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Short communication

Interaction between 5-HT_{1A} and 5-HT_{1B} receptors: effects of 8-OH-DPAT-induced hypothermia in 5-HT_{1B} receptor knockout mice

Alain M. Gardier [a,b,*], Bérangère Gruwez [a], Anne-Cécile Trillat [a], Christian Jacquot [a], René Hen [c], Michel Bourin [d]

[a] *Laboratoire de Neuropharmacologie UPRES EAD MENRT, Faculté de Pharmacie IFR-ISIT Institut de Signalisation et Innovation Thérapeutique, Université Paris-Sud, F92296 Châtenay-Malabry Cedex, France*

[b] *Laboratoire de Pharmacologie, Faculté de Pharmacie, Université de Picardie, F80037 Amiens, France*

[c] *Center for Neurobiology and Behavior, Columbia University, New York, NY 10032, USA*

[d] *Laboratoire de Pharmacologie, Faculté de Médecine, Université de Nantes, F44035 Nantes Cedex 1, France*

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Abstract

To test for adaptive compensatory changes that may have occurred in the functional activity of somatodendritic 5-HT_{1A} receptors during the development of constitutive "knockout" mice lacking the 5-HT_{1B} receptor subtype (5-HT_{1B} −/− KO), we assayed for decrease in body temperature induced by an acute subcutaneous injection of the 5-HT_{1A} receptor agonist, 8-hydroxy 2(di-*n*-propyl(amino)tetralin (8-OH-DPAT), either alone or in the presence of a selective 5-HT_{1A} receptor antagonist, *N*-[4-(2-methoxyphenyl)-1-piperazinyl]-*N*-(2-pyridinyl) cyclo-hexanecarboxamide (WAY 100635). We compared dose–response curves, time course study, calculated ED_{50} values (potency), maximal response to 8-OH-DPAT (efficacy) as well as measurements of the dose-dependent blockade of this response by WAY 100635 between wild-type controls and mutant mice. We found a higher efficacy of 8-OH-DPAT-induced hypothermia in 5-HT_{1B} −/− KO compared to wild-type mice suggesting that an adaptive thermoregulatory process involving the functional activity of somatodendritic 5-HT_{1A} receptors is altered in mutant mice lacking 5-HT_{1B} receptors.

Keywords: 5-HT_{1B} receptor; Knockout mice; 5-HT_{1A} receptor; Hypothermia

1. Introduction

Knockout (5-HT_{1B} −/− KO) mice lacking the serotonin 5-hydroxytryptamine (5-HT_{1B}) receptor subtype have been generated by homologous recombination (Saudou et al., 1994). Neurochemical studies comparing mutants to wild-type (WT) littermates have confirmed the inhibitory role exerted by 5-HT_{1B} autoreceptors on 5-HT release at brain serotonergic nerve terminals (Piñeyro et al., 1995, Trillat et al., 1997; Malagié et al., 2001). The mutants develop and mature normally. However, since the gene is missing throughout the development of these mutants, adaptive compensatory changes may occur in the expression and function of other receptors of neurotransmitters. For example, it has recently been shown that 5-HT_{1B} −/− KO mice have heightened dopaminergic activity in the brain which may underlie their increased vulnerability to drugs of abuse (Scearce et al., 1998).

Another serotonergic receptor subtype, the 5-HT_{1A} receptor could also be involved since, when located presynaptically, it exerts a role similar to that of the terminal knocked out 5-HT_{1B} receptor, i.e., an inhibitory feedback control on the release of 5-HT. Binding studies have failed to reveal such compensatory changes regarding the density of 5-HT_{1A} receptors (Saudou et al., 1994). However, evaluation of the functional activity of somatodendritic 5-HT_{1A} receptors may be more appropriate to check for putative compensatory modifications in these mutants. These autoreceptors are located on cell bodies and dendrites of serotonergic neurons in the midbrain raphe nuclei (Vergé et al., 1985). It is widely accepted that in the mouse, activation of these somatodendritic autoreceptors mediates the thermoregulatory response to 5-HT_{1A} receptor agonists,

* Corresponding author. Laboratoire de Neuropharmacologie UPRES EAD MENRT, Faculté de Pharmacie Tour D1 2ème étage, 5 rue J-B. Clément, Université Paris-Sud, F92296 Châtenay-Malabry Cedex, France. Tel.: +33-1-4683-5416; fax: +33-1-4683-5355.

