A comparative study of the plasma membrane permeabilization and fluidization induced by antipsychotic drugs in the rat brain

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Abstract

We compared the potency of the interaction of three antipsychotic drugs, i.e., chlorpromazine (CPZ), haloperidol (HAL) and sulpiride (SUL), with the plasma membrane in the rat brain. CPZ loading (≥ 100 μM) dose-dependently increased both membrane permeability (assessed as [^{18}F]2-fluoro-2-deoxy-D-glucose-6-phosphate release from brain slices) and membrane fluidity (assessed as the reduction in the plasma membrane anisotropy of 1,6-diphenyl-1,3,5-hexatriene). On the other hand, a higher concentration of HAL (1 mM) was required to observe these effects. However, SUL failed to change membrane permeability and fluidity even at a high concentration (1 mM). These results indicated the following ranking of the potency to interact with the membrane: CPZ > HAL > SUL. The difference among antipsychotic drugs in the potency to interact with the plasma membrane as revealed in the present study may be partly responsible for the difference among the drugs in the probability of inducing extrapyramidal side effects such as parkinsonism and tardive dyskinesia.

Key words: Plasma membrane, Chlorpromazine; Haloperidol, Sulpiride, Extrapyramidal side effects.

Introduction

Antipsychotic drugs have been widely used to treat schizophrenia as well as other psychiatric disorders, but the use of these drugs is limited by their tendency to produce extrapyramidal movement disorders such as tardive dyskinesia (TD) (Gerlach and Casey, 1988) and parkinsonism (Marsden and Jenner, 1980). Although receptors dominate most research on antipsychotic drugs, such small molecular weight, lipid-soluble compounds would easily distribute into the plasma membrane and affect the membrane environment (Meltzer et al., 1996; Tharmapathy et al., 2000). However, little is known about the effects of these drugs on plasma membrane integrity in the central nervous system. While most previous studies compared the cytotoxic effect of antipsychotic drugs on non-neuronal cell types (Boelsterli et al., 1987; Dwyer et al., 2003), little is known about the difference among these drugs in the potency to induce neurotoxicity, especially in the potency to interact with the plasma membrane in the central nervous system.

Radio-labeled 2-deoxy-D-glucose (2DG) have been used as a probe for cell membrane permeability alterations detected by monitoring the leakage of the phosphorylated 2DG from the cells (Andreoli et al., 1985; Walum and Peterson, 1982). Membrane fluidity is measured as the rotational mobility of a fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) which partitions into the membrane bilayer (Shinitzky and Barenholz, 1978). A decrease in the fluorescence polarization of DPH indicates an increase in the membrane fluidity (Ohyashiki et al., 1992).

In the present study, the potency of the interaction of three antipsychotic drugs, i.e.,

chlorpromazine (CPZ; a phenothiazine antipsychotic drug), haloperidol (HAL; a butyrophenone antipsychotic drug), and sulpiride (SUL; a benzamide antipsychotic drug), with the plasma membrane in the rat brain was examined and compared. To investigate the effects of these antipsychotic drugs on plasma membrane permeability, [^{18}F]2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) uptake in fresh rat brain slices was serially and two-dimensionally measured using a dynamic positron autoradiography technique (Murata et al., 1999; Omata et al., 2000). Also, to investigate the effects of these agents on plasma membrane fluidity, plasma membrane anisotropy in the rat brain was measured spectrofluorometrically using DPH as a fluorescent probe.

Methods

Dynamic positron autoradiography technique

All animal procedures were approved by the Animal Care and Use Committee of University of Fukui in accordance with Guidelines for Animal Experiments, University of Fukui. Male Wistar rats (250–300 g) were decapitated, and their brains were removed. Sagittal brain slices (300 μm in thickness) were prepared with a microslicer (DTK-2000, Dosaka EM, Kyoto, Japan), and incubated as previously described (Murata et al., 1999; Omata et al., 2000). ^{18}F was produced by ^{18}O (p,n) ^{18}F nuclear reactions, and [^{18}F]FDG was produced by the method of Hamacher et al. (1986) using an automated [^{18}F]FDG synthesis system (NKK Co. Ltd., Tokyo, Japan). The specific radioactivity of [^{18}F]FDG was 1–2 Ci/mmol at the end of the synthesis, and the total concentration (labeled plus unlabeled) used in the experiment was 0.51–1.07 μg/ml (2.8–5.9 μM). After 1 hour of

pre-incubation, the slices were incubated in Krebs-Ringer solution containing [^{18}F]FDG diluted to 150 kBq/ml. The slices were then incubated with various concentrations of antipsychotic drugs (CPZ, HAL or SUL). Drugs were dissolved in dimethyl sulfoxide (DMSO). The final concentration of the vehicle (DMSO) in the incubation medium was 0.5%. DMSO at this concentration had no effect on [^{18}F]FDG uptake.

