ELSEVIER

Biochimica et Biophysica Acta 1375 (1998) 93–100

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Asymmetrical membrane fluidity of bovine adrenal chromaffin cells and granules and effect of trichosporin-B-VIa

Shuji Kitagawa [a,*], Eiichi Tachikawa [b], Takeshi Kashimoto [b], Yasuo Nagaoka [c], Akira Iida [c], Tetsuro Fujita [d]

[a] *Niigata College of Pharmacy, Kamishin'ei-cho 5-13-2, Niigata 950-2081, Japan*
[b] *Department of Pharmacology, School of Medicine, Iwate Medical University, Uchimaru 19-1, Morioka 020-8505, Japan*
[c] *Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan*
[d] *Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Osaka 573-0101, Japan*

Received 18 June 1998; accepted 10 August 1998

Abstract

We examined membrane fluidity of bovine adrenal chromaffin cells and chromaffin granules using cationic trimethylammonium derivative of diphenylhexatriene (TMA-DPH) as a fluorescence probe. After adding TMA-DPH to the suspension of chromaffin cells and that of granules, it first bound to the outer layer of the plasma membrane of the cells and that of the granule membrane, then gradually penetrated the inner layer of each membrane and distributed to both leaflets of the respective membranes. Accompanying increases in the ratio of incorporated probe on the cytoplasmic side of the chromaffin cell membrane, its fluorescence anisotropy gradually decreased. However, in chromaffin granules, the fluorescence anisotropy gradually increased with increases in the ratio of incorporated probe. These findings suggest that the inner layer of the plasma membrane and outer layer of the granular membrane are more fluid than the corresponding side of each membrane, which is suitable for the fusion between both membranes. We also examined the effect of trichosporin-B-VIa, a fungal ion channel forming α-aminoisobutyric acid-containing peptide, on the fluidity of chromaffin cells using TMA-DPH. The peptide decreased the fluorescence anisotropy and increased the fluorescence intensity in the concentration range that induced Ca^{2+} dependent catecholamine secretion, suggesting that a change in lipid dynamics of the lipid bilayer of the plasma membrane was induced by this peptide.

Keywords: Membrane fluidity; Diphenylhexatriene; Fluorescence; Trichosporin; Chromaffin cell; Chromaffin granule

1. Introduction

The asymmetrical distribution of membrane phospholipids across the lipid bilayer is well known [1–4]. This asymmetry seems to be responsible for cellular functions such as coagulation and membrane fusion and cellular stability [1,2,5,6]. Moreover, associated with this compositional asymmetry, the fluidity of cellular membranes also seems to be asymmetric. In a previous paper we revealed the asymmetrical fluid-

Abbreviations: TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPH-PA, 3-(*p*-(6-phenyl)-1,3,5-hexatrienyl)phenyl propionic acid; KRH, Krebs-Ringer-HEPES; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid

* Corresponding author. Fax: +81 (25) 268-1238; E-mail: kitagawa@niigata-pharm.ac.jp

0005-2736/98/$ – see front matter
PII: S0005-2736(98)00143-6

ity of platelet membrane by using gradual transbilayer incorporation of the quaternary ammonium fluorescence probe, trimethylammonium derivative of diphenylhexatriene (TMA-DPH), into the cytoplasmic side of the membrane [7]. TMA-DPH first binds to the outer layer of the plasma membrane. Therefore, its fluorescence anisotropy reflects the fluidity of the outer layer of the plasma membrane. The cationic probe gradually penetrates the cytoplasmic side of the membrane by a flip process [8]. Therefore, in a state of equilibrium its fluorescence anisotropy reflects the fluidity of whole membrane. If negatively charged lipids are asymmetrically distributed, the equilibrium fluorescence anisotropy mainly reflects that of the negatively charged lipid layer on which the cationic probe preferentially binds [7].

Adrenal chromaffin cells are a good model with which to study the characteristics of both plasma membrane and granular membrane of secretory cells and the relationship between the change in membrane properties and cellular activation. Bovine adrenal chromaffin cells secrete catecholamines via stimulation of the nicotinic receptor by a physiological secretagogue, acetylcholine; binding of acetylcholine to the receptor leads to depolarization of the cell membrane due to an influx of Na^+ through receptor-operated Na^+ channels, causes an influx of Ca^{2+} through voltage-dependent channels, and results in catecholamine secretion by exocytosis [9,10]. In this study we tried to reveal the asymmetry of membrane fluidity of adrenal chromaffin cells and chromaffin granules (adrenomedullary catecholamine storage vesicles) using TMA-DPH. Electrical neutral diphenylhexatriene (DPH) and anionic propionic acid derivative of DPH (DPH-PA) were also used for comparison.

