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8-OH-DPAT acts on both 5-HT_{1A} and 5-HT_7 receptors to induce hypothermia in rodents

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Abstract

Studies using selective drugs and knockout mice have demonstrated that the 5-HT_7 receptor plays an instrumental role in serotonin-induced hypothermia. There is also evidence supporting an involvement of the 5-HT_{1A} receptor, although mainly from studies using 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT), a 5-$HT_{1A/7}$ receptor agonist. Here we studied the effects of 8-OH-DPAT and selective antagonists for the 5-HT_{1A} and 5-HT_7 receptors on body temperature in rats, wild-type (5-$HT_7^{+/+}$) mice and knockout (5-$HT_7^{-/-}$) mice. At lower doses (0.3–0.6 mg/kg, i.p.), 8-OH-DPAT decreased body temperature in 5-$HT_7^{+/+}$ mice but not in 5-$HT_7^{-/-}$ mice. At a higher dose (1 mg/kg, i.p.) 8-OH-DPAT induced hypothermia in both 5-$HT_7^{-/-}$ and 5-$HT_7^{+/+}$ mice. The 5-HT_{1A} receptor antagonist (*S*)-*N*-*tert*-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide (WAY-100135) (10 mg/kg, i.p.) inhibited the effect of 8-OH-DPAT at all doses in rats and mice. In 5-$HT_7^{+/+}$ mice the selective 5-HT_7 receptor antagonist (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulfonyl)phenol (SB-269970) (10 mg/kg, i.p.) fully inhibited the hypothermia induced by 0.3 mg/kg 8-OH-DPAT, but not that of higher doses. In rats, SB-269970 caused a 60% inhibition of the hypothermia induced by 0.3 mg/kg 8-OH-DPAT. Thus, both 5-HT_7 and 5-HT_{1A} receptors are involved in a complex manner in thermoregulation, with the 5-HT_7 receptor being more important at lower, possibly more physiological, concentrations.

Keywords: Thermoregulation; Hypothermia; Serotonin; In vivo; Knockout, mouse

1. Introduction

The recent availability of selective antagonists (Hagan et al., 2000) and knockout mice (Guscott et al., 2003; Hedlund et al., 2003) has considerably advanced our understanding of the 5-HT_7 receptor. However, being one of the most recently described members of the large family of serotonin (5-HT) receptors (Bard et al., 1993; Lovenberg et al., 1993), our knowledge of its function is still limited. All species from which the 5-HT_7 receptor has been isolated show a consistent mRNA distribution pattern within the brain (Gustafson et al., 1996; Lovenberg et al., 1993; Mengod et al., 1996). Ligand binding parameters and distribution is also consistent across species (Bonaventure et al., 2002; Plassat et al., 1993; Ruat et al., 1993; Shen et al., 1993; Tsou et al., 1994). The 5-HT_7 receptor is mainly localized to the thalamus and hypothalamus.

Functionally, the 5-HT_7 receptor has been shown to stimulate cyclic AMP formation (Bard et al., 1993; Lovenberg et al., 1993; Plassat et al., 1993; Ruat et al., 1993). This stimulation has been linked to the activation of a calmodulin-regulated adenylyl cyclase (Baker et al., 1998). The existence of several splice variants of the 5-HT_7 receptor mRNA has been clearly demonstrated (Heidmann et al., 1997; Jasper et al., 1997; Stam et al., 1997). The variants encode receptors that have slightly different lengths of the C-termini, but with no detectable differences in tissue distribution or functional coupling (Krobert and Levy, 2002). The 5-HT_7 receptor has also been detected in the periphery where it is found primarily in smooth muscle cells of blood vessels (Bard et al., 1993; Schoeffter et al., 1996), but also in the gastrointestinal tract (Bard et al., 1993) where it is involved in regulating peristalsis (Tuladhar et al., 2003).

