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Short communication

Effects of 5-HT_2 receptor antagonists on the anti-immobility effects of imipramine in the forced swimming test with mice

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Abstract

The effects of 5-HT_2 receptor antagonists on antidepressant effects of imipramine were investigated in the forced swimming test. Imipramine induced anti-immobility effects in mice dose dependently. Pretreatment with the 5-$HT_{2A/2B/2C}$ receptor antagonist, 4-isopropyl-7-methyl-9-(2-hydroxy-1-methyl-propoxycarbonyl)-4,6*A*,7,8,9,10,10*A*-octahydro-indolo[4,3-FG]quinolone maleate (LY 53857) significantly enhanced the anti-immobility effects of imipramine. The 5-$HT_{2C/2B}$ receptor antagonist, *N*-3-pyridinyl-3,5-dihydro-5-methyl-benzo[1,2-*b*:4,5-*b*′]dipyrrole-1(2*H*)-carboxamide (SB 206553), also enhanced, while the 5-HT_{2A} receptor antagonist, ketanserin, was without effect. These results suggest that blockade of the 5-$HT_{2C/2B}$ receptor leads to potentiation of the antidepressant effects of imipramine. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Imipramine; 5-HT (5-hydroxytryptamine, serotonin); 5-$HT_{2C/2B}$ receptor; 5-HT_{2A} receptor; Forced swimming test; Antidepressant; (Mouse)

1. Introduction

It is recognized that the pathogenesis of depression is closely related to the monoaminergic system, particularly noradrenergic and serotonergic mechanisms. Drugs affecting neurotransmission of serotonin (5-hydroxytryptamine, 5-HT) such as those inhibiting 5-HT reuptake at nerve terminals, or inhibiting 5-HT metabolism (monamine oxidase inhibitors), are effective in depression (Montgomery, 1990; Siever et al., 1991; Briley and Moret, 1993). In addition, the activation of postsynaptic 5-HT_{1A} receptors improves depression. The 5-HT_{1A} receptor partial agonists, buspirone or tandospirone, induce antidepressant effects (Cervo et al., 1988; Chojnacka-Wojcik et al., 1991; Deakin, 1993; Borsini, 1995).

Imipramine is a tricyclic antidepressant and is widely used as therapy for depression. Imipramine shows antidepressant effects by inhibiting the reuptake of both 5-HT and noradrenaline (Briley and Moret, 1993). The forced swimming test is commonly used to evaluate antidepressants, and many antidepressants show the anti-immobility effects (Porsolt et al., 1977; Borsini, 1995). Several reports indicated that a single administration of imipramine reduces immobility in the forced swimming test (Porsolt et al., 1977; Borsini et al., 1991; Redrobe and Bourin, 1997). Although antidepressant effects of imipramine are connected with 5-HT, the involvement of the 5-HT receptor subtypes in the anti-immobility effects of imipramine is not fully understood. It was reported that imipramine has an affinity with 5-HT_2 receptors and that this receptor may be involved in the antidepressant effects of imipramine (Hyttel, 1994; Palvimaki et al., 1996). However, it is not clear whether the 5-HT_2 receptor antagonists inhibit or enhance the antidepressant effects of imipramine. Borsini et al. (1991) reported that mesulergine, which has an affinity with 5-HT_{2C} receptors, can inhibit the anti-immobility effects of imipramine at a high dose. On the contrary, Redrobe and Bourin (1997) showed that combined treatment with 5-HT_2 receptor antagonists and imipramine at a sub-active dose induces apparent anti-immobility effects. Therefore, in this study, we investigated the influences of 5-HT_2 receptor antagonists on the effects of several doses of imipramine in the forced swimming test.

2. Materials and methods

2.1. Animals

Male ddY mice weighing 25–30 g were purchased from SLC Japan (Japan). The mice were given free access to

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food and water and were housed under a controlled 12-h/12-h light–dark cycle (light from 7:00 a.m. to 7:00 p.m.), with room temperature 23 ± 1 °C and humidity 55 ± 5%.