The trichosporin-B-VIa, an α-aminoisobutyric acid (Aib)-containing peptide, which was isolated from the culture broth of *Trichoderma polysporum* as one of 11 kinds of fungal peptides with similar sequences [11], causes Ca^{2+}-dependent catecholamine secretion from bovine adrenal chromaffin cells [12]. However, the precise mechanism of activation of chromaffin cells by trichosporin-Bs is still unknown. Since this group of peptides show various membrane modifying actions [13–17], the change in membrane properties by this peptide may be related to the activation of chromaffin cells. Therefore, in this study we examined the effect of trichosporin-B-VIa on the lipid dynamics of the chromaffin cell membrane using TMA-DPH.

2. Materials and methods

2.1. Materials

1-(4-Trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) and 3-(*p*-(6-phenyl)-1,3,5-hexatrienyl)phenylpropionic acid (DPH-PA) were purchased from Molecular Probe (Junction City, OR, USA). 1,6-Diphenyl-1,3,5-hexatriene (DPH) and bovine serum albumin (essentially fatty acid free) were obtained from Sigma (St. Louis, MO, USA). Trichosporin-B-VIa was synthesized and supplied [18]. Other reagents were all from Wako (Osaka, Japan).

2.2. Preparation of adrenal chromaffin cells and granules

Bovine adrenal glands were kindly provided by the Center of Iwate Livestock Industry. Adrenal chromaffin cells were isolated by collagen digestion, using a method described previously [19]. Chromaffin granules (catecholamine storage vesicles) were prepared essentially according to the method of Kirshner [20]. Chromaffin cells were suspended in oxygenated Krebs-Ringer-HEPES (KRH) buffer (125 mM NaCl, 4.8 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM HEPES-NaOH, 5.6 mM glucose, pH 7.4) at a concentration of 3.0×10^6 cells/ml, and chromaffin granules were suspended in 0.3 M sucrose and 10 mM HEPES-NaOH (pH 7.4) at a concentration of 220 μg protein/ml.

2.3. Measurement of binding and transmembrane incorporation of TMA-DPH

Binding of TMA-DPH to chromaffin cells or granules was quantified by measuring fluorescence intensity in the cells or granules after centrifugation. After incubation of chromaffin cell suspension or granule suspension, which is described above, with a 1.5 μM concentration of TMA-DPH at 37°C, 0.5 ml of the suspension was collected periodically. The samples

were centrifuged at 1000×*g* for 2 min (chromaffin cells) or 35 000×*g* (chromaffin granules) for 4 min at 4°C. The precipitated cells or granules were then solubilized with 1.5% sodium dodecyl sulfate and fluorescence intensity of TMA-DPH in the solubilized sample was measured using excitation and emission wavelengths of 365 nm and 428 nm, respectively, and the amounts of bound probe were calculated.

Reorientation of TMA-DPH into the inside membrane layer of chromaffin cells and granules was quantified by measuring fluorescence intensity remaining after albumin extraction. The albumin extraction method has been used by many investigators to evaluate the amount of amphiphiles incorporated in the membrane inner layer [21,22]. After incubation of chromaffin cell suspension or granule suspension described above with a 1.5 μM concentration of TMA-DPH at 37°C, 0.5 ml of the suspension was collected periodically and transferred to 1 ml of 3% albumin solution which was kept at 4°C. The samples were centrifuged as described above and the precipitated cells or granules were washed twice more with 1.5 ml 1% bovine serum albumin solution at 4°C and then solubilized with 1.5% sodium dodecyl sulfate. Fluorescence intensity of TMA-DPH in the solubilized sample was measured as described above and the amounts of the probes incorporated were calculated.

