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Short communication

Effect of zotepine on dopamine, serotonin and noradrenaline release in rat prefrontal cortex

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Abstract

The present study examined the effect of zotepine, an atypical antipsychotic, on the in vivo release of monoamines in the prefrontal cortex of rats using microdialysis. Local perfusion of zotepine at 10 μM increased extracellular levels of serotonin (5-HT), as well as dopamine and noradrenaline, in the prefrontal cortex. However, systemic administration of zotepine did not affect 5-HT release, although it increased the dopamine and noradrenaline release. These results suggest that the prefrontal 5-HT system does not contribute to the antidepressant effect of zotepine. The difference in the effect of zotepine between local and systemic treatment is discussed.

Keywords: Zotepine; Serotonin (5-HT) release; Microdialysis

1. Introduction

Zotepine (2-[(8-chlorodibenzo[b,f]thiepine-10-yl)oxy]-*N*,*N*-dimethylethylamine) is an antipsychotic drug with an atypical profile, effective against both negative and positive symptoms of schizophrenia and with a low propensity to induce extrapyramidal side effects (Fleischhacker et al., 1989; Petit et al., 1996). Zotepine possesses affinity not only for dopamine D_2-like receptors but also for the noradrenaline and serotonin (5-HT) transporters in vitro (Schotte et al., 1996; Tatsumi et al., 1999; Richelson and Souder, 2000). These in vitro binding experiments suggest that the possible inhibition by zotepine of noradrenaline and 5-HT transporters may contribute to its antidepressant effect. Rowley et al. reported that systemic administration of zotepine increased in vivo release of noradrenaline (1998) and dopamine (2000) in the prefrontal cortex of rats. These effects are considered to be due to an inhibition of the noradrenaline transporter, since local perfusion of nisoxetine, a specific inhibitor of the noradrenaline transporter, increases extracellular noradrenaline and dopamine levels in the prefrontal cortex (Rowley et al., 1998, 2000). However, it is not known whether local perfusion of zotepine increases noradrenaline and dopamine release in the brain regions. Furthermore, it is not known whether zotepine affects in vivo release of 5-HT in the prefrontal cortex. In this study, we examine, using a brain microdialysis technique, the effects of systemic and local treatment of zotepine on the dopamine, 5-HT and noradrenaline release in the prefrontal cortex of rats. The present study shows that 5-HT release in the prefrontal cortex is increased by local perfusion, but not systemic administration, of zotepine. We also examine some possibilities which may explain the reason why the effect of zotepine on 5-HT release differs between systemic administration and local perfusion, although the exact mechanism is not known.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 250–350 g at the beginning of the experiments were used. The animals were maintained under

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controlled environmental conditions (22±1 °C; 12:12-h light–dark cycle, lighting on at 08:00 h; food and water ad libitum) for at least 1 week before use in the experiments. The procedures for handling the animals and for their care were conducted according to Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society.

2.2. Surgery and microdialysis procedures

Each rat was anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and stereotaxically implanted with a guide cannula (one site per animal) for dialysis probe (Eicom, Kyoto, Japan) in the prefrontal cortex (A+3.2 mm, L − 0.6 mm, V − 5.2 mm, from the bregma and skull) as reported previously (Ago et al., 2003, 2005). The cannula was cemented in place with dental acrylic, and the animal was kept warm and allowed to recover from anesthesia. Postoperative analgesia was conducted by a single injection of buprenorphine (0.1 mg/kg, i.p.) (Lu et al., 2005). The active probe membrane was 3 mm long. On the day after surgery, the probe was perfused with Ringer's solution (147.2 mM NaCl, 4.0 mM KCl, and 2.2 mM $CaCl_2$; Fuso Pharmaceutical Industries, Ltd., Osaka, Japan) at a constant flow rate of 2 μl/min. A stabilization period of 3 h was established before the onset of the experiments. Two separate experiments were conducted to determine dopamine, 5-HT, and noradrenaline release, as previously reported (Ago et al., 2003, 2005). In one experiment, samples (20 μl) for 10-min microdialysis were collected and injected immediately into the high-performance liquid chromatography (HPLC) column for simultaneous assay of dopamine and 5-HT. In another experiment, samples (60 μl) for 30-min microdialysis were collected and injected immediately into the HPLC column for noradrenaline assay. After the experiments, Evans Blue dye was microinjected through the cannula to histologically verify the probe position.

