effects of methoxetamine are stronger than those of ketamine [3].

Dysfunction of prefrontal cortex (mPFC) is regarded as a key element in the pathogenesis of schizophrenia. Most notably, cortical dopaminergic and glutamatergic mechanisms are involved in the expression of cognitive impairment in schizophrenia [10, 11]. Therefore, it appears that changes in dopamine concentrations in the mPFC are related to the schizophrenia-like symptoms caused by the drug. In addition to dopamine and glutamate, serotonin is believed to be involved in hallucinations via 5-HT_2 receptors [12]. However, to date, there have been few reports showing the influence of methoxetamine on these amines.

To reveal the influence of methoxetamine on dopamine and serotonin in the mPFC, we explored the dopamine and serotonin concentrations in this region of mice after administration of methoxetamine. Then we investigated these same effects in the striatum and nucleus accumbens (NAC) as a common site of action of psychostimulants such as amphetamines [13]. We also compared the effects of methoxetamine and ketamine by administering the same molar dose of these drugs to mice.

To monitor dopamine and serotonin concentrations, brain microdialysis was used. In this method, a probe with a dialyzer membrane is inserted in a target tissue of a living animal and circulated with perfusate; consequently, biological samples are easily taken as dialysates. When applied to animal brain, we can assess the detailed profiles of objective substances in the brain. Therefore, it is important to evaluate effects of the abused drug on neurotransmitters in order to clarify the phenomena and/or influence caused by the drug. In the present study, this method was used to obtain samples from mouse brain, and dopamine and serotonin concentrations in the samples, as indicators of the effects caused by methoxetamine, were determined by high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) using a method described in our previous reports [14, 15].

Materials and methods

Animals

Male ddY mice were purchased from Kyudo (Saga, Japan). They were housed in an animal room with a 12-h light–dark cycle under standard environmental conditions (ambient temperature, 22 ± 1 °C; humidity, 55 ± 5 %) and had free access to food and water. The animals were used for experiments at a weight of 25–35 g. All animal procedures were approved by the Nagasaki University Animal Care and Use Committee.

Drugs and reagents

Methoxetamine and ketamine were purchased from Seishin-syoji (Kobe, Japan) and Daiichi Sankyo (Tokyo, Japan), respectively. Dopamine, HPLC-grade methanol, 1-decanesulfonic acid sodium salt (SDS), sodium hydrogenphosphate dehydrate, and disodium hydrogenphosphate dodecahydrate were obtained from Wako Pure Chemical (Osaka, Japan). Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA·2Na) was obtained from Dojindo Laboratories (Kumamoto, Japan) and serotonin was purchased from Kanto Chemical (Tokyo, Japan). Dopamine was dissolved in 0.1 M HCl containing 100 mg/l EDTA·2Na for a 10 mM stock solution and 0.1 M acetic acid containing 100 mg/l EDTA·2Na was used for serotonin. The stock solutions were stored at −20 °C. In each experiment, we prepared working solutions at the desired concentrations by serial dilution of the stock solutions.

Brain microdialysis

Surgery for microdialysis was performed on mice, as described below. Mice anesthetized by ethyl carbamate [1.5 g/kg, intraperitoneal (i.p.)] were shaved. Through the exposed bone of the skull, a microdialysis probe, with a 1-mm-long membrane (artificial cellulose, 50 kDa cutoff) (Eicom, Kyoto, Japan), was inserted into the mPFC (coordinates: A, +1.8; L, +0.3; H, −3.0), striatum (coordinates: A, +1.2; L, +1.4; H, −3.2), or NAC (coordinates: A, +1.2; L, +1.4; H, −5.0) following a brain atlas by Franklin and Paxinos (2007) [16]. Perfusion with artificial cerebrospinal fluid was performed at a flow rate of 2.0 μl/min for 90 min to achieve a steady state for monoamine levels [17]. Then samples were collected at intervals of 10 min. Baselines were taken for the first 30 min, and then mice were intraperitoneally injected with a drug. After administration, the dialysates were collected at 10-min intervals up to 100 min. The collected dialysates were applied to HPLC analysis without pretreatment.