The 5-HT_7 receptor has been linked to a number of physiological and pathophysiological phenomena. An early

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hypothesis suggested that the 5-HT_7 receptor mediates the 5-HT-induced phase resetting of the circadian clock within the suprachiasmatic nucleus of the hypothalamus (Lovenberg et al., 1993). The ability of 5-HT_7 receptors to mediate smooth muscle relaxation (Eglen et al., 1997; Schoeffter et al., 1996) has led to the suggestion that ligands may have therapeutic value in migraine (Terron and Falcon-Neri, 1999). The 5-HT_7 receptor has also been implicated in endocrine regulation and neuropsychiatric disorders (Hoyer et al., 2002; Vanhoenacker et al., 2000). Especially intriguing is the possible involvement in disregulated circadian rhythms and depression, since antidepressants induce c-*fos* expression and downregulate 5-HT_7 receptor binding in the suprachiasmatic nucleus (Mullins et al., 1999).

It is well established that 5-HT plays a role in thermoregulation and that systemic administration of 5-HT leads to hypothermia in rats, mice, guinea pigs and rabbits (Sugimoto et al., 1991; Won and Lin, 1988; Yamada et al., 1988). Several studies have attempted to establish which 5-HT receptors mediate this effect. Early evidence suggested involvement of a 5-HT_1 receptor subtype (Yamada et al., 1988). Peripheral and central 5-HT_2 receptors have also been suggested to be involved (Sugimoto et al., 1991), a notion that was disputed in a later study demonstrating that the 5-HT_{2A} receptor most likely is not involved (Morishima and Shibano, 1995). At the time it was instead speculated that 5-HT_{1A} and 5-HT_{2A} receptors had opposite effects, at least in the rat. The evidence supporting a role for the 5-HT_{1A} receptor in 5-HT-induced hypothermia was largely based on data from using 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT) as a ligand (Hjorth, 1985). Since 8-OH-DPAT has affinity also for the 5-HT_7 receptor, it could be hypothesized that this receptor is involved in 5-HT-mediated thermoregulation. The first evidence supporting a role for the 5-HT_7 receptor in thermoregulation was presented in a study using the selective 5-HT_7 receptor antagonist, (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulfonyl)phenol (SB-269970) (Hagan et al., 2000). In that report it was demonstrated that hypothermia induced by 5-carboxamidotryptamine (5-CT) could be antagonized by the selective antagonist, but not by antagonists selective for 5-$HT_{1A/1B}$ and 5-$HT_{1B/1D}$ receptors. Furthermore, the unselective antagonist metergoline, which has high affinity for the 5-HT_7 receptor, was able to block the hypothermia induced by 5-CT. Two subsequent reports using two different 5-HT_7 receptor knockout mouse strains have since confirmed the hypothesis that the 5-HT_7 receptor is important for serotonin-mediated hypothermia (Guscott et al., 2003; Hedlund et al., 2003). The first study showed that both 5-HT itself and 5-CT were unable to induce hypothermia in 5-$HT_7^{-/-}$ mice (Hedlund et al., 2003). The second study demonstrated that 5-CT-induced hypothermia can be reversed by 5-HT_7 receptor antagonists, but not by 5-HT_{1A} receptor antagonists in 5-$HT_7^{+/+}$ mice, and that 5-CT fails to induce hypothermia in 5-$HT_7^{-/-}$ mice (Guscott et al., 2003). However, studies using knockout mice have also provided additional evidence that the 5-HT_{1A} receptor is involved in thermoregulation, since it has been shown that 8-OH-DPAT fails to induce hypothermia in 5-$HT_{1A}^{-/-}$ mice (Heisler et al., 1998). In addition to its action on 5-HT receptors, there is evidence suggesting that 8-OH-DPAT may also act on α_2 adrenoceptors since 8-OH-DPAT has been shown to mediate anti-nociception via α_2 receptors in vivo (Millan and Colpaert, 1991).

In order to clarify the respective involvement of the 5-HT_{1A} and the 5-HT_7 receptor in thermoregulation, we have studied the effects of 8-OH-DPAT and antagonists selective for the two receptors in both rats and mice, including 5-$HT_7^{-/-}$ mice.