2.3. Chemicals and drugs

The following drugs were used: zotepine (Fujisawa Pharmaceutical Co., Osaka, Japan); *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY100635) (Mitsubishi Pharma Co., Yokohama, Japan); 2-[2-(2-methoxy-1,4-benzodioanyl)]-imidazoline (RX821002) (Sigma, St Louis, MO, USA). Zotepine was dissolved in saline containing less than 0.1% v/v acetic acid. WAY100635 and RX821002 were dissolved in saline (0.9% solution of NaCl). The drugs were intraperitoneally injected at 1 ml/kg.

2.4. Statistics

All microdialysis data were calculated as percent change from dialysate basal concentrations, with 100% defined as the average of three fractions before administration. Analyses were made using two-way analysis of variance (ANOVA) for treatment as the intersubject factor and repeated measures with time as the intrasubject factor. Statistical analyses were made using a software package Statview 5.0J for Apple Macintosh computer (SAS Institute Inc., Cary, NC, USA). A value of $P<0.05$ was considered statistically significant.

3. Results

In the brain microdialysis, basal levels of dopamine, 5-HT and noradrenaline in dialysate (not corrected for in vitro probe recovery) were expressed as picogram/fraction. The basal extracellular dopamine, 5-HT and noradrenaline levels (the mean±S.E.M.) in the prefrontal cortex were 0.38±0.04 pg/20 μl, 0.60±0.07 pg/20 μl and 2.61±0.25 pg/60 μl, respectively ($n=32$ for dopamine and 5-HT, $n=24$ for

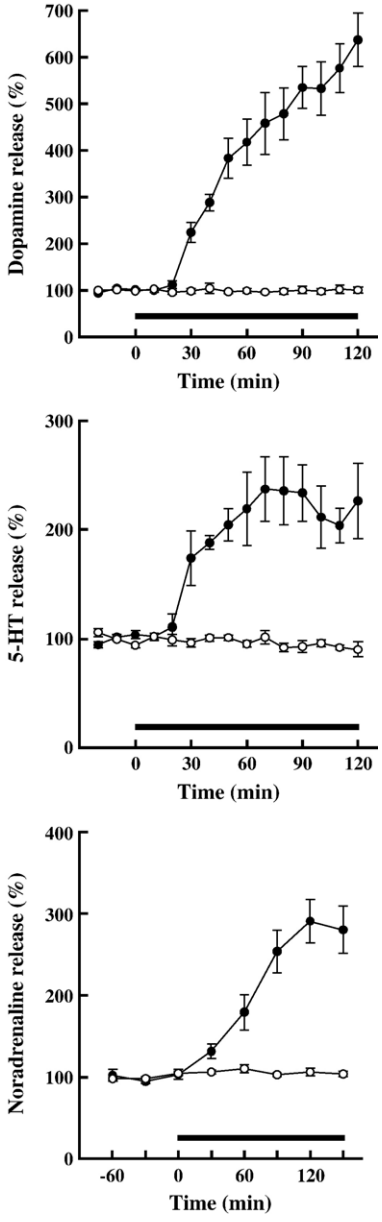

Fig. 1. Effect of local perfusion of zotepine on the in vivo release of dopamine, 5-HT and noradrenaline in rat prefrontal cortex. Vehicle (open circles) or 10 μM of zotepine (closed circles) were perfused into the cortex via the dialysis probe for the time indicated by the horizontal bar. Values are expressed as the mean ±S.E.M. of 4 rats.

noradrenaline). We first examined the effects of local perfusion and systemic administration of zotepine on the in vivo release of dopamine, 5-HT and noradrenaline in rat prefrontal cortex. A pilot experiment showed that local perfusion of 1 μM of zotepine via the microdialysis probe did not affect 5-HT release, although it caused 2-fold increase in dopamine release (data not shown). Zotepine at 10 μM increased the release of dopamine [F (14,119)=47.368; P<0.0001], 5-HT [F (14,119)=9.305; P<0.0001] and noradrenaline [F (7,63) =22.101; P<0.0001] (Fig. 1). When zotepine 1.0 and 3.0 mg/kg was injected intraperitoneally, it increased the release of DA [F (28,194)=11.389; P<0.0001] and noradrenaline [F (14,127)=12.904; P<0.0001], but did not affect the release of 5-HT [F (28,194)=1.411; n.s.] (Fig. 2).