E-mail address: alain.gardier@cep.u-psud.fr (A.M. Gardier).

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8-hydroxy-2(di-*n*-propyl(amino)tetralin (8-OH-DPAT) ipsapirone, flesinoxan (Goodwin et al., 1985; Martin et al., 1992; Forster et al., 1995; Olivier et al., 1998). Indeed, lesioning central serotonergic neurons either by the intracerebroventricular infusion of the neurotoxin 5,7-dihydroxytryptamine or by *p*-chloro-phenylalanine-induced 5-HT depletion abolished the hypothermic response to 8-OH-DPAT (Goodwin et al., 1985; Martin et al., 1992).

In the present study, we investigated whether or not the changes in thermoregulatory responses mediated by the functional activity of somatodendritic 5-HT_{1A} receptors could have compensated for the absence of terminal 5-HT_{1B} autoreceptors in adult constitutive 5-HT_{1B} $-/-$ KO mice.

2. Materials and methods

2.1. Animals

The founders of the wild-type and mutant colonies used in the present study were the product of heterozygous mattings made at the animal facility of Columbia University. These founders were shipped to France and their offsprings were bred and reared in independent colonies. Wild-type and 5-HT_{1B} receptor knockout mice are male and female mice, 9–11 weeks old, and obtained on a pure 129/Sv genetic background. Group-housed mice were kept in standard cages on a 12:12-h light–dark cycle with light onset at 7 a.m. with a free access to food and water. Mice were housed in groups of eight at an ambient temperature of 22 ± 1°C for at least 2 h before measurement of body temperature and drug administration.

2.2. Drugs

The following drugs and chemicals were used in this study: 8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide, the racemic mixture ($\pm$8-OH-DPAT) (Sigma–Aldrich–RBI, L'Isle D'Abeau Chesnes, France); {*N*-[4-(2-methoxyphenyl)-1-piperazinyl]-*N*-(2-pyridinyl) cyclo-hexanecarboxamide, 3HCl} WAY 100635 from Wyeth Research (Maidenhead, UK). WAY 100635 (0.01 and 0.1 mg/kg) or vehicle was administered subcutaneously (s.c.) 30 min before the s.c. injection of standard challenge dose of 8-OH-DPAT (0.05, 0.1, 0.2, 0.4 or 0.6 mg/kg) as previously described (Trillat et al., 1998).

2.3. 8-OH-DPAT-induced hypothermia in mice

Body temperature was measured in gently restrained mice by inserting a thermistor probe (Electrotherm M99, Paris, France), lubricated with petroleum jelly before each use, for a length of 2 cm into the rectum of mice. Digital recordings of the temperature were obtained with an accuracy of $\pm$0.1°C as indicated in technical specifications of the instrument. The measurement was performed from 13:00 to 17:00 h. The results are expressed as means $\pm$ S.E.M.

To set up the receptor agonist–antagonist study, vehicle or WAY 100635 was administered first (*at* t_0), then 8-OH-DPAT 30 min later (*at* t_{30}). Temperatures were then measured immediately before each drug administration and at 15, 30, 45 and 60 min after 8-OH-DPAT injection. The hypothermic response to 8-OH-DPAT was measured as the maximal decrease in body temperature recorded in this latter period as previously described (Bill et al., 1991; Trillat et al., 1998).

2.4. Statistical analysis

All the statistical analyses were performed by using the computer software Statview 4.02 (Abacus concepts, Berkeley, CA, USA). The effects of drugs on rectal temperature were calculated to yield a theoretical maximum effect (efficacy) as described by Tallarida and Murray (1987). The ED_{50} values (doses of 8-OH-DPAT that elicits a half-maximal reduction of basal body temperature) and 95% confidence intervals were determined by calculating the functional response for each 8-OH-DPAT dose (based on the maximum effect being 100%), converting the data to log values by using the computer software Prism 2.0 (GraphPad Software, San Diego, USA). Tests for parallelism and potency ratios were calculated according to the method of Tallarida and Murray (1987).

