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Short communication

# 5-$HT_{2C}$ receptors mediate penile erections in rats: actions of novel and selective agonists and antagonists

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**Abstract**

The mixed 5-$HT_{2A}$/5-$HT_{2B}$/5-$HT_{2C}$ receptor agonist, *m*-(chlorophenyl)piperazine (mCPP), elicited penile erections in rats, an action mimicked by the selective 5-$HT_{2C}$ receptor agonist, RO 60-0175 (*S*)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine), whereas the preferential 5-$HT_{2B}$ receptor agonist, BW 723C86 (1-[5-(thienylmethoxy)-1*H*-3-indoyl] propan-2-amine) was ineffective. The actions of mCPP and RO 60-0175 were dose-dependently abolished by the novel 5-$HT_{2B}$/5-$HT_{2C}$ receptor antagonists, SB 200,646 (1-(1-methylindol-5-yl)-3-(3-pyridyl) urea) and SB 206,553 (5 methyl-1-(3-pyridil-carbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole). In contrast, penile erections were not significantly affected by the selective 5-$HT_{2B}$ receptor antagonist, SB 204,741 (1-(1-methylindol-5-yl)-3-(3-methylisothiazol-5-yl)-urea) nor by the selective 5-$HT_{2A}$ receptor antagonist, MDL 100,907 ([*R*(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol]). These data provide rigorous pharmacological evidence that activation of 5-$HT_{2C}$ receptor elicits penile erections in the rat. This model should, thus, be of use for characterising novel ligands at this site.

*Keywords:* 5-HT (5-hydroxytryptamine, serotonin); 5-$HT_{2C}$ receptor; Penile erection

## 1. Introduction

Serotonin receptors modulate several physiological functions including, appetite, mood, motor behaviour and nociception (see Bloom and Kupfer, 1995; Molineaux et al., 1989; Tecott et al., 1995). Several functional parameters have been proposed for the characterization of actions at 5-$HT_{2C}$ sites, including hypolocomotion, hyperthermia and the induction of penile erections (Berendsen et al., 1990; Kennett and Curzon, 1988; Lucki et al., 1991). However, owing to a lack of selective ligands at 5-$HT_{2C}$ receptors, it has proven difficult to differentiate actions mediated at 5-$HT_{2C}$ receptors from those mediated by 5-$HT_{2A}$ and, in particular, 5-$HT_{2B}$ receptors (Baxter et al., 1995; Bonhaus et al., 1995; Boess and Martin, 1994). Indeed, such widely used ligands as the agonist, *m*-(chlorylphenyl)piperazine (mCPP), and the antagonists, ritanserin and mesulergine, show similar affinities at each of these sites and exert marked activity at other receptor types, such as $\alpha_1$-adrenoceptors and dopamine $D_2$ receptors (Baxter et al., 1995; Boess and Martin, 1994). Recently, however, novel ligands were described which lack marked affinity at other 5-HT receptor types, the use of which should permit the unequivocal identification of actions mediated by 5-$HT_{2C}$ receptors. That is, the selective 5-$HT_{2C}$ receptor agonist, RO 60-0175 (*S*)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine) (Martin et al., 1995); the 5-$HT_{2B}$/5-$HT_{2C}$ receptors antagonists, SB 200,646 (1-(1-methylindol-5-yl)-3-(3-pyridyl) urea) (Wood et al., 1995) and SB 206,553 (5 methyl-1-(3-pyridil-carbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole) (Kennett et al., 1996b), the selective 5-$HT_{2B}$ receptor antagonist, SB 204,741 (1-(1-methylindol-5-yl)-3-(3-methylisothiazol-5-yl)-urea) (Forbes et al., 1996), the preferential 5-$HT_{2B}$ receptor agonist, BW 723C86 (1-[5-(thienylmethoxy)-1*H*-3-indoyl] propan-2-amine) (Kennett et al., 1996a) and the selective 5-$HT_{2A}$ receptor antagonist, MDL 100,907 ([*R*(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol]) (Sörensen et al., 1993). Employing these ligands, we now provide evidence that 5-$HT_{2C}$ receptors mediate penile erections in the rat.