2.4. Measurement of fluorescence intensity and anisotropy

The fluorescence intensities and anisotropies of TMA-DPH and other analogs in chromaffin cells and granules were measured as described [7]. Suspension of adrenal chromaffin cells or granules was mixed with 14 vol of KRH buffer (chromaffin cells) or 9 vol of 0.3 M sucrose and 10 mM HEPES-NaOH (pH 7.4) (granules), respectively. The suspension was then incubated with a final concentration of 0.5 μM TMA-DPH or other analogs for a period of time between 2 and 120 min at 37°C and the fluorescence intensities of these probes were measured at the same temperature in an F-4010 spectrofluorometer (Hitachi Seisakusho, Tokyo, Japan) equipped with excitation and emission polarizers. The excitation and emission wavelengths used for TMA-DPH were as described above, and those of DPH and DPH-PA were 363 and 428 nm, and 366 and 430 nm, respectively. Steady-state fluorescence anisotropy, γ, was calculated according to the equation

$$\gamma = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH})$$

where I_{VV} and I_{VH} are the fluorescence intensities measured with a vertical polarizer and analyzer mounted vertically and horizontally, respectively. $G = I_{HV}/I_{HH}$ is the correction factor [23].

3. Results

3.1. Binding and transbilayer incorporation of TMA-DPH in chromaffin cells and granules

As previously revealed in platelet membrane, the quaternary ammonium cation TMA-DPH is expected to slowly penetrate the cytoplasmic side of the cellular membrane by a flip process [7,8]. Therefore, we first examined the binding and transbilayer incorporation of TMA-DPH in adrenal chromaffin cells during their incubation with the cationic probe.

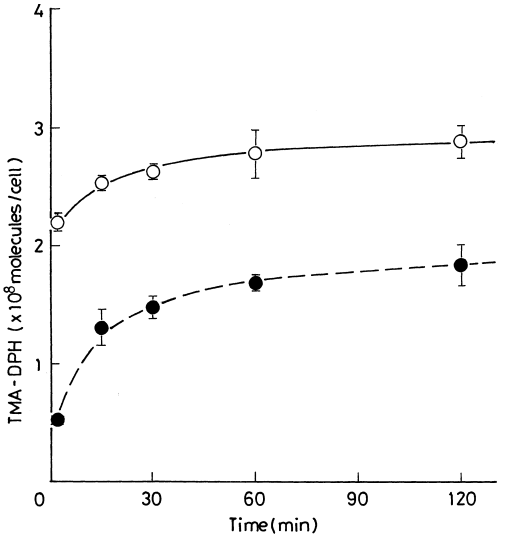

Fig. 1. Time courses of total binding (○) and amount of TMA-DPH incorporated into the inside layer (●) of adrenal chromaffin cells. After incubation with 1.5 μM TMA-DPH, the cells were centrifuged with or without albumin washing. Then the cells were solubilized with sodium dodecyl sulfate and the amounts of total binding and incorporated probe were determined by measuring the fluorescence intensity. Data are means ± S.D. for three experiments.

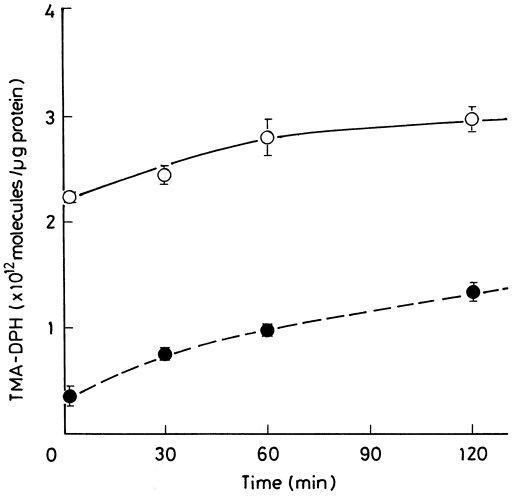

Fig. 2. Time courses of total binding (○) and amount of TMA-DPH incorporated into the inside layer (●) of adrenal chromaffin granules. After incubation with 1.5 μM TMA-DPH, the granules were centrifuged with or without albumin washing. Then the cells were solubilized with sodium dodecyl sulfate and the amounts of total binding and incorporated probe were determined by measuring the fluorescence intensity. Data are means ± S.D. for three experiments.