2. Materials and methods

2.1. Animals

All the studies have been carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health.

2.1.1. Rat study

The telemetry experiments described below were performed in female Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 300–325 g. The animals were housed in a controlled environment with a 12-h light–dark cycle. They had free access to water and food pellets. A colony of 40 animals were used for the present study.

2.1.2. 5-$HT_7^{-/-}$ mice

Additional experiments measuring body temperature rectally were performed in 8- to 10-week-old male 5-$HT_7^{-/-}$ mice and their 5-$HT_7^{+/+}$ siblings. The generation of 5-$HT_7^{-/-}$ mice has been described previously (Hedlund et al., 2003). Briefly, a construct was designed with an insertion at an MluI site within exon II of the 5-HT_7 receptor gene, at a location encoding the fifth transmembrane domain of the receptor protein. This interruption should inactivate all splice forms of the receptor. The construct was transfected into 129Sv/Ev embryonic stem cells, and breeding was then done on a C57BL/6J background. Successive mating of heterozygous progeny to the inbred C57BL/6J strain (backcrossing) had been performed for at least 10 generations before 5-$HT_7^{+/+}$ and 5-$HT_7^{-/-}$ homozygous offspring were used in the present study. The mice were housed in a controlled environment and had free access to water and food pellets. A total of 48 mice were used for the present study.

2.2. Telemetry

Sterile telemetric devices (TA10TA-F40, DataSciences International, St. Paul, MN) were implanted into the peritoneal cavity of isoflurane-anesthetized rats. Following surgery, all rats were single housed. After a 7-day recovery

period, core body temperature was measured noninvasively by radiotelemetry. On the day of experimentation, the baseline temperature was monitored for 60 min prior to drug injection. All experiments were started between 0900 and 1000 h. Rat core body temperatures were continuously recorded, before and after injection, and averaged each 2 min for a 7-h period.

2.3. Rectal temperature

For the experiments in 5-$HT_7^{+/+}$ and 5-$HT_7^{-/-}$ mice core body temperature was measured using a rectal probe thermometer (YSI 4000A Thermometer, YSI, Yellow Springs, OH). A basal value was measured immediately before any injection and measurements were then made every 30 min for 2 h. All experiments were started at 0900 h.

2.4. Drug treatments

2.4.1. Rats

The 5-HT_{1A} receptor antagonists (*S*)-*N*-*tert*-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide (WAY-100135) (Cliffe et al., 1993)) and pindolol (Hoyer et al., 2002), the 5-HT_7 receptor antagonists SB-269970 and 2*a*-(4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl)-2*a*,3,4,5-tetrahydrobenzo[cd]indol-2(1H)-one (DR-4004) (Kikuchi et al., 1999), or the α_2 receptor antagonist atipamezole (Newman-Tancredi et al., 1998), or vehicle were given at different doses 20 min prior to 0.3 mg/kg 8-OH-DPAT. All drugs except pindolol were prepared in 5% dextrose. Pindolol was prepared in 0.2% HCl in 2 × phosphate-buffered saline. All drugs were given as single intra-peritoneal injections in a volume of 1 ml/kg. The dose 0.3 mg/kg 8-OH-DPAT was chosen for these studies because it has been found to consistently cause a significant decrease in core body temperature as compared to vehicle treated rats.

2.4.2. Mice

The mice were injected with 8-OH-DPAT intra-peritoneally in the doses indicated in a total volume of 0.25 ml. In certain cases either WAY-10035 (10 mg/kg) or SB-269970 (10 mg/kg) was given as a single intra-peritoneal injection in a volume of 0.25 ml 30 min prior to the 8-OH-DPAT injection. All drugs were dissolved in 0.9% saline. Control experiments were performed with 0.9% saline, WAY-100135 (10 mg/kg), or SB-269970 (10 mg/kg) alone.