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## 2. Materials and methods

Penile erections were measured in male Wistar rats (120–140 g) essentially as described previously (Jenck et al., 1993). Briefly, immediately following treatment with vehicle, mCPP or RO 60-0175, they were placed in transparent, Plexiglas observation cages behind which was situated a mirror to facilitate observation. Penile erections were recorded over 45 min. Antagonists were administered 30 min prior to the agonists. Drugs were dissolved in sterile water and injected subcutaneously (s.c.). Drug doses are in terms of the base.

## 3. Results

mCPP and RO 60-0175 elicited penile erections over a similar dose range with their dose–response curves inflecting at the highest dose tested (Fig. 1). They displayed a similar maximal effect. BW 723C86 was inactive at doses of 0.16 ($n = 4$) and 2.5 ($n = 8$) mg/kg, s.c.; penile erections = $0.5 \pm 0.5$ and $0.3 \pm 0.2$, respectively: not significantly ($P > 0.05$) different to vehicle ($n = 8$), penile erections = $0.1 \pm 0.1$. SB 200,646 dose-dependently inhibited the induction of penile erections by mCPP with an inhibitory dose$_{50}$ (95% confidence limits) ($ID_{50}$ (95% C.L.)) of 3.6 (1.4–9.3) mg/kg, s.c. (Fig. 2). It also blocked the action of RO 60-0175 at similar doses although, as supplies were exhausted, a complete dose–response curve could not be constructed. SB 206,553 more potently and completely abolished the actions of both mCPP and RO 60-0175 with $ID_{50}$ values (95% C.L.s) of 0.06 (0.02–0.24) and 0.18 (0.07–0.47) mg/kg, s.c., respectively (Fig. 2). SB 204,741 (10.0 mg/kg, s.c.) was ineffective. Vehicle/mCPP = $2.7 \pm 0.3$ vs. SB 204,741/mCPP = $2.3 \pm 0.2$, $P > 0.05$ and vehicle/RO 60-0175 = $1.8 \pm 0.2$ vs. SB 204,741/RO 60-0175 = $1.3 \pm 0.3$, $P > 0.05$. MDL 100,907 (0.04 mg/kg, s.c.) was also inactive. Vehicle/mCPP = $3.4 \pm 0.2$ vs. MDL 100,907/mCPP = $2.7 \pm 0.5$, $P > 0.05$. Vehicle/RO 60-0175 = $1.7 \pm 0.2$ vs. MDL 100,907/RO 60-0175 = $1.7 \pm 0.2$, $P > 0.05$. The