The exposed radioluminography plates (BAS-MP 2040S, Fuji Photo Film Co., Tokyo, Japan) were scanned using a BAS-1500 (Fuji Photo Film Co.). The pixel size was 100 μm. The regions of the brain slices were identified by referring to the brain map of the rat (Paxinos and Watson, 1998). The obtained image data were quantitatively analyzed as follows. The radioactivity of ^{18}F decreases with the same time course (half-life = 109.7 min) in both the brain slices and the surrounding bathing medium, and it is not necessary to compensate for the radioactive decay when the radioactivity pixel value of a region of interest is divided by that of the bathing medium. Thus, the relative increment in the [^{18}F]FDG uptake in the region of interest can be expressed in decay-corrected form by the following ratio:

$$\text{Relative Uptake Ratio} = (\text{RI} - \text{BM}) / \text{BM}$$

where RI is defined as the radioactivity signal [photostimulated luminescence (PSL)/mm^2] on the radioluminography plate detected beneath the region of interest, and BM as the average radioactivity signal (PSL/mm^2) on the radioluminography plate detected beneath the bathing medium solution surrounding each brain slice.

[^{18}F]FDG metabolite analysis

In order to estimate the [^{18}F]FDG metabolites released from the brain slices as a result of the administration of antipsychotic drugs, metabolite analysis was performed by thin-layer chromatography (TLC) on Whatman LK6DF silica gel plates (Clifton, NJ, USA) with a solvent system of acetonitrile/water (95:5). The metabolites in the incubation medium were sampled after the administration of antipsychotic drugs and separated by TLC. The TLC plates were exposed to a radioluminography plate.

Fluorescence anisotropy measurement

The synaptosome fraction was prepared as previously reported (Gray and Whittaker, 1962). Briefly, the rat brain tissue was homogenized in 10 vol of 0.32 M sucrose using an Ultrasonic Disrupter (UR-20P, Tomy Seiko Co. Ltd., Tokyo, Japan). Each homogenate was centrifuged at 1000 g and 4°C for 10 min. The supernatant was removed and centrifuged at 12000 g and 4°C for 20 min. The resultant pellet was suspended in 20 vol of 50 mM Tris-HCl buffer, pH 7.4, and washed twice by centrifugation at 12000 g and 4°C for 20 min. The final pellet, resuspended in the same buffer, was used for the measurement of membrane anisotropy.

Membrane anisotropy was measured by the method of Shinitzky and Inbar (1976) and Ohyashiki et al. (1992). Briefly, membranes were diluted in 50 mM Tris-HCl buffer, pH 7.4, and mixed with the fluorescent probe DPH (0.8 mg protein/ml, 3.3 μM DPH). The DPH stock solution (1 mM) was prepared in tetrahydrofuran. The mixture was incubated at 25°C for 10 min, the reaction was stopped by the addition of a large volume of buffer, and the mixture was centrifuged at 15000 g and 4°C for 20 min. The

obtained pellet was washed twice with the buffer and resuspended in the same buffer. Various concentrations of antipsychotic drugs were added to DPH-labeled membrane solutions (0.07 mg protein/ml) and incubated at 36°C for 10 min. The final concentration of the vehicle (DMSO) in the reaction mixture was 0.5%. DMSO alone at this concentration had no effect on membrane fluidity. Fluorescence measurements were carried out at 36°C with a Beacon 2000 fluorescence polarization system (Invitrogen Corporation, San Diego, CA, USA). The excitation and emission wavelengths were 330 and 420 nm, respectively. The steady-state fluorescence polarization (P) was expressed using the formula (Shinitzky and Barenholz, 1978):

$$P = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + I_{\perp})$$

where $I_{\parallel}$ and $I_{\perp}$ are the emission intensities parallel and perpendicular, respectively, to the plane of the excitation light. “Anisotropy” is a term often used in the fluorescence polarization field, and is also inversely related to fluidity. The fluorescence anisotropy (A) was calculated from the fluorescence polarization value using the formula (Shinitzky and Barenholz, 1978):

$$A = 2P / (3 - P)$$

Protein amounts were measured with a Bio-Rad protein assay kit (Hercules, CA, USA) using bovine serum albumin as the standard.