2.5. Drugs

(*R*)-(+)-8-OH-DPAT (HBr salt) and SB-269970 (HCl salt) were purchased from Sigma (Saint Louis, MO). (*S*)-(−)-Pindolol (free base) and (*S*)-WAY-100135 (2HCl salt) were obtained from Tocris (Ellisville, MO). DR-4004 and atipamezole were synthesized and prepared in their free base form at Johnson & Johnson Pharmaceutical Research and Development. All doses are expressed as free base.

2.6. Data analysis and statistical analysis

For both studies (rat and mouse) analysis of variance (either one-way or two-way analysis of variance (ANOVA) as appropriate) was used to analyze the body temperature data. Dunnett's test was used for post hoc comparisons using GraphPad Prism software (GraphPad Software, San Diego, CA). Differences were considered significant at $P<0.05$. In the rat study, change in core body temperature was calculated for all animals by comparing baseline prior to dosing to the minimum temperature reached following drug administration. For the mouse study, data analysis was performed by comparing baseline values with the time point 30 min after 8-OH-DPAT injection as maximal effects were seen at this point.

3. Results

3.1. Telemetry in rats

In the telemetry experiments 8-OH-DPAT (0.3 mg/kg) induced a significant hypothermia (-2.05 ± 0.55 °C, $P<0.0001$) as observed 30 min after the injection. The body temperature had in all cases returned to basal values after 2 h (Fig. 1).

3.1.1. 8-OH-DPAT and 5-HT_{1A} receptor antagonists

The 5-HT_{1A} receptor antagonists WAY-100135 and pindolol dose-dependently inhibited the hypothermia induced by 8-OH-DPAT (0.3 mg/kg) (Figs. 1 and 2A,B). Statisti-

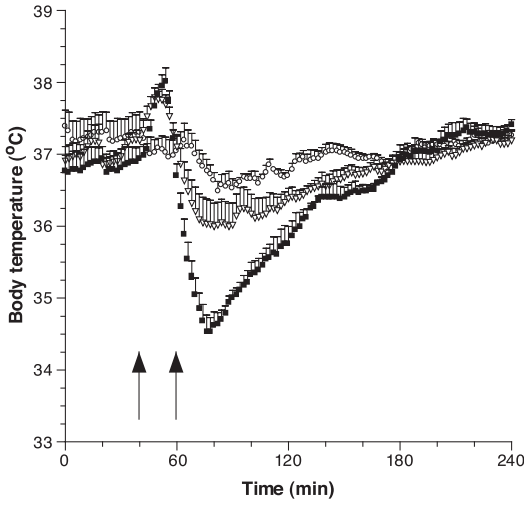

Fig. 1. Pretreatment with WAY-100135 or SB-269970 antagonizes thermic responses to 8-OH-DPAT in rats. The graph shows the time course response to 8-OH-DPAT (0.3 mg/kg) alone (■) and following pretreatment with WAY-100135 (10 mg/kg, ○) or SB-269970 (3 mg/kg, ▽). A rat telemetric in vivo system was used to measure core body temperature. Baseline temperature was monitored for 60 min prior to 8-OH-DPAT administration (indicated by right arrow). WAY-100135, SB-269970 or vehicle was given 20 min prior to 8-OH-DPAT (left arrow). Data represent the mean for $n=7$ (vehicle) or $n=4$ (pretreatment with WAY-100135 or SB-269970) ± S.E.M.).

cally significant inhibition of 8-OH-DPAT induced hypothermia by WAY-100135 or pindolol was observed starting at a dose of 1 mg/kg (Fig. 2A,B). At 10 mg/kg WAY-100135 or pindolol, the recorded body temperature was not different from baseline, thus completely blocking the effect of 8-OH-DPAT.

3.1.2. 8-OH-DPAT and 5-HT$_7$ receptor antagonists

The 5-HT$_7$ receptor antagonists SB-269970 and DR-4004 dose-dependently inhibited the hypothermia induced by 8-OH-DPAT (0.3 mg/kg) (Figs. 1 and 2C,D). A plateau was reached at 0.3 mg/kg SB-269970 and 3 mg/kg DR-4004 where an approximately 60% inhibition of the 8-OH-DPAT-induced hypothermia was obtained (Fig. 2C,D).