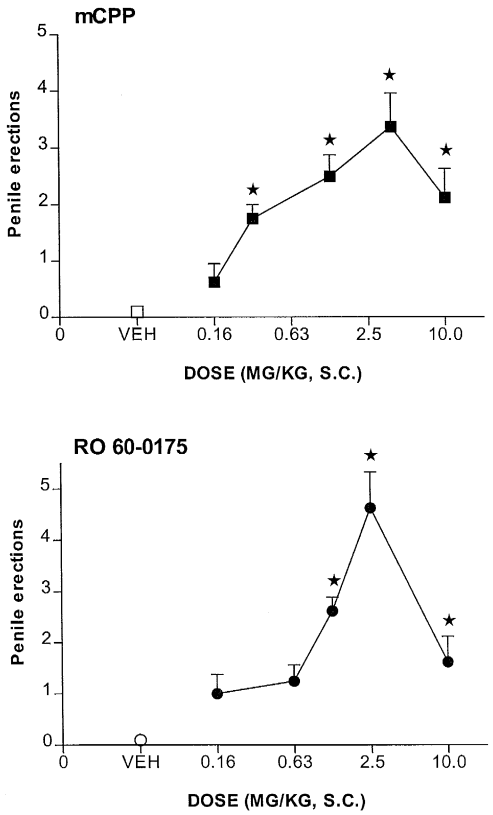


Fig. 1. Induction of penile erections by the 5-$HT_{2C}$ agonists, mCPP and RO 60-0175, in rats. Means ± S.E.M. shown. $n = 8$ per group. ANOVA as follows: mCPP, $F(5,42) = 8.0$, $P < 0.001$ and RO 60-0175, $F(5,42) = 14.6$, $P < 0.001$. Asterisks indicate significance of differences to vehicle values in Dunnett's test ($P < 0.05$).

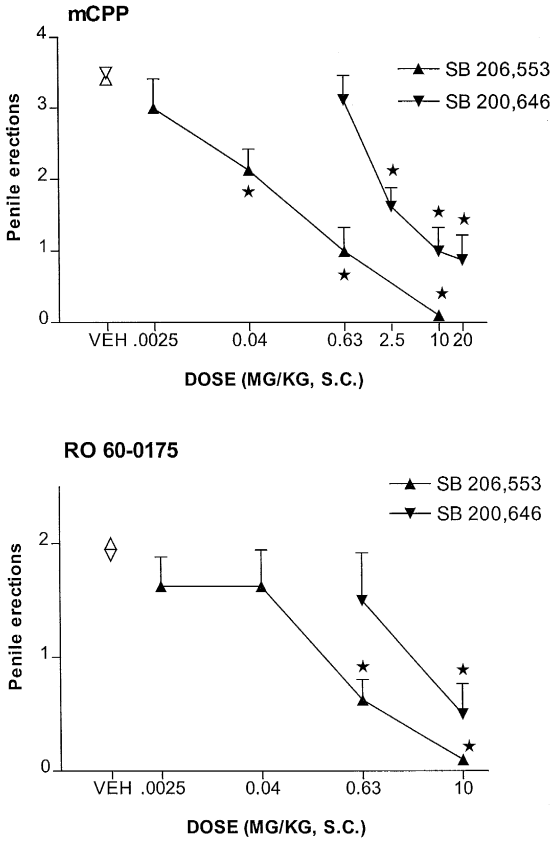


Fig. 2. Inhibition of mCPP- and RO 60-0175-induced penile erections by the 5-$HT_{2C}$ antagonists, SB 200,646 and SB 206,553. The dose of mCPP and RO 60-0175 was 2.5 mg/kg, s.c. in each case. Means ± S.E.M. shown. $n = 4$–12 per group. ANOVA as follows: for mCPP, SB 200,646, $F(4,37) = 16.6$, $P < 0.001$ and SB 206,553, $F(4,35) = 26.5$, $P < 0.001$ and for RO 60-0175, SB 200,646, $F(2,21) = 5.4$, $P < 0.05$ and SB 206,553, $F(4,31) = 6.3$, $P < 0.001$. Asterisks indicate significance of differences to vehicle values in Dunnett's test ($P < 0.05$).

antagonists did not induce penile erections alone (not shown).