Materials

CPZ hydrochloride, HAL, (–)-SUL and DPH were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The Bio-Rad protein assay kit was obtained from

Bio-Rad Laboratories Inc. (Hercules, CA, USA). All other chemicals were from Nacalai Tesque Inc. (Kyoto, Japan).

Statistical analysis

The presented values are shown as the means ± SD. The Mann-Whitney U-test was used to evaluate the significance of differences, and a *p* value of less than 0.05 was considered significant.

Results

Figure 1 shows time-resolved images of [^{18}F]FDG uptake of two typical brain slices before and after the loading of 100 μM CPZ (a) and under the control condition (b). To depict dynamic changes of [^{18}F]FDG uptake, the relative uptake ratio at 10-min intervals in the striatum before and after loading various concentrations (30, 100, 300 μM and 1 mM) of antipsychotic drugs was plotted against time (Figure 2). The slope of the graph indicates the rate of [^{18}F]FDG uptake.

The slope of the graph for 30 μM CPZ was definitely increased during the entire time course. However, when slices were loaded with buffer containing ≥ 100 μM CPZ, the slope of the graph initially increased, then gradually decreased and finally became negative (this finding may reflect the outflow of [^{18}F]FDG metabolites from brain slices). The time it took to reach a negative value was shortened as the drug concentration was increased (results for 30, 100, and 300 μM are shown in Figure 2(a)). Similar results were obtained in each of the regions examined (data not shown). The

slope of the graph for ≤ 100 μM HAL was similar to that for the control, but that for 300 μM HAL was definitely increased during the entire time course. However, when slices were loaded with buffer containing 1 mM HAL, the slope of the graph initially increased, then gradually decreased and finally became negative (results for 100, 300 μM and 1 mM are shown in Figure 2(b)). Similar results were obtained in each of the regions examined (data not shown). The slope of the graph for all the concentrations of SUL tested was similar to that for the control (results for 1 mM are shown in Figure 2(c)). Similar results were obtained in each of the regions examined (data not shown). The TLC data suggested that the major [^{18}F]FDG metabolite released from the slices as a result of the administration of ≥ 100 μM CPZ or 1 mM HAL was [^{18}F]FDG-6-phosphate, not [^{18}F]FDG (data not shown). Because [^{18}F]FDG-6-phosphate cannot be transported via glucose transporters, it is likely that the efflux of [^{18}F]FDG-6-phosphate was not mediated by glucose transporters, and that the increased plasma membrane permeability of the cells allowed intracellular [^{18}F]FDG-6-phosphate to leak from the cells.

Relatively low concentrations of CPZ (30 μM) induced no significant changes in anisotropy, while relatively high concentrations of CPZ (≥ 100 μM) induced a dose-dependent decrease in fluorescence anisotropy (i.e., an increase in membrane fluidity) (Figure 3(a)). Upon treatment with HAL, a significant decrease in anisotropy was observed only at the concentration of 1 mM (results for 300 μM and 1mM are shown in Figure 3(b)). However, the addition of SUL did not induce any significant change in anisotropy at any of the concentrations tested (results for 300 μM and 1 mM

are shown in Figure 3(b)).

Discussion

Our findings indicated the following ranking of the potency to induce plasma membrane permeabilization and fluidization: CPZ > HAL > SUL. This order of ranking was in good accord with the ranking of the relative cytotoxicity induced by these agents in various types of cells (Boelsterli et al., 1987; Dwyer et al., 2003).

Both CPZ and HAL are amphiphilic drugs (Pappu and Hauser, 1981). Amphiphilic molecules are reported to penetrate into membrane, make disordered regions in lipid packing and enhance the permeability of membrane (Katsu et al., 1987, 1989, 1990, 1993). Therefore, in the present study, the amphiphilic properties of the drugs may have enhanced permeability and fluidity of the plasma membrane of brain cells. The lipophilicity of the drugs is indicated as one of the non-receptor-mediated mechanisms (Goosey and Doggett, 1983). These raise the possibility that the differences among the drugs in the potency to interact with the plasma membrane revealed in our study would partly underlie the differences among the drugs in the potency to induce cytotoxic effects reported in previous studies (Boelsterli et al., 1987; Dwyer et al., 2003).