2.2. Drug treatment

Imipramine HCl was obtained from Nacalai Tesque (Japan). 4-Isopropyl-7-methyl-9-(2-hydroxy-1-methyl-propoxycarbonyl) - 4, 6*A*,7,8,9,10, 10*A* - octahydro - indolo [4, 3-FG]quinolone maleate (LY 53857), *N*-3-pyridinyl-3,5-dihydro-5-methyl-benzo[1,2-*b*:4,5-*b*′]dipyrrole-1(2*H*)-carboxamide HCl (SB 206553), and ketanserin tartrate were obtained from Research Biochemicals (USA). 5-HT_2 receptor antagonists were administered 30 min before the injection of imipramine

2.3. Forced swimming test

The forced swimming test was performed according to the methods described by Porsolt et al. (1977). Each mouse was placed in a 25-cm glass cylinder (10 cm diameter) containing 15 cm of water maintained at 23 ± 1 °C. Immobility was recorded during a 6-min swimming test.

2.4. Measurement of locomotor activity

Locomotor activity for 10 min was measured by a digital counter with an infrared sensor (Neuroscience, Japan) 30 min after the injection of 5-HT_2 receptor antagonists. The apparatus detects and records a digital count of the horizontal movements of animals.

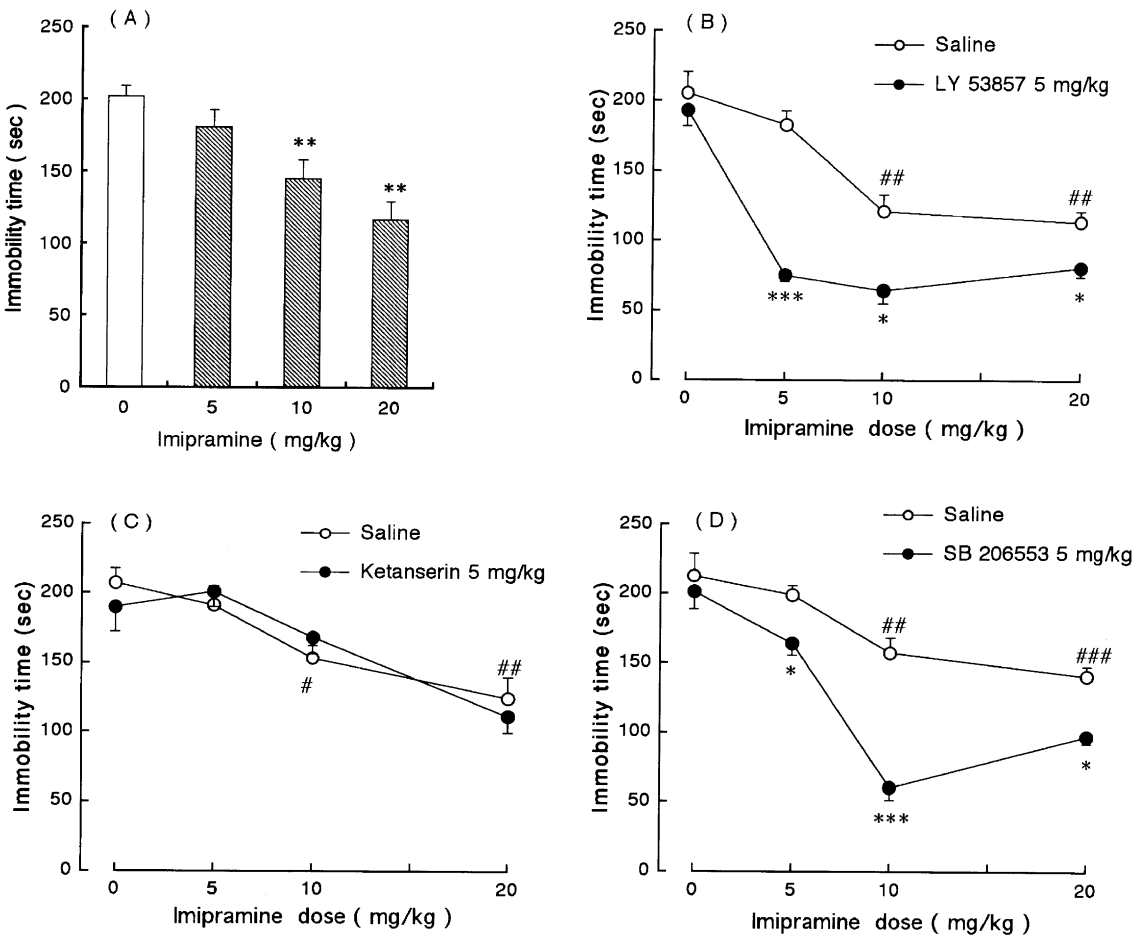

Fig. 1. Effects of imipramine on immobility in forced swimming test and effects of LY 53857, ketanserin and SB 206553 on anti-immobility effects of imipramine in mice. (A) Effects of imipramine on immobility in forced swimming test. Results are shown as means ± S.E. ($N = 7–9$). Imipramine was given i.p. $^{**}P < 0.01$. (B) Effects of LY 53857 on anti-immobility effects of imipramine in mice. Results are shown as means ± S.E. ($N = 6–10$). Imipramine was given i.p. LY 53857 at 5 mg/kg was injected i.p. 30 min before imipramine. $^{*}P < 0.05$, $^{***}P < 0.0001$ vs. respective saline-pretreated group. $\#\#P < 0.01$ vs. saline + saline-treated group. (C) Effects of ketanserin on anti-immobility effects of imipramine in mice Results are shown as means ± S.E. ($N = 7–8$). Imipramine was given i.p. Ketanserin at 5 mg/kg was injected i.p. 30 min before imipramine. $\#P < 0.05$, $\#\#P < 0.01$ vs. saline + saline-treated