Drug administration

Saline or 20 mg/kg methoxetamine was injected to evaluate the effects of methoxetamine on dopamine and serotonin in the striatum, NAC, and mPFC in mice. To compare the effects of methoxetamine and ketamine, 20 mg/kg of methoxetamine or ketamine (19 or 38 mg/kg) was injected. A dose of 19 mg/kg of ketamine is equivalent in 20 mg/kg of methoxetamine. Each group consisted of three mice. All drugs were administered i.p. to mice at 0.1 ml/10 g body weight. After i.p. administration of the drugs, conditions and behaviors of the mice were monitored.

HPLC–ECD conditions for determination of dopamine and serotonin

HPLC–ECD conditions for determination of dopamine and serotonin concentrations were similar to those described in previous reports [14, 15]. Briefly, the HPLC system was an HTEC-500 (Eicom) equipped with an ECD. A graphite working electrode was used (WE-3G), and the applied voltage of the conditioning cell was set at +400 mV. Dopamine and serotonin were separated with a PP-ODSII column (4.6 × 50 mm, i.d., 2.5 μm). The column temperature was set at 25 °C, and elution was performed with a mobile phase composed of 1.5 % methanol in 0.1 M phosphate buffer (pH 5.4) containing 50 mg/l EDTA·2Na and 500 mg/l SDS, at a flow rate of 0.5 ml/min. Under these conditions, the retention times of dopamine and serotonin were about 1.4 and 4.5 min, respectively. The concentrations were determined from calibration curves with concentration ranges of 0.025–50 nM for dopamine and 0.02–50 nM for serotonin.

Statistical analysis

Dopamine and serotonin concentrations in the samples were used to calculate the individual baseline levels. The baseline concentrations of dopamine and serotonin in the control group were 0.05–0.18 nM (dopamine) and 0.07–0.14 nM (serotonin) in mPFC, 0.05–0.46 nM (dopamine) and 0.03–0.10 nM (serotonin) in striatum, and 0.31–1.42 nM (dopamine) and 0.03–0.06 nM (serotonin) in NAC. The peak concentration (C_{max}) was obtained from the original data, and the area under the time–concentration curve up to 100 min (AUC_{0-100}) and the mean residence time up to 100 min (MRT_{0-100}) were calculated by moment analysis [18].

Significant differences were evaluated by Student's *t*-test for comparison between the control group and methoxetamine group, by Dunnett's test for comparison with baseline concentrations, or by the Tukey–Kramer test for comparison between more than three groups. A *P* value less than 0.05 was considered statistically significant. Statistical calculations were performed using JMP Pro 10 (SAS Institute, Tokyo, Japan).

Results and discussion

Effects of methoxetamine on dopamine and serotonin in different brain areas

Time–concentration profiles of dopamine and serotonin in the striatum, NAC, and mPFC for up to 100 min after i.p. administration of 20 mg/kg methoxetamine are shown in Fig. 1. After i.p. administration of methoxetamine, each mouse appeared sallow during the experiment. Methoxetamine caused no change in C_{max} of dopamine and serotonin in the striatum or NAC from the baseline ($P > 0.05$, Dunnett's test) (Fig. 1). The AUC_{0-100} values of striatal dopamine in the control and methoxetamine groups were 6.5 and 8.4 ($\times 10^3$ baseline %·min), respectively, and those of striatal serotonin in the control and methoxetamine groups were 8.0 and 9.0 ($\times 10^3$ baseline %·min), respectively. Similarly, in the NAC, AUC_{0-100} values of dopamine in the control and methoxetamine groups were 8.1 and 10.5 ($\times 10^3$ baseline %·min), respectively, and those of serotonin in the control and methoxetamine groups were 9.2 and 10.5 ($\times 10^3$ baseline %·min), respectively. There was no significant difference between the AUC_{0-100} values for dopamine and serotonin in the striatum or NAC