Our data showed that glycolytic activity was enhanced by relatively low concentrations of CPZ (≥ 30 μM) and high concentrations of HAL (300 μM and 1 mM). This would arise due to the amphiphilic property of CPZ and HAL, since an increase in glycolytic activity as a consequence of increased ATP consumption and ATP depletion due to the amphiphilic action on the plasma membrane has been reported (Rissanen et

al., 2003). Another possible explanation for the enhancement of glycolytic activity is that a compensation for the inhibition of oxidative phosphorylation by CPZ and HAL at the mitochondrial respiratory chain level, since these drugs are known to inhibit mitochondrial complex I activity (Burkhardt et al., 1993).

One of the limitations of our findings is that the concentrations of antipsychotic drugs used in our study were higher than the therapeutic concentrations in human plasma, which range from 0.2 to 2.0 μM (Curry et al., 1970; Ulrich et al., 1998). Besides, in vivo, greater than 90% of the drug is bound to the red blood cells and plasma protein and 10% is the free drug (Brinkschulte et al., 1982), which is the pharmacologically active portion. However, due to their large distribution volumes, these drugs can accumulate at 20- to 30-fold higher concentrations in brain tissue than in serum (Baldessarini et al., 1993; Kornhuber et al., 1999). The high lipophilicity of these drugs also enables them to concentrate in the membrane as high as 1000-fold greater than the concentration in aqueous solution (Seeman, 1977). Therefore, these drugs may reach neurotoxic levels in the brain.

The membrane fluidization induced by antipsychotic drugs could result in the fusion between the synaptic vesicle membrane and the presynaptic membrane, which may enhance spontaneous secretion of dopamine and thus underlie TD (Seeman et al., 1974). Membrane permeabilization could cause neuronal degeneration and death, which has been proposed as a toxic mechanism of Parkinson's disease (Volles et al., 2001). These considerations lead us to speculate that membrane permeabilization and fluidization by antipsychotic drugs may play at least a partial role in the pathogenesis of

drug-induced extrapyramidal side effects such as parkinsonism and TD.

At present, little has been reported about the comparative potencies of different antipsychotic drugs for inducing extrapyramidal adverse effects. SUL is considered to be a drug with a documented lower risk of these unwanted effects in human (Gerlach and Casey, 1984; Spila-Alegiani et al., 1995) and in an animal model (Gunne et al., 1986). Although the mechanisms responsible for the differential incidence of extrapyramidal adverse effects among antipsychotic drugs are uncertain, these observations prompt us to speculate that SUL may have a lower probability of inducing extrapyramidal side effects such as parkinsonism and TD than HAL and CPZ, partly due to its lower potency for interacting with the plasma membrane, as revealed in our study. Further studies will be needed to clarify the relationship between the membrane actions of antipsychotic drugs and the pathogenesis of antipsychotic drug-induced extrapyramidal side effects.

In conclusion, we compared for the first time the potency of the interaction of three antipsychotic drugs, CPZ, HAL and SUL, with the plasma membrane in the rat brain. CPZ permeabilized and fluidized plasma membrane at relatively low concentrations ($\geq$ 100 μM). HAL required a higher concentration (1 mM) to induce these effects. SUL did not act on the membrane even at a high concentration (1 mM). These findings implied the following order of the potency to interact with the plasma membrane: CPZ > HAL > SUL, which may be partly responsible for the difference among the drugs in the probability of inducing extrapyramidal side effects such as parkinsonism and TD.

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Statement of Interest

None.

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Figure legends

Figure 1. Time-resolved pseudocolor images of [^{18}F]FDG uptake in sagittally sectioned rat brain slices. Time zero is when CPZ was added to the incubation medium. Two typical slices before and after the loading of 100 μM CPZ (a) and under the control condition with its diagram (b) for three representative time periods (–10–0 min, 200–210 min, and 550–560 min) are shown. The filled regions in the diagram represent the five brain regions assessed in the present study (frontal cortex, caudate putamen, thalamus, hippocampus, and cerebellum). For decay correction, the color-coding was based on the relative uptake ratio (see text for further explanation).