group. (D) Effects of SB 206553 on anti-immobility effects of imipramine in mice. Results are shown as means ± S.E. ($N = 7–9$). Imipramine was given i.p. SB 206553 at 5 mg/kg was injected i.p. 30 min before imipramine. $^{*}P < 0.05$, $^{***}P < 0.0001$ vs. respective saline-pretreated group. $\#\#P < 0.01$, $\#\#\#P < 0.001$ vs. saline + saline-treated group.

2.5. Statistics

Dose-related effects of imipramine on immobility were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. Other results were analyzed by two-way ANOVA followed by Tukey's test.

3. Results

3.1. Effects of imipramine on immobility in forced swimming test

Fig. 1A shows that imipramine dose dependently reduced immobility in mice.

3.2. Effects of 5-HT_2 receptor antagonists on anti-immobility effects of imipramine

Fig. 1B shows the immobility after the co-administration of various doses of imipramine and the 5-$HT_{2A/2B/2C}$ receptor antagonist, LY 53857. Apparent anti-immobility was observed following the administration of LY 53857 at 5 mg/kg and a sub-active dose of imipramine (5 mg/kg). LY 53857 significantly enhanced the anti-immobility effects of imipramine at doses of 10 and 20 mg/kg.

Effects of imipramine and the 5-HT_{2A} receptor antagonist, ketanserin (5 mg/kg), on immobility are shown in Fig. 1C. Ketanserin did not change the duration of immobility due to imipramine at any dose.

Fig. 1D shows the effects of the 5-$HT_{2C/2B}$ receptor antagonist, SB 206553 (5 mg/kg), on the duration of immobility in imipramine-treated mice. Pretreatment with SB 206553 at 5 mg/kg significantly reduced immobility in imipramine (5–20 mg/kg)-treated mice.

LY 53857, ketanserin and SB 206553 alone did not affect immobility.

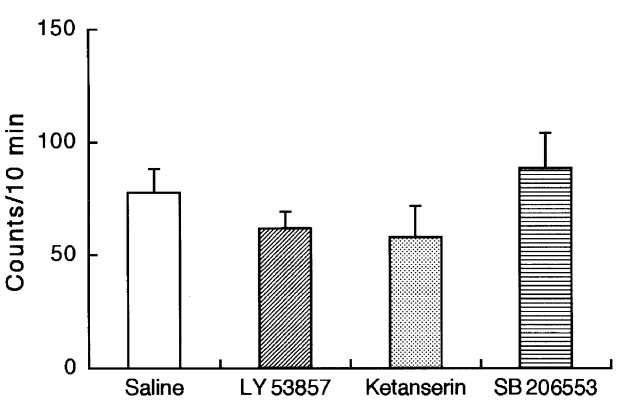

Fig. 2. Effects of LY 53857, ketanserin and SB 206553 on locomotor activity in mice. Results are shown as means ± S.E. ($N = 5–7$). Locomotor activity was measured for 10 min. LY 53857 at 5 mg/kg, ketanserin 5 mg/kg and SB 206553 at 5 mg/kg was injected i.p. 30 min before measurement of locomotor activity.