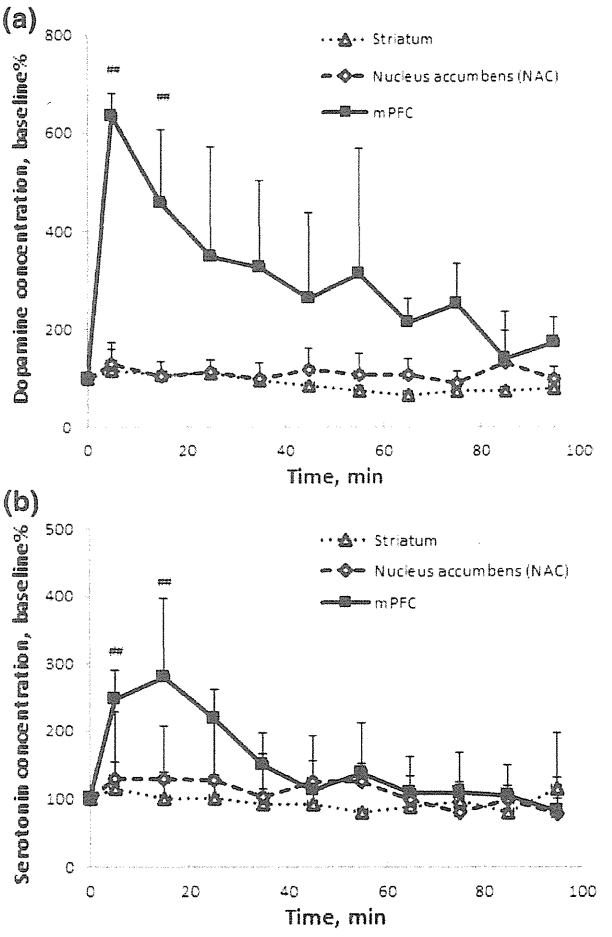

Fig. 1 Time–concentration profiles of dopamine (**a**) and serotonin (**b**) in the striatum, nucleus accumbens (NAC), and prefrontal cortex (mPFC) after i.p. administration of 20 mg/kg of methoxetamine. Each point plotted as mean + SD ($n = 3$). *P* values were calculated by Dunnett's test in comparison with baseline. *Double hash* indicates $P < 0.01$ vs baseline

between the control group and the methoxetamine group ($P > 0.05$, Student's t-test). It has been reported that the pharmacological effects, side effects, and physical symptoms of methoxetamine, a ketamine analogue, are similar to ketamine [3, 4, 8, 9]. Koshikawa et al. [19] reported that ketamine had no influence on the striatal dopamine system in rats. Therefore, the observation concerning ketamine may partly support our results that methoxetamine caused no change in dopamine concentrations in the striatum (Fig. 1). As far as the NAC is concerned, there was no increase in dopamine concentration following methoxetamine administration (Fig. 1). This finding is in line with a previous report that 50 mg/kg ketamine had little increase of dopamine concentrations in the NAC of rats [20].

The striatum and NAC are known to be the sites of action of stimulants, such as amphetamines, by increased dopamine and serotonin concentrations [13]. Previously, we showed that i.p. methamphetamine (10 mg/kg) and 3,4-methylenedioxymethamphetamine (MDMA) (10 mg/kg) increased dopamine concentration in the striatum of rats up to 6000 and 1620 % from baseline, respectively [14, 15]. In addition, these drugs both increased serotonin concentrations in the striatum by up to 1300 %. Baumann et al. [21] showed that MDMA (3 mg/kg) significantly increased dopamine and serotonin concentrations in the striatum, NAC, and mPFC of rats. In this study, we showed that methoxetamine caused a large increase in dopamine and serotonin concentrations only in the mPFC, but not in the striatum or in the NAC (Fig. 1). These results suggest that the mechanism of pharmacological effects caused by methoxetamine is different from those of amphetamines in rats. Further studies about the neurochemical effects of methoxetamine need to be validated.