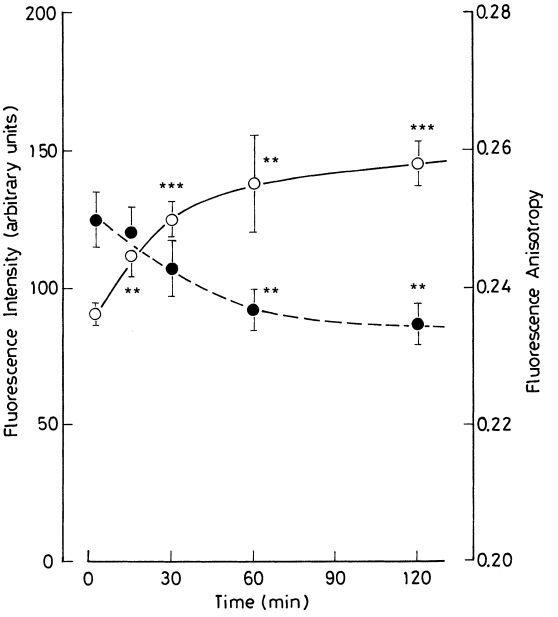

Fig. 3. Time courses of change in fluorescence intensity (○) and fluorescence anisotropy (●) of TMA-DPH in bovine adrenal chromaffin cells. Chromaffin cells were incubated with 0.5 μM TMA-DPH at 37°C, and the fluorescence intensity and anisotropy were measured as described in Section 2. Data are means ± S.D. for four experiments. The significance of differences between initial values (values after 2 min incubation) and values after longer incubation were determined by Student's *t*-test: $^{**}P < 0.01$, $^{***}P < 0.001$.

As shown in Fig. 1, the amount of TMA-DPH bound to the chromaffin cells increased during incubation. As was also shown in the same figure, incorporated TMA-DPH into the inner sides markedly increased during the incubation as observed in bovine platelets [7]. The incorporated TMA-DPH quickly increased during the first 15 min and then slowly increased over the 2 h of the experiment. Of the molecules of the cationic probe bound to the chromaffin cells, more than 60% was present on the cytoplasmic side after 120 min incubation when the binding equilibrium of the cationic probe seemed to have been almost obtained both between medium and outer membrane leaflet and between outer leaflet and inner leaflet.

Likewise, TMA-DPH was gradually incorporated to the inner membrane layer of catecholamine storage granules as shown in Fig. 2. About 45% of the cationic probe bound to the granules was found in

Table 1
Effect of incubation time on fluorescence intensity and fluorescence anisotropy of different concentrations of TMA-DPH in adrenal chromaffin cells

TMA-DPH (μM)	Time (min)	Fluorescence intensity	Fluorescence anisotropy
0.1	2	25.1 ± 2.4	0.246 ± 0.002
	60	34.8 ± 2.8**	0.235 ± 0.003**
0.2	2	42.8 ± 3.7	0.246 ± 0.003
	60	57.2 ± 9.4*	0.233 ± 0.003**
0.5	2	94.7 ± 3.6	0.248 ± 0.003
	60	128.7 ± 15.5*	0.236 ± 0.004**

The experimental procedure was the same as described in Fig. 3. Data are expressed as the means ± S.D. of four experiments. Fluorescence intensity is expressed in arbitrary units. The significance of differences between initial values (values after 2 min incubation) and values after 60 min incubation were determined by Student's *t*-test: $^{*}P < 0.05$, $^{**}P < 0.01$.

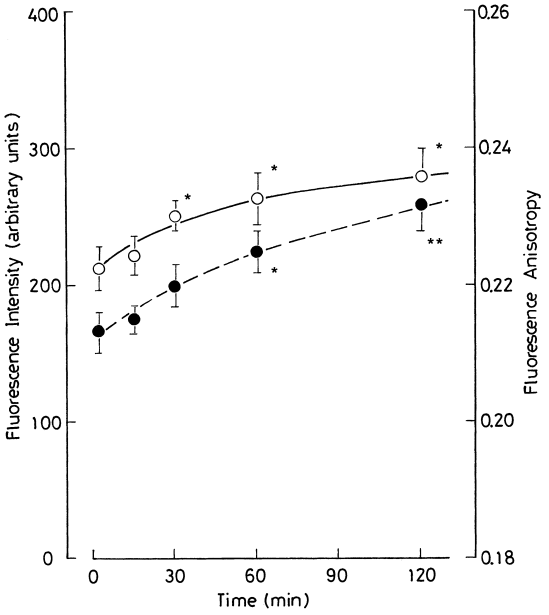

Fig. 4. Time courses of change in fluorescence intensity (○) and fluorescence anisotropy (●) of TMA-DPH in bovine chromaffin granules. Chromaffin granules were incubated with 0.5 μM TMA-DPH at 37°C, and the fluorescence intensity and anisotropy were measured as described in Section 2. Data are means ± S.D. for three experiments. The significance of differences between initial values (values after 2 min incubation) and values after longer incubation were determined by Student's *t*-test: $*P < 0.05$, $**P < 0.01$.

the inner layer after 120 min incubation. However, in chromaffin granules the transbilayer incorporation of the cationic probe proceeded more slowly than that in the cells, according to a comparison between the rates of increase in the ratio of probe incorporated into the inner layer in the cells and granules.