3.3. Effects of 5-HT_2 receptor antagonists on locomotor activity

As shown in Fig. 2, LY 53857 (5 mg/kg), ketanserin (5 mg/kg) and SB 206553 (5 mg/kg) were without effect on locomotor activity.

4. Discussion

It has been reported that, in mice and rats, the tricyclic antidepressant imipramine reduces immobility in the forced swimming test (Porsolt et al., 1977; Borsini et al., 1991; Borsini, 1995; Redrobe and Bourin, 1997). As shown in Results, imipramine induced dose-dependent anti-immobility effects in mice, which is consistent with previous findings. Differential effects of 5-HT_2 receptor antagonists on the duration of immobility in imipramine-treated mice have been reported. Although anti-immobility effects of imipramine at an active dose are antagonized by mesulergine, co-administration of a sub-active dose of imipramine with the 5-HT_2 receptor antagonist, ritanserin or ketanserin, induces an apparent anti-immobility effect (Borsini et al., 1991; Redrobe and Bourin, 1997). This discrepancy may be explained by differences in the imipramine dosages used. However, there is no report on the combined effects of the 5-HT_2 receptor antagonists and imipramine at various doses in the forced swimming test.

Our results indicated that the anti-immobility effects of imipramine were significantly enhanced by pretreatment with the non-selective 5-HT_2 receptor antagonist, LY 53857. LY 53857 reduced the duration of immobility in mice treated with all doses of imipramine, that is, sub-active and active doses. It has been reported that LY 53857 has a high affinity with the 5-$HT_{2A/2B/2C}$ receptor subtypes (Baxter et al., 1995). The dose of LY 53857 in the present study was that inhibiting 5-HT_{2A} and 5-HT_{2C} receptor-mediated head shake responses and hypophagia (Kennett and Curzon, 1991), although it is not clear whether LY 53857 inhibits 5-HT_{2B} receptor-related effects in vivo. Redrobe and Bourin (1997) reported that ritanserin, another 5-$HT_{2A/2B/2C}$ receptor antagonist, enhances anti-immobility effects of imipramine at a sub-active dose in mice, which is consistent with our results. Therefore, it indicates that blockade of 5-HT_2 receptors potentiates anti-immobility effects of imipramine.

We further examined which 5-HT_2 receptor subtypes are involved in the effects of imipramine. As shown in Results, the 5-HT_{2A} receptor antagonist, ketanserin (5 mg/kg), did not affect the duration of immobility in mice treated with imipramine, regardless of the dose of imipramine. The anti-immobility effect of imipramine was not affected by ketanserin at the same doses as LY 53857. In contrast, the 5-$HT_{2C/2B}$ receptor antagonist, SB 206553, augmented the anti-immobility effects of imipramine and was effective at both sub-active and active doses of

imipramine. It was reported that SB 206553 at the dose used in the present study can block the hyperphagia elicited by the 5-HT_{2B} receptor agonist, BW 723C86, and the hypolocomotion elicited by activation of the 5-HT_{2C} receptor (Kennett et al., 1996, 1997). This finding suggests that inhibition of the 5-$HT_{2C/2B}$ receptor, but not of the 5-HT_{2A} receptor, enhances the anti-immobility effects of imipramine.

Ketanserin has a higher affinity for 5-HT_{2A} receptors than for other 5-HT_2 receptor subtypes (Baxter et al., 1995). The dose of ketanserin used in the present study was higher than those inhibiting the 5-HT_{2A} receptor-mediated hyperglycemia induced by the 5-HT_{2A} receptor agonist or head shake responses elicited by 5-hydroxytryptophan (Kennett and Curzon, 1991; Sugimoto et al., 1996). Therefore, it is suggested that the 5-HT_{2A} receptor is not related to enhancement of the effects of LY 53857 on anti-immobility. A previous report indicated that administration of imipramine at a sub-active dose along with ketanserin (8 mg/kg) showed anti-immobility effects. However, the dosage of ketanserin (8 mg/kg) used was higher than that in the present study. Ketanserin has lower affinity for the 5-HT_{2C} receptor than for the 5-HT_{2A} receptor. However, at high doses, ketanserin can block the 5-HT_{2C} receptor-mediated hypophagia elicited by mCPP, although its effects are not strong (Kennett and Curzon, 1991). Therefore, a high-dose treatment with ketanserin may block the 5-HT_{2C} receptor and enhance the anti-immobility effects of imipramine.