Following ingestion of ketamine, the changes in monoamine concentrations in the mPFC are considered to be interrelated with schizophrenia [10], and hallucinations may be associated with anomalies in serotonin [12]. As shown in Fig. 1, methoxetamine increases the C_{max} of dopamine in the mPFC significantly up to 634 % ($P < 0.05$, Dunnett's test), and that of serotonin also rose significantly up to 279 % from baseline after an administration of methoxetamine ($P < 0.05$, Dunnett's test). In addition, AUC_{0-100} ($\times 10^3$ baseline %·min) values of dopamine and serotonin in the methoxetamine group were much higher than in the control group (dopamine, 29.0 vs 5.2; serotonin, 14.7 vs 5.8; $P < 0.05$, Student's t-test). Ketamine was reported to enhance the release of dopamine and serotonin in mPFC following antagonization to NMDA receptor [10]. Taking these observations into consideration, these results suggest that methoxetamine causes the release of dopamine and serotonin in the mPFC by antagonizing NMDA receptor, similar to ketamine.

Comparison between the effects of ketamine and methoxetamine on dopamine and serotonin in mPFC

Time–concentration profiles of dopamine and serotonin in mPFC after administration of saline (control), 19 or 38 mg/kg ketamine, or 20 mg/kg methoxetamine are shown in Fig. 2. The parameters for dopamine and serotonin in each group are shown in Table 1. As shown in Table 1, 19 mg/kg ketamine, which is an equivalent molar concentration to 20 mg/kg methoxetamine, did not significantly affect the C_{max} or AUC_{0-100} values of dopamine and serotonin compared with the control group. When