Table 2
Effect of incubation time on fluorescence intensity and fluorescence anisotropy of DPH-PA in adrenal chromaffin cells and granules

Sample	Time (min)	Fluorescence intensity	Fluorescence anisotropy
Cell	2	105.2 ± 5.5	0.234 ± 0.002
	60	158.4 ± 13.3	0.237 ± 0.003
	120	142.9 ± 10.4	0.235 ± 0.005
Granule	2	107.8 ± 18.3	0.238 ± 0.002
	60	86.9 ± 10.7	0.239 ± 0.002

The experimental procedure was as for Figs. 3 and 4. Data are means ± S.D. for four experiments. Fluorescence intensity is expressed in arbitrary units.

3.2. *Fluorescence intensity and anisotropy of TMA-DPH and its analogs in adrenal chromaffin cells and granules*

We next examined the change of fluorescence intensity and anisotropy of TMA-DPH in both chromaffin cells and granules that accompanied the increase of incorporated probe during incubation. TMA-DPH is virtually non-fluorescent in aqueous medium and becomes fluorescent by binding to membranes like other DPH derivatives. As shown in Fig. 3 for the results on the chromaffin cells, fluorescence intensity of TMA-DPH quickly increased during the first 15 min and then slowly increased. The time course of the increase in this fluorescence intensity is similar to that of the binding of the cationic probe shown above. Corresponding to the increase of fluorescence intensity, the fluorescence anisotropy of the probe decreased as observed in the platelets [7]. Similar changes in the fluorescence intensity and anisotropy were also found in different concentrations of TMA-DPH as shown in Table 1. From these results on the gradual decrease in fluorescence anisotropy and corresponding increase in the ratio of cationic probe incorporated into the cytoplasmic side shown in Fig. 1, it is suggested that binding sites for TMA-DPH on the cytoplasmic side of chromaffin cells are more fluid than those in the outer leaflet of the plasma membrane.

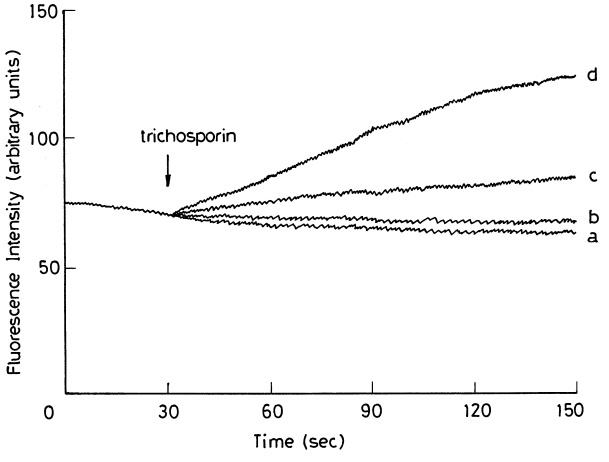

Fig. 5. Effect of trichosporin-B-VIa on fluorescence intensity of TMA-DPH in adrenal chromaffin cells. Chromaffin cells were incubated with 0.5 μM TMA-DPH at 37°C for 2 min and trichosporin was added. Concentration of trichosporin (μM) was: a: 0; b: 1.7; c: 5; d: 10.

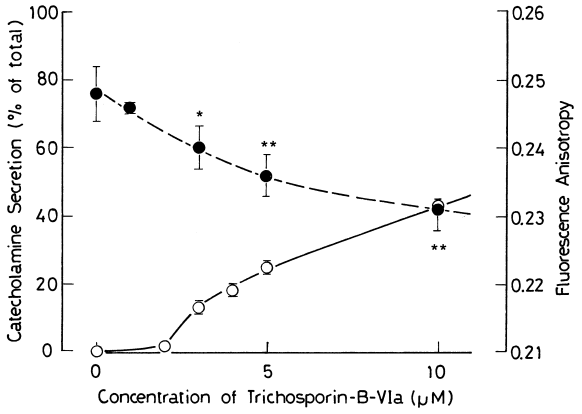

Fig. 6. Effect of trichosporin-B-VIa on fluorescence anisotropy of TMA-DPH (●) and its relationship with induction of catecholamine secretion (○). Chromaffin cells were incubated with 0.5 μM TMA-DPH at 37°C for 2 min and trichosporin was added. After further incubation for 3 min, fluorescence anisotropy was measured. Data of catecholamine secretion induced by trichosporin-B-VIa is cited from Tachikawa et al. [12]. Values of fluorescence anisotropy were means ± S.D. for four experiments and the significance of differences between control values and those in the presence of trichosporin-B-VIa were determined by Student's *t*-test: $*P < 0.05$, $**P < 0.01$.