Figure 2. Effect of treatment with various concentrations of CPZ (a), HAL (b) and SUL (c) on the time-course of [^{18}F]FDG uptake in the striatum. Ordinate: relative uptake ratio of ^{18}F-radioactivity (see text for further explanation). Abscissa: time in minutes. Time zero is defined as the time when the antipsychotic drug (CPZ, HAL or SUL) was introduced into the bathing medium containing brain slices. The point at which each drug was applied (= time zero) is indicated by the arrow. Values are the means obtained for six slices (SD is omitted).

Figure 3. Effects of treatment with various concentrations of CPZ, (a), HAL and SUL (b) on the DPH fluorescence anisotropy as an index of membrane fluidity. Data represent the means ± SD obtained for six samples. $*p < 0.05$ compared with control values.

Figure 1

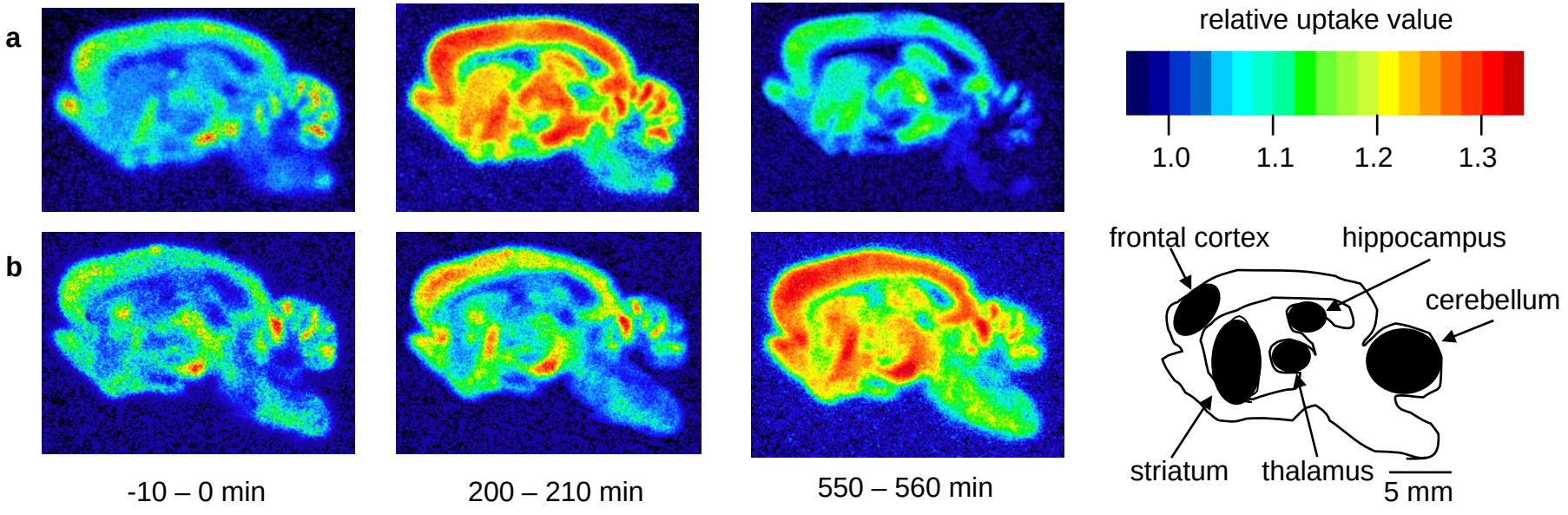
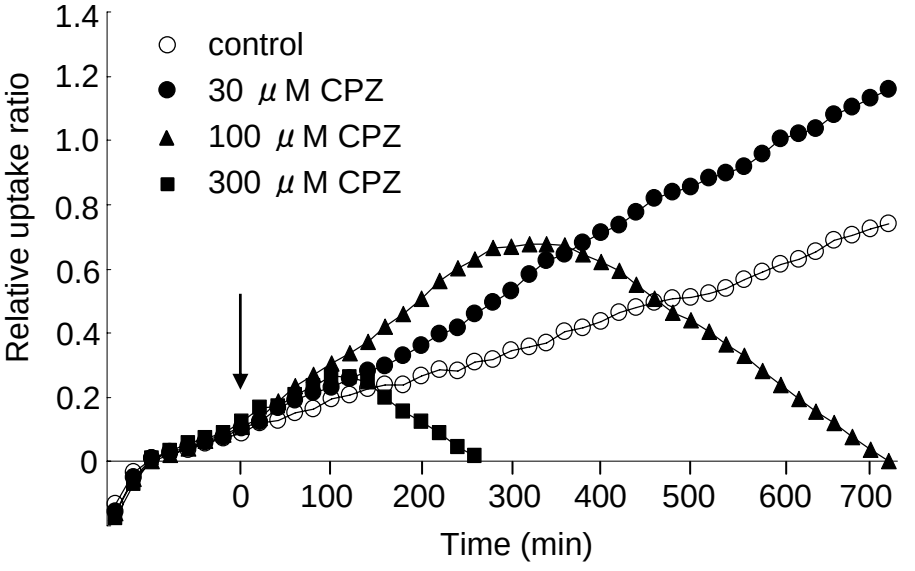
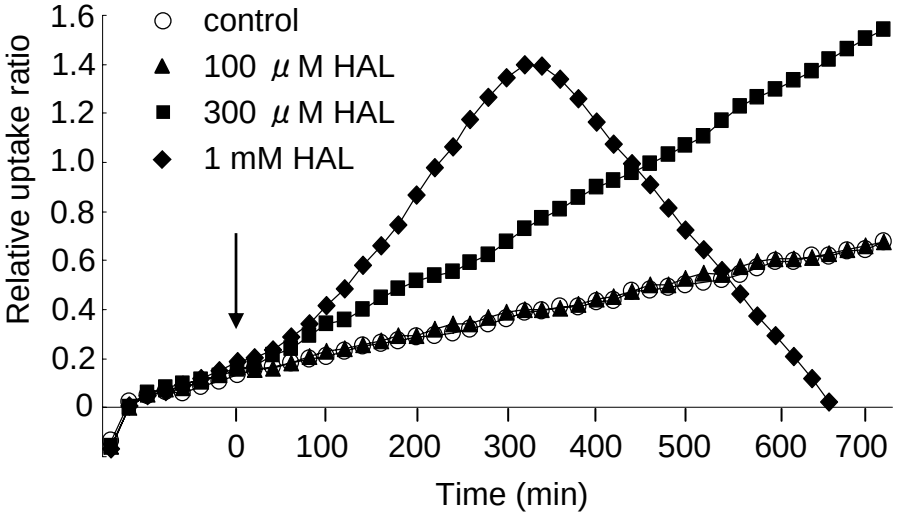