## 4. Discussion

The selective 5-$HT_{2C}$ receptor agonist, RO 60-0175 (Martin et al., 1995), mimicked mCPP in dose-dependently eliciting penile erections. RO 60-0175 shows (> 100-fold) lower affinity at 5-$HT_{2B}$ and 5-$HT_{2A}$ vs. 5-$HT_{2C}$ receptors: $pK_i$ values = 6.0 and 5.8 vs. 8.8, respectively (Martin et al., 1995; Congress of the European College of Neuropsychopharmacology; Newman-Tancredi, A. and Millan, M.J., unpublished observations). Thus, it is likely that the common actions of RO 60-0175 and mCPP at 5-$HT_{2C}$ receptors underlie this penile erection response (Martin et al., 1995). The selective 5-$HT_{2A}$ receptor antagonist, MDL 100,907, at a dose known to abolish actions mediated by 5-$HT_{2A}$ receptors (Schreiber et al., 1995), did not modify the actions of mCPP or RO 60-0175 suggesting that 5-$HT_{2A}$ receptors are not involved in the induction of penile erections, consistent with the low affinity of RO 60-0175 at 5-$HT_{2A}$ sites. In distinction, SB 200,646 and SB 206,553, which behave as potent antagonists at 5-$HT_{2C}$ vs. 5-$HT_{2A}$ receptors (Kennett et al., 1996b; Wood et al., 1995) blocked the actions of both mCPP and RO 60-0175. Over the dose range required for inhibition of penile erections, SB 200,646 and SB 206,553 do not significantly modify motor behaviour in the rotarod test, indicating that non-specific, motor actions are not involved in this antagonism (unpub. obs.). Further, the potency ratios of SB 200,646 and SB 206,553 in blocking penile erections correspond to their relative affinities at 5-$HT_{2C}$ receptors (Kennett et al., 1996b; Wood et al., 1995). Since RO 60-0175 possesses only low affinity at 5-$HT_{2B}$ receptors, at which it behaves as a weak agonist (Martin et al., 1995), the antagonist actions of SB 200,646 and SB 206,553 at 5-$HT_{2B}$ receptors are unlikely to underlie their blockade of penile erections. In support of the contention that 5-$HT_{2B}$ receptors are not involved in the induction of penile erections, the novel and selective antagonist at 5-$HT_{2B}$ receptors, SB 204,741 (Forbes et al., 1996) was ineffective in modifying the action of mCPP or RO 60-0175. Further, the novel agonist at 5-$HT_{2B}$ receptors, BW 723C86 (Kennett et al., 1996a), did not evoke penile erections.

Dose–response curves for both mCPP and RO 60-0175 were biphasic. Inasmuch as agonists at 5-$HT_{1A}$ or 5-$HT_{2A}$ receptors inhibit induction of penile erections, the weak affinity of RO 60-0175 at 5-$HT_{1A}$ ($pK_i$ = 5.6, unpub. obs.) or 5-$HT_{2C}$ receptors ($pK_i$ = 5.8, Martin et al., 1995) might interfere with the 5-$HT_{2C}$ agonist actions of RO 60-0175 at high doses. On the other hand, the onset of motor-suppressive actions of RO 60-0175 at higher doses might also be involved (Kennett and Curzon, 1988).

In conclusion, employing several, novel and selective ligands, the present data provide compelling evidence that 5-$HT_{2C}$ receptors mediate penile erections in the rat. The response of penile erections should, thus, provide a useful model for the discovery and characterization of novel ligands at 5-$HT_{2C}$ receptors. To our knowledge, this is the first study to simultaneously employ these novel, selective ligands for the identification of actions mediated by 5-$HT_{2C}$ receptors. These drugs should facilitate the further exploration of the physiological significance of 5-$HT_{2C}$ receptors and permit an improved understanding of their implication in psychiatric disease and other disorders.

## References


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

Berendsen, H.G., Jenck, F., Broekkamp, C.L.E., 1990. Involvement of 5-$HT_{1C}$ receptors in drug-induced penile erections in rats. Psychopharmacology 101, 57–61.

Bloom, F.E., Kupfer, D.J. (Eds.), 1995. Psychopharmacology: The Fourth Generation of Progress. Raven Press, New York, 2002 pp.

Boess, F.G., Martin, I.L., 1994. Molecular biology of 5-HT receptors. Neuropharmacology 33, 275–317.

Bonhaus, D.W., Bach, C., De Souza, A., Salazar, F.H.R., Matsuoka, B.D., Zuppan, P., Chan, H.W., Eglen, R.M., 1995. The pharmacology and distribution of human 5-hydroxytryptamine$_{2B}$ (5-$HT_{2B}$) receptor gene products: comparison with 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors. Br. J. Pharmacol. 115, 622–628.