3.1.3. 8-OH-DPAT and atipamezole

The α_2-adrenoceptor antagonist atipamezole in doses from 0.3 to 10 mg/kg did not block the hypothermia induced by 8-OH-DPAT (Fig. 2E).

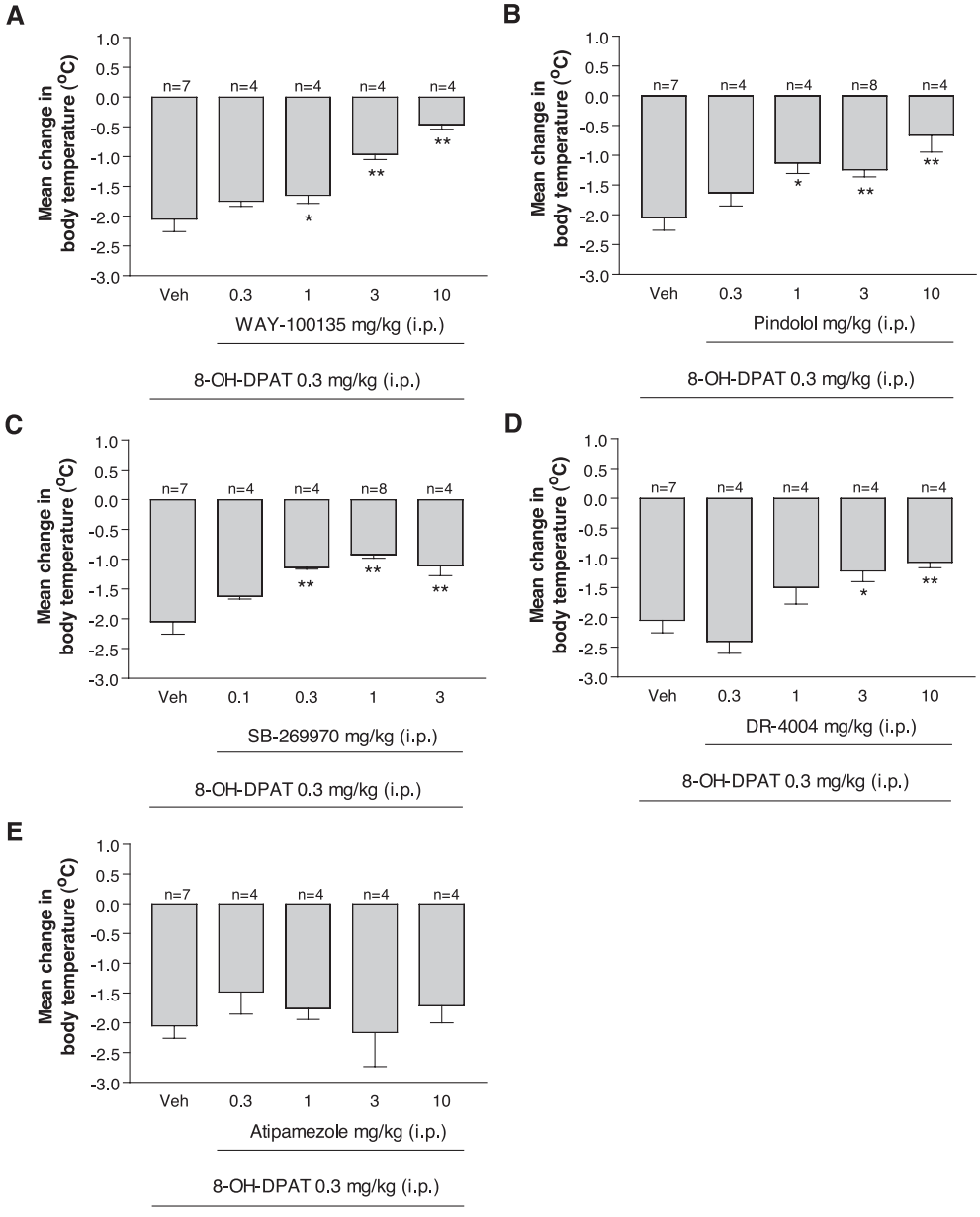

Fig. 2. Effect of WAY-100135 (A), pindolol (B), SB-269970 (C), DR-4004 (D), and atipamezole (E) on 8-OH-DPAT induced hypothermia in rats. All drugs were given i.p. 20 min before 8-OH-DPAT (0.3 mg/kg, i.p.). A rat telemetric in vivo system was used to measure core body temperature. Change in body temperature was calculated for each animal by comparing baseline to the minimum temperature reached following drug administration. Data bars represents the mean ± S.E.M. for $n=4$ to 8. *$P<0.05$, **$P<0.001$, compared to 8-OH-DPAT alone group. Statistical analysis was performed using analysis of variance followed by Dunnet's post-hoc test.