a
b
relative uptake value
1.0
1.1
1.2
1.3
frontal cortex
hippocampus
cerebellum
striatum
thalamus
5 mm
-10 – 0 min
200 – 210 min
550 – 560 min

Figure 2

a


control
30 μM CPZ
100 μM CPZ
300 μM CPZ
Relative uptake ratio
Time (min)

b


control
100 μM HAL
300 μM HAL
1 mM HAL
Relative uptake ratio
Time (min)

C

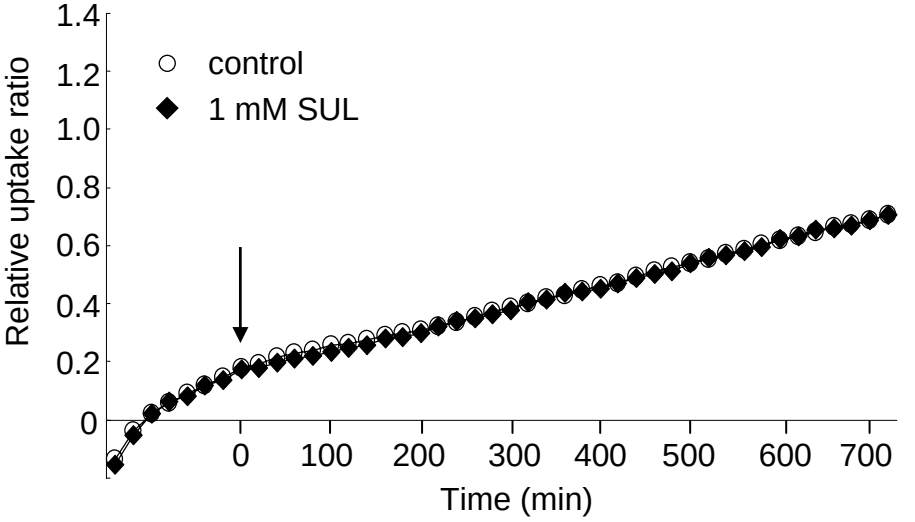
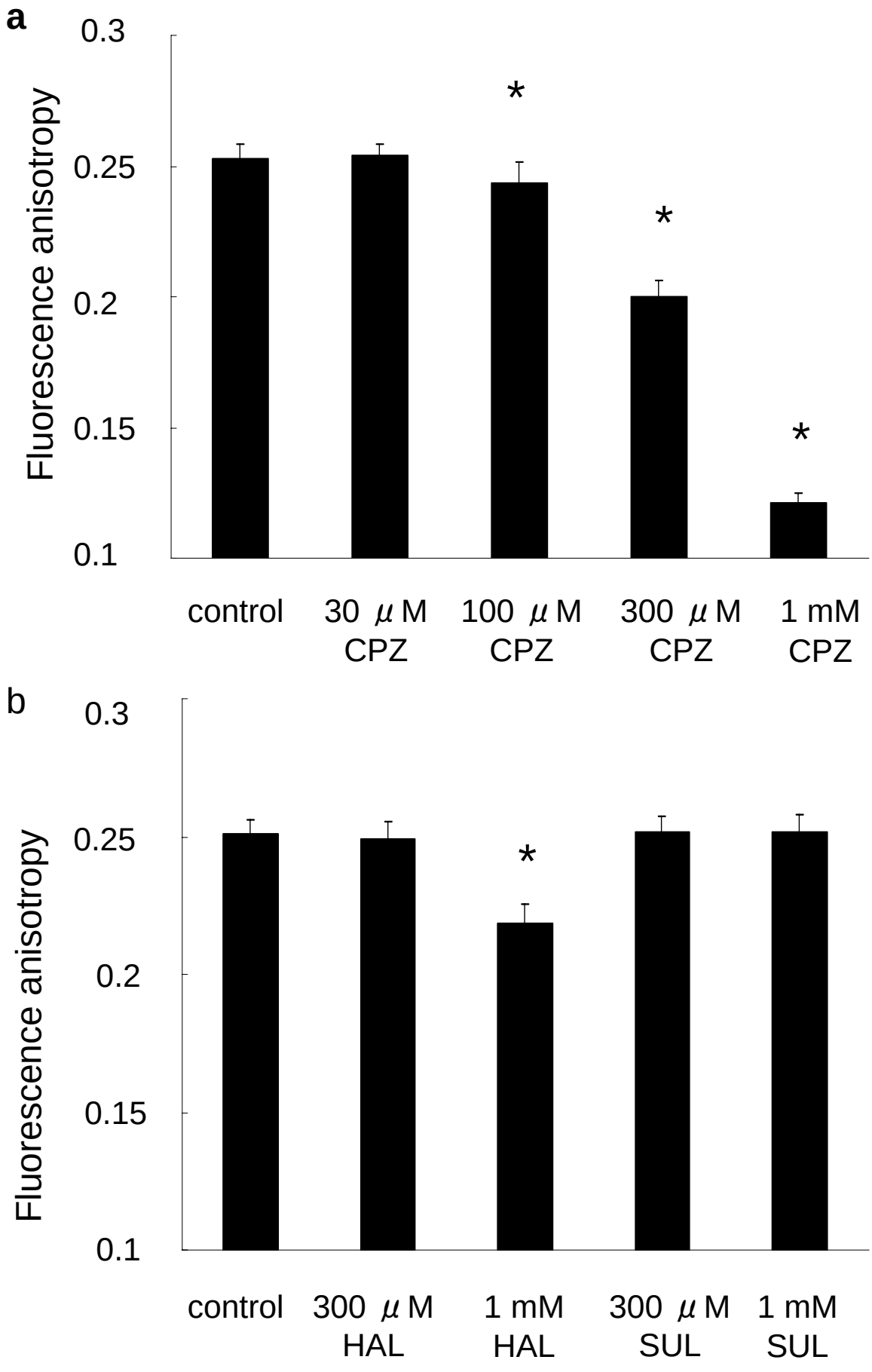

control
1 mM SUL
Relative uptake ratio
Time (min)

Figure 3


a
0.3
0.25
0.2
0.15
0.1
Fluorescence anisotropy
*
*
*
control
30 μM CPZ
100 μM CPZ
300 μM CPZ
1 mM CPZ
b
0.3
0.25
0.2
0.15
0.1
Fluorescence anisotropy
*
control
300 μM HAL
1 mM HAL
300 μM SUL
1 mM SUL