Forbes, I.T., Jones, G.E., Murphy, O.E., Holland, V., Baxter, G.S., 1996. *N*-(1-Methyl-5-indolyl)-*N*′-3-methyl-5-isothiazolyl)urea: a novel, high-affinity 5-$HT_{2B}$ receptor antagonist. J. Med. Chem. 38, 855–857.

Jenck, F., Moreau, J.-L., Mutel, V., Martin, J.R., Haefely, W.E., 1993. Evidence for a role of 5-$HT_{1C}$ receptors in the antiserotonergic properties of some antidepressant drugs. Eur. J. Pharmacol. 231, 223–226.

Kennett, G.A., Curzon, G., 1988. Evidence that mCPP may have behavioural effects mediated by central 5-$HT_{1C}$ receptors. Br. J. Pharmacol. 94, 137–147.

Kennett, G.A., Bright, F., Trail, B., Baxter, G.S., Blackburn, T.P., 1996a. Effects of the 5-$HT_{2B}$ receptor agonist, BW 723C86, on three rat models of anxiety. Br. J. Pharmacol. 117, 1443–1448.

Kennett, G.A., Wood, M.D., Bright, F., Cilia, J., Piper, D.C., Gager, T., Thomas, D., Baxter, G.S., Forbes, I.T., Ham, P., Blackburn, T.P., 1996b. In vitro and in vivo profile of SB 206,553, a potent 5-$HT_{2C}$ /5-$HT_{2B}$ receptor antagonist with anxiolytic-like properties. Br. J. Pharmacol. 117, 427–434.

Lucki, U., Ward, H.R., Frazer, A., 1991. Effect of 1-(*m*-chlorophenyl)piperazine and 1-(*m*-trifluoromethylphenyl) piperazine on locomotor activity. J. Pharmacol. Exp. Ther. 249, 155–164.

Martin, J.R., Bös, M., Jenck, F., Köhler, C., Moreau, J.-L., Sleight, A.J., Stadler, H., Wichmann, J., 1995. 5-$HT_{2C}$ receptor agonists and antagonists in animal models of anxiety. Eur. Neuropsychopharmacol. Abstr. 5, 209.

Molineaux, S.M., Jesseli, T.M., Axel, R., Julius, D., 1989. 5-$HT_{1C}$ receptor is a prominent serotonin receptor subtype in the central nervous system. Proc. Natl. Acad. Sci. USA 86, 6793–6797.

Schreiber, R., Brocco, M., Audinot, V., Gobert, A., Veiga, S., Millan, M.J., 1995. [1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane]-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT)$_{2A}$ receptors: modulation by novel 5-$HT_{2A/2C}$ antagonists, $D_1$ antagonists and 5-$HT_{1A}$ agonists. J. Pharmacol. Exp. Ther. 273, 101–112.

Sörensen, S.M., Kehne, J.H., Fadayel, G.M., Humphreys, T.M., Ketteler, H.J., Sullivan, C.K., Taylor, V.L., Schmidt, C.J., 1993. Characterization of the 5-$HT_2$ receptor antagonist MDL 100,907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. J. Pharmacol. Exp. Ther. 266, 684–691.

Tecott, L.H., Sun, L.M., Akana, S.F., Strack, A.M., Lowenstein, D.H., Dallman, M.F., Jullius, D., 1995. Eating disorder and epilepsy in mice lacking 5-$HT_{2C}$ serotonin receptors. Nature 374, 542–546.

Wood, M.D., Gager, T.L., Thomas, D.R., Newton, R.A., Phipps, S.L., Elliott, J.M., 1995. The 5-$HT_{2C/2B}$ receptor antagonist, SB 200,646A, is a potent and selective antagonist of the human 5-$HT_{2C}$ receptor. Br. J. Pharmacol. 114, 155.