The dose response of 8-OH-DPAT was also analyzed by a two-way analysis of variance (ANOVA) with the drug treatment (vehicle or 8-OH-DPAT) and the mice's genotype (wild-type or knockout) as main factors. The effects of WAY 100635 on the 8-OH-DPAT-induced hypothermia were analyzed by using a three-way ANOVA with 5-HT_{1A} receptor antagonist (pre-treatment: vehicle or WAY 100635), 5-HT_{1A} receptor agonist (treatment: vehicle or 8-OH-DPAT) and the mice's genotype (wild-type or knockout) as main factors, followed by a Fisher's "Protected Least Significant Difference" (PLSD) post-hoc test. Statistical significance was set at $P < 0.05$.

3. Results

Comparison of the effects of various doses of selective 5-HT_{1A} receptor agonist 8-OH-DPAT on body temperature between wild-type and 5-HT_{1B} receptor knockout mice indicates that, in both genotypes, 8-OH-DPAT (0.05–0.6 mg/kg s.c.) produced a significant dose-dependent decrease in body temperature in the range of 8-OH-DPAT doses studied (Fig. 1A). Statistical analysis of the hypothermic response to 8-OH-DPAT by a two-way ANOVA revealed significant main effects of drug treatment ($F(5, 112) = 46.07$, $P < 0.001$) and genotype factor ($F(1,112) = 6.61$, $P < 0.05$), and a significant interaction ($F(5,112) = 2.21$, $P < 0.05$). In each strain, post-hoc comparisons

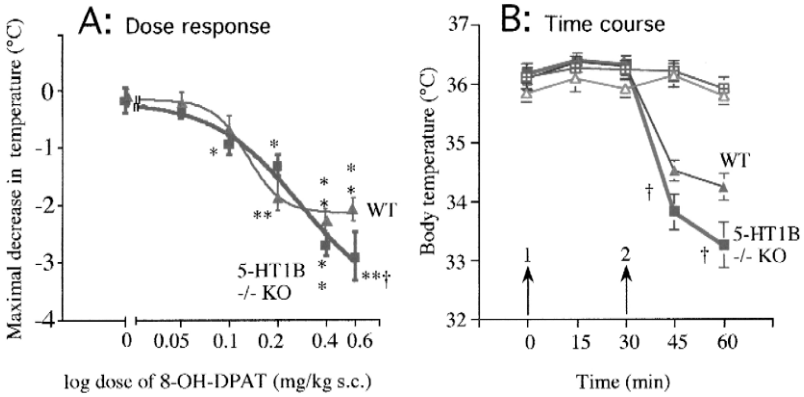

Fig. 1. Comparison of the maximal changes in body temperature induced by various doses of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT in wild-type (WT; ▲) and 5-HT_{1B} receptor knockout (5-HT_{1B} −/− KO; ■) mice. (**A**) Dose–response curves of 8-OH-DPAT induced hypothermia (0, 0.05, 0.1, 0.2, 0.4 and 0.6 mg/kg, s.c.). Results are expressed at the maximal decrease in rectal temperature, i.e., 30 min after 8-OH-DPAT injection. (**B**) Time course of the hypothermic response to vehicle (WT: open triangle; KO: open square) or 8-OH-DPAT (0.6 mg/kg, s.c.; WT: filled triangle; KO: filled square). Values are means ± S.E.M. of body temperature for 10–12 mice per group. Vehicle was administered at t_0 (*first arrow*) and 8-OH-DPAT 30 min later (*second arrow*). †$P < 0.05$ for comparison between 5-HT_{1B} −/− KO and WT mice at either 15 or 30 min post-8-OH-DPAT injection (two-way ANOVA followed by post-hoc Fisher's PLSD t test). $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to vehicle-treated mice.