We also examined the change of fluorescence intensity and anisotropy of TMA-DPH in chromaffin granules. As shown in Fig. 4, fluorescence intensity of TMA-DPH in the granules increased gradually, which is consistent with the increase in binding of the probe to the granules as shown in Fig. 2. In contrast to the change of the fluorescence anisotropy in chromaffin cells mentioned above, the fluorescence anisotropy of TMA-DPH in the granules gradually increased with increases in the ratio of cationic probe incorporated into the inner side as shown in Fig. 2, suggesting that binding sites for TMA-DPH in the inner leaflet of chromaffin granules are more solid than those in the outer leaflet of the granule membrane.

We also observed changes of fluorescence anisotropy of anionic DPH-PA during incubation in both adrenal chromaffin cells and chromaffin granules to compare them with the changes of TMA-DPH. In contrast to the cationic probe, transbilayer movement of DPH-PA quickly occurs because most of the anionic probe can be easily extracted by albumin as previously reported [7]. As shown in Table 2, the fluorescence intensity of this probe gradually increased during 60 min in chromaffin cells, but the fluorescence anisotropy did not change during the incubation in either chromaffin cells or granules.

3.3. *Effects of trichosporin-B-VIa on fluorescence properties of TMA-DPH and its analogs in chromaffin cells*

Trichosporin-B-VIa, an α-aminoisobutyric acid (Aib)-containing peptide, is known to induce catecholamine secretion by causing Ca^{2+} influx into the cells and an increase in intracellular free Ca^{2+} concentration [12]. The secretion was completely dependent on the external Ca^{2+} at concentrations of 2∼5 μM of trichosporin-B-VIa, while partly independent on Ca^{2+} at higher concentrations (10∼30 μM) [12]. From the characteristics of Ca^{2+} influx it

Table 3
Effects of 5 μM trichosporin-B-VIa and of 100 μM acetylcholine on fluorescence intensity and fluorescence anisotropy of TMA-DPH and its analogs in chromaffin cells

Probe	Stimulant	Fluorescence intensity	Fluorescence anisotropy
TMA-DPH	None	72.3 ± 1.2	0.248 ± 0.003
	Trichosporin	122.1 ± 2.7***	0.236 ± 0.003**
	Acetylcholine	74.8 ± 4.4	0.248 ± 0.003
DPH-PA	None	107.5 ± 4.3	0.231 ± 0.002
	Trichosporin	134.8 ± 2.2***	0.232 ± 0.002
DPH	None	94.7 ± 9.7	0.167 ± 0.004
	Trichosporin	127.1 ± 11.8*	0.164 ± 0.003

The experimental procedure was as for Figs. 5 and 6. Data are means ± S.D. for four experiments. Fluorescence intensity is expressed in arbitrary units. The significance of differences between control values and values in the presence of trichosporin-B-VIa or acetylcholine were determined by Student's *t*-test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

was proposed that activation of chromaffin cells by lower concentration of trichosporin-B-VIa was due to the formation of Ca^{2+}-permeable ion channels [12]. However, the precise mechanism of activation of the chromaffin cells is still unknown. Since Aib-containing peptides show membrane-modifying actions such as induction of hemolysis [13], fusion of lipid vesicles [14] and ion channel formation [15–17], we examined the change in dynamics in the membrane lipid bilayer in the chromaffin cells by observing the fluorescence intensity and anisotropy of TMA-DPH together with its analogs for comparison.

As shown in Fig. 5, fluorescence intensity of TMA-DPH gradually increased on addition of trichosporin-B-VIa at the concentration that induced catecholamine secretion as shown in Fig. 6. Corresponding with this increase, as also shown in Fig. 6, there was a decrease in fluorescence anisotropy in the concentration range that induced catecholamine secretion. Significant decrease in fluorescence anisotropy was observed even at the concentration (3 and 5 μM) that induced catecholamine secretion, completely dependent on the external Ca^{2+}. The decrease was not induced in chromaffin cell membrane prepared by hypotonic lysis (data not shown). These changes in fluorescence properties seemed to have been partly due to the increased incorporation of the cationic probe into the cytoplasmic side, because about 15% of the increased incorporation of TMA-DPH was observed after a 3-min incubation of the chromaffin cells with 5 μM trichosporin-B-VIa. Furthermore, these changes in fluorescence were not common for stimulants to the chromaffin cells. Although acetylcholine also induces catecholamine secretion [9], no significant change was observed in either fluorescence intensity or fluorescence anisotropy at the concentration of 100 μM, as shown in Table 3, at which maximal activity (induction of about 30% catecholamine secretion) was obtained.