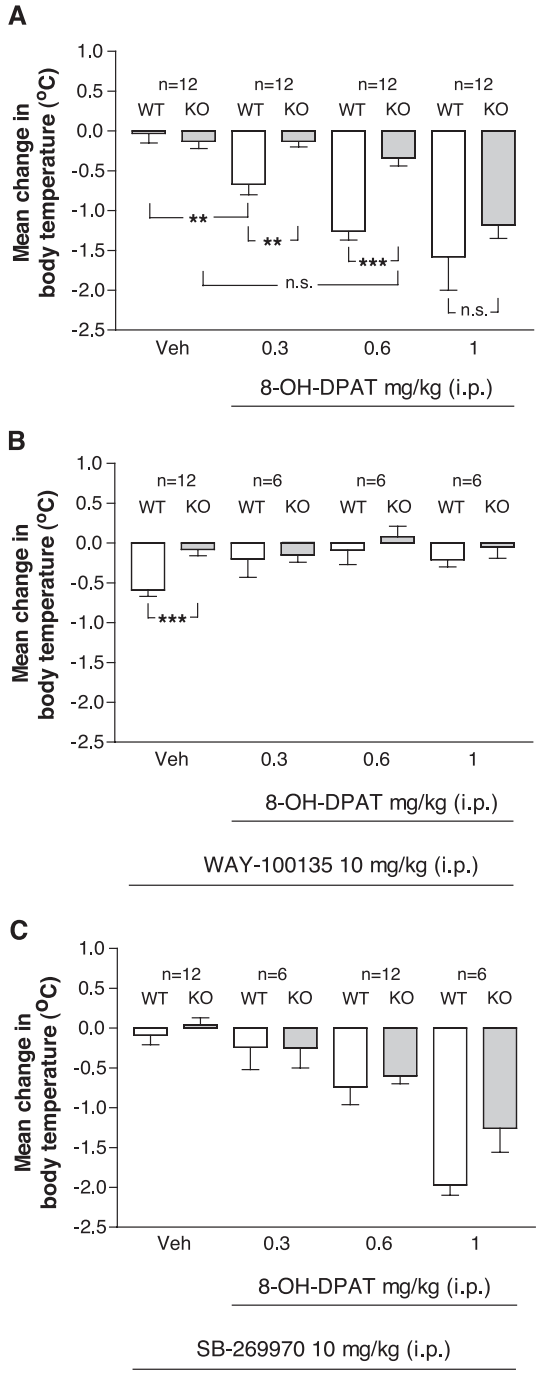

Fig. 3. (A) Effects of 8-OH-DPAT on core body temperature in 5-HT$_7^{+/+}$ (WT) and 5-HT$_7^{-/-}$ (KO) mice. The vehicle 0.9% saline was used as control. (B) Ability of the 5-HT$_{1A}$ receptor agonist WAY-100135 (10 mg/kg) to inhibit the hypothermic effect of 8-OH-DPAT. (C) Ability of the 5-HT$_7$ receptor antagonist SB-269970 (10 mg/kg) to inhibit the hypothermic effect of 8-OH-DPAT. $n=6-12$ animals per genotype per treatment group. Data is presented as mean ± S.E.M. Select relevant comparisons are indicated. *$P<0.05$, **$P<0.01$, ***$P<0.001$, n.s.: not significant. Statistical analysis was performed using analysis of variance followed by Dunnett's post-hoc test.

