenyl)propane (Py-3-Py). Values are represented as the mean ± SEM of 5 determinations.

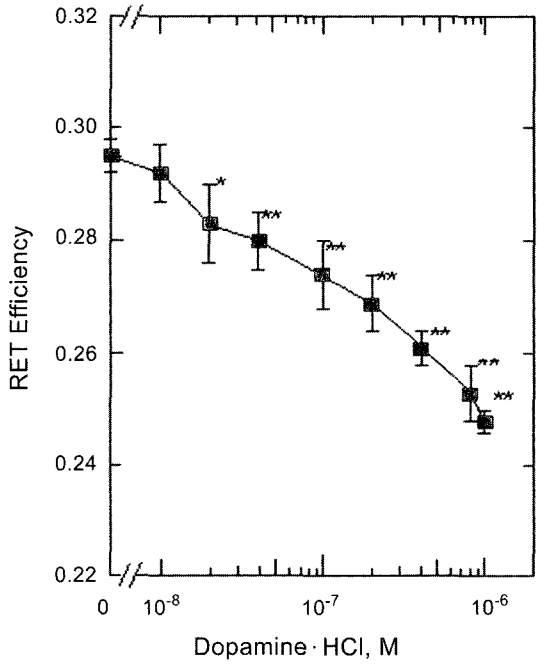

Fig. 3. Effects of dopamine·HCl on protein clustering in SPMV. Efficiency of RET from tryptophan to Py-3-Py was taken as a measure of protein clustering and was calculated by eq. 4. Fluorescence measurements were performed at 37°C (pH 7.4). Each point represents the mean ± SEM of 5 determinations. An asterisk and double asterisk signify $P<0.05$ and $P<0.01$, respectively, compared to the control by Student's *t*-test.

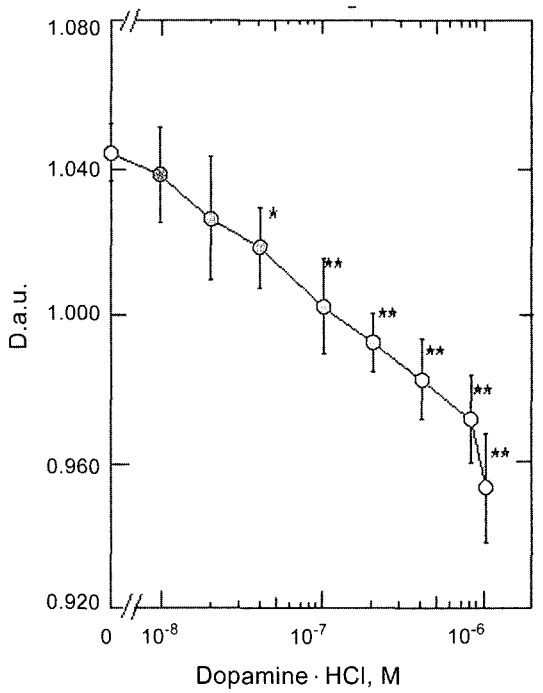

Fig. 4. Effects of dopamine·HCl on lipid bilayer thickness in SPMV. D (a measure of bilayer thickness) values were calculated by eq. 6 and were expressed in arbitrary units (a.u.). Fluorescence measurements were performed at 37°C (pH 7.4). Values are represented as the mean ± SEM of 5 determinations. An asterisk and double asterisk signify $P<0.05$ and $P<0.01$, respectively, compared to the control by Student's *t*-test.

ness (D) in arbitrary units was 1.044±0.008 (37°C, pH 7.4). Dopamine·HCl decreased the thickness (D) in a dose-dependent manner (Fig. 4) (37°C, pH 7.4). The significant decreases in the thickness (D) were observed even at 4×10^{-8} M (Fig. 4).

It is a natural consequence that the thickness (D) of the SPMV lipid bilayer has been decreased by dopamine·HCl, which indicates that the membrane has been expanded. It was discussed above that the drug increased the bulk lateral mobility of the SPMV lipid bilayer, and the significant increase in the bulk lateral mobility by the drug was observed at 2×10^{-8} M. Nevertheless, the thickness (D) of the membrane lipid bilayer has not been altered by the same concentration (2×10^{-8} M) of dopamie·HCl. It is only when it reaches a concentration point of 4×10^{-8} M that the thickness (D) of the membrane lipid bilayer was significantly decreased. This illustrates that the thickness (D) of the lipid bilayer can be changed and affected only by a considerable change of lipid mobility. Or it could possibly be that the measuring fluorescence probe technique employed in the present research cannot catch the infinitesimal change of the membrane thickness (D).

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