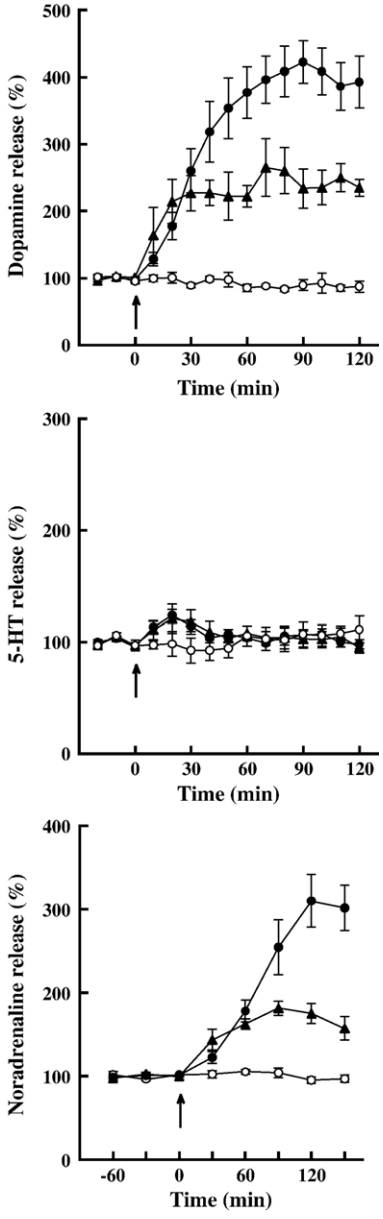

Fig. 2. Effect of systemic zotepine on the in vivo release of dopamine, 5-HT and noradrenaline in rat prefrontal cortex. Vehicle (open circles) or 1.0 mg/kg (closed triangles) and 3.0 mg/kg (closed circles) of zotepine were intraperitoneally administered at 0 min (arrow). Values are expressed as the mean±S.E.M. of 4 to 7 rats.

In view of the difference in the effect of zotepine on 5-HT release between local perfusion and systemic administration, we examined the possible involvement of the regulation of prefrontal 5-HT release by 5-HT_{1A} receptors or α_2-adrenoceptors in the effect of systemic administration of zotepine. The effect of systemic zotepine on the 5-HT release was not changed by pretreatment (30 min before zotepine) with the 5-HT_{1A} receptor antagonist WAY100635 (0.1 mg/kg, i.p.) (n=4, F (14,119)=1.235; n.s.) or the α_2-adrenoceptor antagonist RX821002 (3.0 mg/kg, i.p.) (n=3–4, F (17,125)= 1.588; n.s.) (data not shown).

4. Discussion

The present study confirmed the previous finding that systemic zotepine increased in vivo release of dopamine and noradrenaline in the prefrontal cortex (Rowley et al., 1998). In addition, we showed that local perfusion of zotepine increased extracellular levels of dopamine and noradrenaline in the prefrontal cortex. In view of the previous in vitro finding that zotepine has affinity for the noradrenaline transporter (Schotte et al., 1996; Tatsumi et al., 1999; Richelson and Souder, 2000), this finding suggests that the effect of systemic zotepine is due to the inhibition of the noradrenaline transporter, although it is not excluded that the 5-HT_{2A} receptor blockade may play a role in the increase of dopamine release, since zotepine also has affinity for 5-HT_{2A} receptors (Schotte et al., 1996; Richelson and Souder, 2000). The present study further showed that local perfusion of zotepine increased not only the dopamine and noradrenaline release but also the 5-HT release in the prefrontal cortex. This result, taken together with the previous finding that zotepine has affinity for the human 5-HT transporter (K_d=45 nM, Tatsumi et al., 1999), suggests that zotepine inhibits the 5-HT transporter. In contrast, systemic administration of zotepine did not increase 5-HT release in the prefrontal cortex. This finding suggests that the in vitro profile of zotepine concerning the interaction with 5-HT system is not reflected in its antipsychotic and antidepressant effects.

The release of 5-HT, unlike dopamine and noradrenaline, was not affected by systemic administration of zotepine, although it was increased by the local application. 5-HT release is predominantly controlled by 5-HT_{1A} autoreceptors on the cell bodies of serotonergic neurons (Hjorth and Auerbach, 1994; Gundlah et al., 1997), whereas noradrenaline release is predominantly regulated by terminal α_2 autoreceptors (Worley et al., 1999) and dopamine release is regulated by both mechanisms (Santiago and Westerink, 1991; Westerink et al., 1994; Adell and Artigas, 2004). Thus, 5-HT_{1A} autoreceptor function may contribute to the negative effect of systemic zotepine on 5-HT release. It is also considered that zotepine increases extracellular levels of noradrenaline and the increased noradrenaline interacts with α_2-adrenoceptor. These effects via 5-HT_{1A} or α_2 autoreceptors should inhibit 5-HT release. However, zotepine even in combination with pretreatment of the 5-HT_{1A} receptor antagonist WAY100635 or the α_2-adrenoceptor antagonist RX821002 did

not increase the 5-HT release in the prefrontal cortex. In this study, the doses of WAY100635 and RX821002 were chosen to block 5-HT_{1A} autoreceptors (Ago et al., 2003) and presynaptic α_2-adrenoceptors (Worley et al., 1999), respectively. These results may exclude the possible involvement of the regulation mechanism of 5-HT release in the effect of systemic administration of zotepine. It is not known why the effect of zotepine on 5-HT release differs between local perfusion and systemic administration.