3.2. Rectal temperature in 5-HT$_7^{-/-}$ mice

There was no difference in basal body temperature between 5-HT$_7^{+/+}$ and 5-HT$_7^{-/-}$ mice (36.39 ± 0.09 and 36.38 ± 0.07 °C, respectively). In all experiments maximal responses where seen 30 min after injection of 8-OH-DPAT and the following analysis is based on the values at this time point. The body temperature had in all cases returned to basal values after 2 h.

3.2.1. 8-OH-DPAT-induced hypothermia

A two-way ANOVA showed significant effects for dose ($F(3,88)=19.34$, $P<0.001$), genotype ($F(1,88)=11.93$, $P<0.001$), and an interaction between dose and genotype ($F(3,88)=2.74$, $P<0.05$). Thus, a dose-dependent hypothermia was observed in 5-HT$_7^{+/+}$ mice when 8-OH-DPAT was injected alone, in concentrations ranging from 0.3 to 1 mg/kg. In the 5-HT$_7^{-/-}$ mice, doses up to 0.6 mg/kg 8-OH-DPAT failed to induce hypothermia (Fig. 3A). The 1 mg/kg dose of 8-OH-DPAT induced significant hypothermia also in the 5-HT$_7^{-/-}$ mice (Fig. 3A), although to a lesser degree than in the 5-HT$_7^{+/+}$ mice.

3.2.2. 8-OH-DPAT and WAY-100135

Here a two-way ANOVA showed a significant effect only for genotype ($F(1,52)=6.06$, $P<0.05$), since when injected alone 10 mg/kg of the 5-HT$_{1A}$ receptor antagonist WAY-100135 caused a small but significant hypothermia in the 5-HT$_7^{+/+}$ mice, but not in the 5-HT$_7^{-/-}$ mice. In combination with 8-OH-DPAT, however, a dose of 10 mg/kg WAY-100135 was able to fully inhibit the hypothermia induced by 8-OH-DPAT at all doses tested (Fig. 3B). This was true for both 5-HT$_7^{+/+}$ and 5-HT$_7^{-/-}$ mice.

3.2.3. 8-OH-DPAT and SB-269970

The two-way ANOVA showed a significant effect for concentration ($F(3,64)=28.00$, $P<0.001$). The 5-HT$_7$ receptor antagonist SB-269970 (10 mg/kg) did not affect body temperature in 5-HT$_7^{+/+}$ or 5-HT$_7^{-/-}$ mice (Fig. 3C). SB-269970 was able to block the hypothermia induced by 0.3 mg/kg 8-OH-DPAT in 5-HT$_7^{+/+}$ mice, and to partially block the effect of 0.6 mg/kg 8-OH-DPAT (Fig. 3C), thus eliminating the difference between 5-HT$_7^{+/+}$ and 5-HT$_7^{-/-}$ mice. However, SB-269970 had no inhibitory effect on the hypothermia induced by 1 mg/kg 8-OH-DPAT in either the 5-HT$_7^{-/-}$ or 5-HT$_7^{+/+}$ mice. In addition, SB-269970 had no effect on 8-OH-DPAT-induced hypothermia in the 5-HT$_7^{-/-}$ mice (Fig. 3C).

4. Discussion

The major finding of the present study was that both 5-HT$_{1A}$ and 5-HT$_7$ receptors are involved in 5-HT-mediated hypothermia with the important observation that the

5-HT_7 receptor is exclusively involved at lower agonist concentrations.