Borsini et al. (1991) reported that mesulergine, the 5-HT_{2C} receptor antagonist inhibited the anti-immobility effects of imipramine. However, in their study, ritanserin, which blocks the 5-HT_{2C} receptor, did not affect the anti-immobility effects of imipramine. In addition, the authors demonstrated that the dopamine D_2 receptor antagonist, sulpiride, also antagonized some effects of imipramine (Borsini et al., 1991). Recently, it was reported that antisense of dopamine D_2 receptor antagonizes the anti-immobility effects of imipramine in rats (Dziedzicka-Wasylewska et al., 2000). Since mesulergine has an affinity for the dopamine D_2 receptor (Leysen et al., 1981), this D_2 receptor may be related to effects of mesulergine.

The blockade of 5-$HT_{2C/2B}$ receptor increases noradrenaline or dopamine release in the brain, as measured by microdialysis in rats (Di Matteo et al., 1998; Millan et al., 1998). This raises the possibility that the amplifying effects of the 5-$HT_{2C/2B}$ receptor blocker, SB 206553, on anti-immobility effects of imipramine are mediated by increasing noradrenaline or dopamine release. It was reported that, in a receptor binding assay, imipramine has an affinity with the 5-HT_{2C} receptor (Hyttel, 1994; Palvimaki et al., 1996). Together, these findings indicate that the 5-HT_{2C} receptor may be related to the antidepressant effects of imipramine. The involvement of the 5-HT_{2B} receptor in depression remains unclear and further studies are required.

Since drugs increasing locomotor activity may diminish immobility in the forced swimming test, this effect may be related to the effects of 5-HT_2 receptor antagonists on anti-immobility effects. However, our results indicate that LY 53857, ketanserin or SB 206553 did not influence locomotor activity. Therefore, the effects of these antagonists on anti-immobility effects are not related to locomotor activity.

In summary, our present results indicate that the anti-immobility effects of imipramine were enhanced by pretreatment with the LY 53857 and SB 206553, which have an affinity with 5-$HT_{2C/2B}$ receptors. Recently, it was suggested that the 5-HT_{2C} receptor inverse agonist may be useful for therapy of depression (Bromidge et al., 2000). Thus, the 5-HT_{2C} receptor antagonists may potentiate effects of antidepressants.

References

Baxter, G., Kennett, G.A., Blaney, F., Blackburn, T., 1995. 5-HT_2 receptor subtypes: a family re-united? Trends Pharmacol. Sci. 16, 105–110.

Borsini, F., 1995. Role of the serotonergic system in the forced swimming test. Neurosci. Behav. Rev. 19, 377–395.

Borsini, F., Cesana, R., Vidi, A., Mennini, T., 1991. Evidence that imipramine activates 5-HT_{1C} receptor function. Eur. J. Pharmacol. 203, 359–363.

Briley, M., Moret, C., 1993. Neurobiological mechanisms involved in antidepressant therapies. J. Clin. Neuropharmacol. 16, 24–35.

Bromidge, S.M., Dabbs, S., Davies, D.T., Davies, S., Duckworth, D.M., Forbes, I.T., Gaster, L.M., Ham, P., Jones, G.E., King, F.D., Mulholland, K.R., Saunders, D.V., Wyman, P.A., Blaney, F.E., Clarke, S.E., Blackburn, T.P., Blackburn, V., Blackburn, H., Kennett, G.A., Lightowler, S., Middlemiss, D.N., Trail, B., Riley, G.J., Wood, M.D., 2000. Biarylcarbamoylindolines are novel and selective 5-HT2C receptor inverse agonists: identification of 5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-6-trifluoromethylindoline (SB-243213) as a potential antidepressant/anxiolytic agent. J. Med. Chem. 43, 1123–1134.