In conclusion, noradrenaline transporter, but not 5-HT transporter, inhibition may contribute to the antidepressant effect of zotepine.

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References

Adell, A., Artigas, F., 2004. The somatodendritic release of dopamine in the ventral tegmental area and its regulation by afferent transmitter systems. Neurosci. Biobehav. Rev. 28, 415–431.

Ago, Y., Koyama, Y., Baba, A., Matsuda, T., 2003. Regulation by 5-HT_{1A} receptors of the in vivo release of 5-HT and DA in mouse frontal cortex. Neuropharmacology 45, 1050–1056.

Ago, Y., Nakamura, S., Baba, A., Matsuda, T., 2005. Sulpiride in combination with fluvoxamine increases in vivo dopamine release selectively in rat prefrontal cortex. Neuropsychopharmacology 30, 43–51.

Fleischhacker, W.W., Barnas, C., Stuppäck, C.H., Unterweger, B., Miller, C., Hinterhuber, H., 1989. Zotepine vs. haloperidol in paranoid schizophrenia: a double-blind trial. Psychopharmacol. Bull. 25, 97–100.

Gundlah, C., Martin, K.F., Heal, D.J., Auerbach, S.B., 1997. In vivo criteria to differentiate monoamine reuptake inhibitors from releasing agents: sibutramine is a reuptake inhibitor. J. Pharmacol. Exp. Ther. 283, 581–591.

Hjorth, S., Auerbach, S.B., 1994. Further evidence for the importance of 5-HT_{1A} autoreceptors in the action of selective serotonin reuptake inhibitors. Eur. J. Pharmacol. 260, 251–255.

Lu, L., Hope, B.T., Dempsey, J., Liu, S.Y., Bossert, J.M., Shaham, Y., 2005. Central amygdala ERK signaling pathway is critical to incubation of cocaine craving. Nat. Neurosci. 8, 212–219.

Petit, M., Raniwalla, J., Tweed, J., Leutenegger, E., Dollfus, S., Kelly, F., 1996. A comparison of an atypical and typical antipsychotic, zotepine versus haloperidol in patients with acute exacerbation of schizophrenia: a parallel-group double-blind trail. Psychopharmacol. Bull. 32, 81–87.

Richelson, E., Souder, T., 2000. Binding of antipsychotic drugs to human brain receptors. Focus on newer generation compounds. Life Sci. 68, 29–39.

Rowley, H.L., Kilpatrick, I.C., Needham, P.L., Heal, D.J., 1998. Elevation of extracellular cortical noradrenaline may contribute to the antidepressant activity of zotepine: an in vivo microdialysis study in freely moving rats. Neuropharmacology 37, 937–944.

Rowley, H.L., Needham, P.L., Kilpatrick, I.C., Heal, D.J., 2000. A comparison of the acute effects of zotepine and other antipsychotics on rat cortical dopamine release, in vivo. Naunyn-Schmiderberg's Arch. Pharmcol. 361, 187–192.

Santiago, M., Westerink, B.H., 1991. The regulation of dopamine release from nigrostriatal neurons in conscious rats: the role of somatodendritic autoreceptors. Eur. J. Pharmacol. 204, 79–85.

Schotte, A., Janssen, P.F.M., Gommeren, W., Luyten, W.H.M.L., Van Gompel, P., Lesage, A.S., De Loore, K., Leysen, J.E., 1996. Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. Psychopharmacology 124, 57–73.

Tatsumi, M., Jansen, K., Blakely, R.D., Richelson, E., 1999. Pharmacological profile of neuroleptics at human monoamine transporters. Eur. J. Pharmacol. 368, 277–283.

Westerink, B.H., de Boer, P., Santiago, M., De Vries, J.B., 1994. Do nerve terminals and cell bodies of nigrostriatal dopaminergic neurons of the rat contain similar receptors? Neurosci. Lett. 167, 109–112.

Worley, K.E., Hughes, Z.A., Heal, D.J., Stanford, S.C., 1999. Comparison of changes in the extra cellular concentration of noradrenalin in rat frontal cortex induced by sibutramine or *d*-amphetamine: modulation by α_2-adrenoceptors. Br. J. Pharmacol. 127, 1860–1866.