We furthermore observed the effect of trichosporin-B-VIa on the fluorescence properties of anionic DPH-PA and electrical neutral DPH. As also shown in Table 2, increases of fluorescence intensity of DPH-PA and DPH were observed; however, no significant changes in fluorescence anisotropy of these probes were observed.

4. Discussion

DPH and its derivatives have been widely used to monitor membrane fluidity. However, they seem to differ in their localization in the membrane lipid bilayer due to their electric charge [7]. That is, quaternary ammonium cation TMA-DPH binds first to the outer layer of the plasma membrane and then gradually penetrates the cytoplasmic side by a flip process, distributing to both membrane leaflets but mainly to the side with a more negative surface charge. However, anionic DPH-PA quickly penetrates the cytoplasmic side and was mainly located in the layer with less negative surface charge because of electronic repulsion and neutral DPH is localized within the hydrophobic core of the membrane. Therefore, their fluorescence anisotropy seems to reflect mainly the fluidity of the membrane region where they preferentially distribute.

The decrease in the fluorescence anisotropy of TMA-DPH in the chromaffin cells accompanying the increase in the ratio of probe incorporated into the cytoplasmic side of the cellular membrane suggests that the outer layer of the plasma membrane of chromaffin cells is relatively rigid compared to the cytoplasmic side. The present findings on the asymmetry of fluidity in the chromaffin cell membrane may be due to the asymmetric distribution of polyunsaturated fatty acids in phospholipid acyl chains and that of cholesterol as suggested in mammalian erythrocytes and platelets [7,24].

In contrast, the fluorescence anisotropy of TMA-DPH in the chromaffin granules increased accompanied with the increase in the ratio of the incorporated probe into the inner layer. These findings suggest that the outer layer of the chromaffin granules is more fluid than the inner layer. This asymmetry of fluidity of membrane leaflets in both chromaffin cells and granules is suitable for the fusion between plasma membrane and intracellular granule membranes during exocytosis because a fluid lipid is more favorable for membrane fusion [25].

The values of fluorescence anisotropy of DPH-PA in chromaffin cells and granules, which seems to be distributed to both the outer and inner membrane layer because of the quick incorporation into the inner layer [7], did not change during incubation and resembled equilibrium values of fluorescence ani-

sotropy of TMA-DPH in both preparations. Gradual increase in fluorescence intensity of the anionic probe in chromaffin cells may be related to its transfer into cytoplasmic granules.

The values of fluorescence anisotropy of TMA-DPH and DPH-PA in chromaffin granules were smaller than those in platelets [7] and intestinal brush border membrane [26]. This finding suggests that the lipid bilayer in chromaffin granules is relatively fluid compared with the lipid bilayer of other biological membranes. The relatively fluid nature of chromaffin granule membrane is consistent with previous findings by electron spin label study [27]. We also confirmed this by using 5-doxylstearic acid as a spin label (data not shown). The fluid state of the lipid bilayer of the chromaffin granules is possibly due to the lower cholesterol content and high content of lysolecithin in the granule membrane [28] as suggested by Marsh et al. [27].

Addition of trichosporin-B-VIa induced a prompt increase in fluorescence intensity and decrease in fluorescence anisotropy of TMA-DPH. These changes occurred in the concentration range that induced catecholamine secretion completely dependent on the external Ca^{2+}, although the changes were larger at 10 μM of trichosporin-B-VIa, which seems to induce partial cell damage [12]. Since acetylcholine did not induce any change in the fluorescence properties of TMA-DPH, the changes by trichosporin-B-VIa observed here seem to be related to the membrane modifying action of this peptide. In fact trichosporin-B-VIa stimulated transbilayer incorporation of TMA-DPH. Since fluorescence anisotropy of neither anionic DPH-PA nor electrical neutral DPH changed by this peptide, it seems that the mean fluidity of plasma membrane was not changed by this peptide. Therefore, redistribution of membrane lipids may be induced by this peptide, which may be related to the induction of Ca^{2+} influx and stimulation of chromaffin cells.