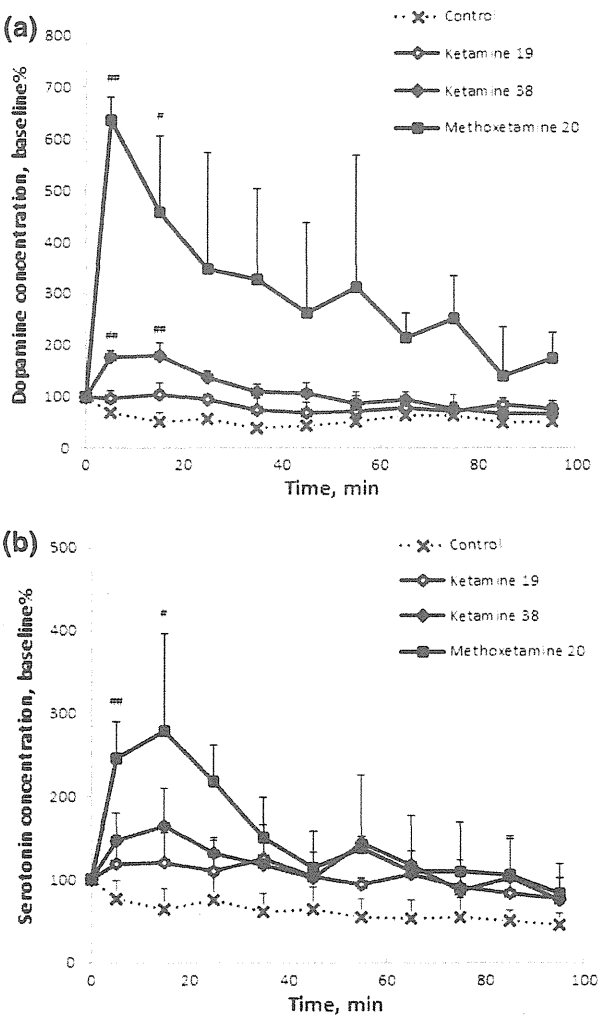

Fig. 2 Time–concentration profiles of dopamine (**a**) and serotonin (**b**) in the prefrontal cortex (mPFC) after administration of saline (control), 19 or 38 mg/kg ketamine (ketamine 19/ketamine 38), or 20 mg/kg methoxetamine (methoxetamine 20). Each point plotted as mean + SD ($n = 3$). P values were calculated by Dunnett's test in comparison to baseline. *Hash* indicates $P < 0.05$ vs baseline; *double hash* indicates $P < 0.01$ vs baseline

Table 1 Moment analysis parameters of dopamine and serotonin in the prefrontal cortex (mPFC) after administration of saline (control), ketamine (19 or 38 mg/kg) (ketamine 19/ketamine 38), or methoxetamine 20 mg/kg (methoxetamine 20)

	Group			
	Control	Ketamine 19	Ketamine 38	Methoxetamine 20
Dopamine				
C_{max} (baseline %)	103 ± 5.6^{c}	115 ± 12.7^{c}	188 ± 19.5^{b}	634 ± 45.9^{a}
AUC_{0-100} ($\times 10^3$ baseline %·min)	5.2 ± 1.3^{b}	7.8 ± 0.5^{b}	10.4 ± 0.2^{b}	29.0 ± 11.7^{a}
MRT_{0-100} (min)	45.9 ± 3.6^{c}	44.8 ± 2.2^{bc}	38.9 ± 1.4^{ab}	37.7 ± 2.4^{a}
Serotonin				
C_{max} (baseline %)	101 ± 1.1^{b}	135 ± 31.1^{ab}	185 ± 40.7^{ab}	286 ± 111^{a}
AUC_{0-100} ($\times 10^3$ baseline %·min)	5.8 ± 2.0^{b}	9.8 ± 1.5^{ab}	11.3 ± 2.9^{ab}	14.7 ± 2.8^{a}
MRT_{0-100} (min)	42.8 ± 1.5^{a}	44.5 ± 2.5^{a}	42.3 ± 6.3^{a}	38.5 ± 5.7^{a}

C_{max} peak concentration, AUC_{0-100} area under time–concentration curve up to 100 min, MRT_{0-100} mean residence time up to 100 min. Data are presented as mean ± SD ($n = 3$). Different lowercase characters indicate significant difference ($P < 0.05$, Tukey–Kramer test)

38 mg/kg ketamine was used, the C_{max} values of dopamine and serotonin were about 1.9 times higher than baseline (Fig. 2; Table 1). It has been reported that the pharmacological effects of methoxetamine in humans can be stronger than those of ketamine [4, 22]. However, the secretion characteristics of dopamine and serotonin are not yet clear. In the 20-mg/kg methoxetamine group, the C_{max} of dopamine increased from 100 (baseline) to 634 %, and the AUC_{0-100} of dopamine was 29.0×10^3 baseline %·min. These parameters were significantly higher than any other group ($P < 0.05$, Tukey–Kramer test). Furthermore, the C_{max} and AUC_{0-100} values of serotonin in the 20-mg/kg methoxetamine group tended to show high values compared with the ketamine groups (Fig. 2; Table 1). The results suggest that methoxetamine causes stronger dopaminergic symptoms (such as cognitive impairment) and stronger serotonergic symptoms (such as hallucinations) than ketamine.

Conclusions

To the best of our knowledge, this is the first study to show changes in dopamine and serotonin concentrations in the mPFC following methoxetamine administration using brain microdialysis in mice. Methoxetamine increased dopamine and serotonin concentrations in the mPFC, and titers of methoxetamine for these compounds, were higher than those of ketamine. We conclude that consumption of methoxetamine may be associated with an increased risk of developing schizophrenia-like symptoms when compared with ketamine.

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Conflict of Interest There are no financial or other relations that could lead to a conflict of interest.

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