indicated that, in wild-types, there is no significant change in body temperature at 0.05 and 0.1 mg/kg 8-OH-DPAT compared to vehicle, while decreases is observed at 0.2 ($^{*}P < 0.05$), 0.4 ($^{**}P < 0.01$) and 0.6 ($^{**}P < 0.01$) mg/kg 8-OH-DPAT. In mutants, there is no significant change in body temperature at 0.05 mg/kg 8-OH-DPAT compared to vehicle, while decreases is observed at 0.1 ($^{*}P < 0.05$), 0.2 ($^{**}P < 0.01$), 0.4 ($^{**}P < 0.01$) and 0.6 ($^{**}P < 0.01$) mg/kg 8-OH-DPAT. The ED_{50} values and 95% confidence intervals are 0.13 (0.07–0.23) and 0.28 (0.06–0.42) (mg/kg, s.c.) when calculated 30 min after drug administration in WT and 5-HT_{1B} −/− KO mice, respectively.

Furthermore, the time course of the hypothermic response to 8-OH-DPAT (0.6 mg/kg, s.c.) indicates that 8-OH-DPAT induced a rapid decrease in body temperature, the largest decrease in body temperature being measured at 30 min after 8-OH-DPAT injection in both WT and mutant mice (Fig. 1B). These hypothermic responses to 8-OH-DPAT were maximal at a dose as low as 0.2 mg/kg in WT mice and at 0.6 mg/kg in 5-HT_{1B} −/− KO mice (Fig. 1A and B: −2.1°C, and −3.1°C, respectively; $P < 0.05$). Time-dependent study of body-temperature in vehicle-treated wild-type and knockout mice (Fig. 1B) shows that (i) basal temperature did not differ between the two strains (conversely to a recent report (Bouwknecht et al., 2001), and (ii) the stress due either to the rectal insertion of the thermistor probe or to the subcutaneous injections did not cause changes in basal body temperature.

Statistical analysis of the hypothermic response to 8-OH-DPAT in the presence of WAY 100635 by a three-way ANOVA revealed significant main effects of pre-treatment ($F(1,220) = 35.9$, $P < 0.001$), treatment ($F(5,220) = 60.9$, $P < 0.001$) and genotype factor ($F(1,220) = 9.17$, $P < 0.01$). Thus, pretreatment with the selective 5-HT_{1A} receptor antagonist, WAY 100635 (0.01 and 0.1 mg/kg) 30 min before 8-OH-DPAT markedly and dose-dependently antagonized the 8-OH-DPAT-induced hypothermia in the two strains of mice studied (Fig. 2). WAY 100635, at the