Acknowledgements

This work was supported by the Research Foundation for Pharmaceutical Sciences of Japan.

References

[1] R.F.A. Zwaal, Biochim. Biophys. Acta 515 (1978) 163–205.
[2] R.F.A. Zwaal, H.C. Hemker, Haemostasis 11 (1982) 12–39.
[3] B.H. Lubin, F.A. Knypers, D.T.-Y. Chiu, S.B. Shohet, Methods Hematol. 19 (1988) 171–201.
[4] A. Zachowski, Biochem. J. 294 (1993) 1–14.
[5] E.M. Bevers, J. Rosing, R.F.A. Zwaal, in: D.E. MacIntyre, J.L. Gordon (Eds.), Platelets in Biology and Pathology: III, Elsevier, Amsterdam, 1987, pp. 127–160.
[6] H. Chap, B. Perret, M. Plantavid, H. Lachachi, L. Douste-Blazy, in: D.E. MacIntyre, J.L. Gordon (Eds.), Platelets in Biology and Pathology: III, Elsevier, Amsterdam, 1987, pp. 191–204.
[7] S. Kitagawa, M. Matsubayashi, K. Kotani, K. Usui, F. Kametani, J. Membr. Biol. 119 (1991) 221–227.
[8] E.M. Bevers, P.F.J. Verhallen, A.J.W.G. Visser, P. Comfurius, R.F.A. Zwaal, Biochemistry 29 (1990) 5132–5137.
[9] A. Wada, S. Takara, F. Izumi, H. Kobayashi, N. Yanagihara, Neuroscience 15 (1985) 283–292.
[10] E. Tachikawa, S. Takahashi, K. Furumachi, T. Kashimoto, A. Iida, Y. Nagaoka, T. Fujita, Y. Takaishi, Mol. Pharmacol. 40 (1991) 790–797.
[11] A. Iida, M. Okuda, S. Uesato, Y. Takaishi, T. Shingu, M. Morita, T. Fujita, J. Chem. Soc. Perkin Trans. I (1990) 3249–3255.
[12] E. Tachikawa, K. Nogimori, S. Takahashi, K. Mizuma, K. Itoh, T. Kashimoto, Y. Nagaoka, A. Iida, T. Fujita, Biochim. Biophys. Acta 1282 (1996) 140–148.
[13] G. Irmscher, G. Jung, Eur. J. Biochem. 80 (1977) 165–174.
[14] A.L.Y. Lau, S.I. Chan, Biochemistry 13 (1974) 4942–4948.
[15] P. Mueller, D.O. Rudin, Nature 300 (1968) 325–330.
[16] G. Boheim, K. Janko, D. Leibfritz, T. Ooka, W.A. Konig, G. Jung, Biochim. Biophys. Acta 433 (1976) 182–199.
[17] O.F. Robert Jr., F.M. Richards, Nature 300 (1982) 325–330.
[18] Y. Nagaoka, A. Iida, T. Fujita, Chem. Pharm. Bull. 42 (1994) 1258–1263.
[19] E. Tachikawa, S. Takahashi, T. Kashimoto, J. Neurochem. 53 (1989) 19–26.
[20] N. Kirshner, J. Biol. Chem. 237 (1962) 2311–2317.
[21] S.L. Schrier, A. Zachowski, P. Herve, J.-C. Kader, P.F. Devaux, Biochim. Biophys. Acta 1105 (1992) 170–176.
[22] C. Schwichtenhővel, B. Deuticke, C.W.M. Haest, Biochim. Biophys. Acta 1111 (1992) 35–44.
[23] R.F. Chen, R.L. Bowman, Science 147 (1965) 729–732.
[24] M. Seigneuret, A. Zachowski, A. Hermann, P.F. Devaux, Biochemistry 23 (1984) 4271–4275.
[25] J. Wilschut, N. Düzgünes, D. Hoekstra, Biochemistry 24 (1985) 8–14.
[26] S. Kitagawa, Y. Sugaya, M. Nishizawa, H. Hirata, J. Membr. Biol. 146 (1995) 193–199.
[27] D. Marsh, G.K. Radda, G.A. Ritchie, Eur. J. Biochem. 71 (1976) 53–61.
[28] H. Blaschko, H. Firemark, A.D. Smith, H. Winkler, Biochem. J. 104 (1967) 545–549.