Cervo, L., Grignaschi, G., Samanin, R., 1988. Different effects of intracerebral and systemic administration of buspirone in the forced swimming test: involvement of a metabolite. Life Sci. 43, 2095–2102.

Chojnacka-Wojcik, E., Tatarczynska, E., Golembiowska, K., Przegalinski, E., 1991. Involvement of 5-HT_{1A} receptors in the antidepressant-like activity of gepirone in the forced swimming test in rats. Neuropharmacology 30, 711–717.

Deakin, J., 1993. A review of clinical efficacy of 5-HT_{1A} agonists in anxiety and depression. J. Psychopharmacol. 7, 283–289.

Di Matteo, V., Di Giovanni, G., Di Mascio, M., Esposito, E., 1998. Selective blockade of serotonin$_{2C/2B}$ receptors enhances dopamine release in the rat nucleus accumbens. Neuropharmacology 37, 265–272.

Dziedzicka-Wasylewska, M., Kolasiewicz, W., Rogoz, Z., Margas, W., Maj, J., 2000. The role of dopamine D_2 receptor in the behavioral effects of imipramine study with the use of antisense oligonucleotide. J. Physiol. Pharmacol. 51, 401–409.

Hyttel, J., 1994. Pharmacological characterization of the selective serotonin reuptake inhibitors(SSRI's). Int. J. Pharmacol. 9, 19–26.

Kennett, G.A., Curzon, G., 1991. Potencies of antagonists indicate that 5-HT_{1C} receptors mediate 1-3(chlorophenyl)piperazine-induced hypophagia. Br. J. Pharmacol. 103, 2016–2020.

Kennett, G.A., Wood, M.D., Bright, F., Clia, J., Piper, D.C., Gager, T., Thomas, D., Baxter, G.S., Forbes, I.T., Ham, P., Blackburn, T.P.,

1996. In vitro and in vivo profile on SB 206553, a potent 5-$HT_{2C/2B}$ receptor antagonist with anxiolytic-like properties. Br. J. Pharmacol. 117, 427–434.

Kennett, G.A., Ainsworth, K., Trail, B., Blackburn, T.P., 1997. BW 723 C86, a 5-HT_{2B} receptor agonist, causes hyperphagia and reduced grooming in rats. Neuropharmacology 36, 233–239.

Leysen, J.E., Awouters, F., Kennis, L., Laduron, P.M., Vandenberk, J., Janssen, P.A.J., 1981. Receptor binding profile of R 41 468, a novel antagonist at 5-HT_2 receptors. Life Sci. 28, 1015–1022.

Millan, M.J., Dekeyne, A., Gobert, A., 1998. Serotonin(5-$HT)_{2C}$ receptors tonically inhibit dopamine(DA) and noradrenaline(NA), but not 5-HT, release in the frontal cortex in vivo. Neuropharmacology 37, 953–955.

Montgomery, S.A., 1990. Clinical efficacy of 5-HT uptake inhibitors. Neuropharmacology 13, 285–286.

Palvimaki, E.P., Roth, B.L., Majasuo, H., Laakso, A., Kuoppamaki, M., Syvalahti, E., Hietala, J., 1996. Interactions of selective serotonin reuptake inhibitors with the serotonin 5-HT_{2C} receptor. Psychopharmacology 126, 234–240.

Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioural despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. 229, 327–336.

Redrobe, J.P., Bourin, M., 1997. Partial role of 5-HT_2 and 5-HT_3 receptors in the activity of antidepressants in the mouse forced swimming test. Eur. J. Pharmacol. 325, 129–135.

Siever, L.J., Kahn, R.S., Lawlor, B.A., Trestman, R.L., Lawrence, T.L., Coccaro, E.F., 1991. Critical issues in defining the role of serotonin in psychiatric disorders. Pharmacol. Rev. 43, 509–525.

Sugimoto, Y., Yamada, J., Yoshikawa, T., Horisaka, K., 1996. Effects of the 5-$HT_{2C/2B}$ receptor agonist 1-(3-chlorophenyl)piperazine on the plasma glucose levels of rats. Eur. J. Pharmacol. 307, 75–80.