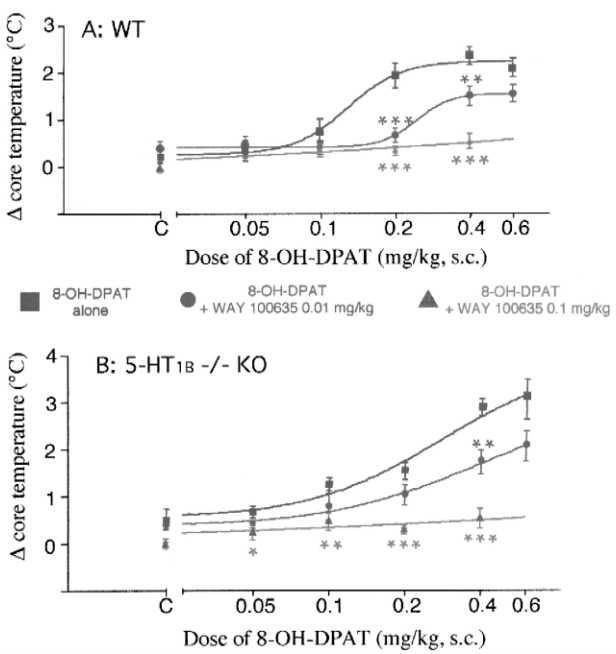

Fig. 2. Effects of the selective 5-HT_{1A} receptor antagonist WAY 100635 on 8-OH-DPAT-induced hypothermia in wild-type and 5-HT_{1B} receptor knockout mice. WAY 100635 (0.01 or 0.1 mg/kg) was administered subcutaneously 30 min before 8-OH-DPAT (0.05, 0.1, 0.2, 0.4 or 0.6 mg/kg). Bars represent means ± S.E.M. of body temperature for 10–12 mice per group. Body temperature was recorded immediately before each drug administration as well as 15 and 30 min following 8-OH-DPAT injection. Mice were treated either with 8-OH-DPAT (square) or [WAY 100635 0.01 mg/kg + 8-OH-DPAT] (circle) or [WAY 100635 0.1 mg/kg + 8-OH-DPAT] (triangle). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ when compared to vehicle-treated controls (one-way ANOVA followed by Fisher's PLSD *t*-test).

highest dose administered (0.1 mg/kg), in 5-HT_{1B} −/− KO mice, but not in wild-type controls, appears to block statistically the response to doses of 8-OH-DPAT as low as 0.05 and 0.1 mg/kg ($P < 0.05$ and $P < 0.01$, respectively). This blockade induced by WAY 100635 had a different profile in WT mice. Indeed, WAY 100635 (0.1 mg/kg) did not block the effects of low doses of 8-OH-DPAT, but blocked those measured when 8-OH-DPAT was administered at a higher dose (0.2 mg/kg; $P < 0.001$).

4. Discussion

The functional activity of somatodendritic 5-HT_{1A} receptors is modified in 5-HT_{1B} −/− KO mice when compared to wild-type littermates. Although basal body temperature is unchanged in mutants, when they are challenged with 8-OH-DPAT, an alteration of thermoregulation becomes apparent. The estimated ED_{50} values for 8-OH-DPAT-induced hypothermia are different (0.13 and 0.28 mg/kg, in WT and mutant mice, respectively), but confidence intervals of these values overlapped. Thus, the potency of 8-OH-DPAT in inducing hypothermia is similar in both strains. However, the maximum of the hypothermic response to 8-OH-DPAT occurred at a 0.2 mg/kg dose in WT mice (−2.1°C) and at 0.6 mg/kg in mutants (−3.1°C). These results suggest that 8-OH-DPAT has a *similar potency* in both mice's strains, but is *more efficacious* in 5-HT_{1B} −/− KO than in WT in inducing hypothermia.

Furthermore, pretreatment with the selective 5-HT_{1A} receptor antagonist, WAY 100635, antagonized dose-dependently the 8-OH-DPAT-induced hypothermia in the two strains in agreement with previous report (Forster et al., 1995; Trillat et al., 1998). In mutants, WAY 100635, at the highest dose tested (0.1 mg/kg), blocked the response to doses of 8-OH-DPAT at doses as low as 0.05 and 0.1 mg/kg. In WT mice, WAY 100635 (0.1 mg/kg) did not block the effects of low doses of 8-OH-DPAT, but blocked those of an intermediate dose (0.2 mg/kg). As for the dose-response study, these data indicate that WAY 100635 was *more efficacious* in antagonizing 8-OH-DPAT-induced hypothermia in mutants than in WT mice.

Thus, in the absence of terminal 5-HT_{1B} autoreceptors in adult constitutive 5-HT_{1B} −/− KO mice, the functional activity of the somatodendritic 5-HT_{1A} receptor subtype is altered. These changes could result from the absence of 5-HT_{1B} receptor throughout the development of KO mice and will influence the phenotype of the adults. These mutants are known to be more reactive, less anxious than WT controls, and display increased vulnerability to stressful situations such as the isolation, the stress generated by the intruder, and novel environments (Saudou et al., 1994; Ramboz et al., 1996; Zhuang et al., 1999). Our assertion regarding the present data must be confirmed by studying 8-OH-DPAT-induced hypothermia following its injection into the dorsal raphe nucleus (Higgins et al., 1988) or intracerebroventricularly (icv) in mice (Goodwin et al., 1985).

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