The data show that 8-OH-DPAT in vivo acts on both 5-HT_{1A} and 5-HT_7 receptors as demonstrated by the use of selective antagonists for these receptors and 5-$HT_7^{-/-}$ mice. The hypothesis that 5-HT_7 receptors are involved in 5-HT-mediated thermoregulation is well supported by data using a selective antagonist (Hagan et al., 2000) and 5-HT_7 receptor knockout mice (Guscott et al., 2003; Hedlund et al., 2003). In fact, those studies hypothesized that the 5-HT_7 receptor by itself was responsible for 5-HT-related thermoregulation, at least when observed as 5-CT-induced hypothermia. This is supported by the fact that the 5-HT_{1A} receptor antagonist WAY-100635 was unable to inhibit the effect of 5-CT. Furthermore, 5-HT and 5-CT failed to induce hypothermia in 5-$HT_7^{-/-}$ mice. To our knowledge, the effect of 5-CT on body temperature in 5-HT_{1A} receptor knockout mice has not been tested. Such an experiment may clarify the mechanism for 5-CT-induced hypothermia. The present data confirm the important involvement of 5-HT_7 receptors in 5-HT-related thermoregulation by using 8-OH-DPAT to reveal that the 5-HT_7 receptor is exclusively activated at lower doses as seen when comparing 5-$HT_7^{-/-}$ mice with 5-$HT_7^{+/+}$ mice. The effect of a low dose of 8-OH-DPAT could also be fully blocked by the 5-HT_7 receptor antagonist SB-269970.

However, at higher doses of 8-OH-DPAT it is evident that at least one other receptor subtype is involved. This is demonstrated by the induction of hypothermia in the 5-$HT_7^{-/-}$ mice by 8-OH-DPAT and by the observation that neither of the two 5-HT_7 receptor antagonists, SB-269970 and DR-4004, were able to completely inhibit the effect of 8-OH-DPAT in rats or mice. Since both pindolol (in rats) and WAY-100135 (in rats and mice) were able to block the effect of 8-OH-DPAT, it may be hypothesized that 5-HT_{1A} receptor is involved in the hypothermia. Based on the earlier assumption that 8-OH-DPAT was selective for the 5-HT_{1A} receptor, there is a large body of data supporting a role for the 5-HT_{1A} receptor in thermoregulation (Hjorth, 1985; Li et al., 1999; McAllister-Williams et al., 1999). Support for the involvement of the 5-HT_{1A} receptor in 8-OH-DPAT-induced hypothermia is also provided by results from 5-HT_{1A} receptor knockout mice where 8-OH-DPAT (0.2–1 mg/kg, s.c.) fails to induce hypothermia (Heisler et al., 1998).

It is interesting to note that WAY-100135 by itself was able to induce hypothermia in 5-$HT_7^{+/+}$ mice but not in 5-$HT_7^{-/-}$ mice. Even though WAY-100135 is generally considered to be a selective antagonist for the 5-HT_{1A} receptor, it has also been suggested to have partial agonist properties since it has been shown to stimulate 5-HT release in vivo (Assie and Koek, 1996). The present data, however, suggest that WAY-100135 in addition or exclusively has agonist properties at the 5-HT_7 receptor. The data also suggest that WAY-100135 is an antagonist at 5-HT_7 receptors since it was able to fully inhibit the effect of 8-OH-DPAT on body temperature at all concentrations tested in both species. Additional studies would be required to clearly establish the properties of WAY-100135, although the effect is strikingly reminiscent of the allosteric properties of oleamide on the 5-HT_7 receptor, at which it acts as an agonist in the absence of 5-HT but as an insurmountable antagonist when 5-HT is present (Hedlund et al., 1999; Thomas et al., 1997).

Although the data support the involvement of the 5-HT_{1A} receptor in addition to the 5-HT_7 receptor, one might have hypothesized that the α_2-adrenoceptor activity plays a role since in vivo 8-OH-DPAT has been found to have α_2-adrenoceptor activity (Millan and Colpaert, 1991; Prow et al., 1996; Winter, 1988). We tested this hypothesis by studying the effect of the α_2-adrenoceptor antagonist atipamezole on 8-OH-DPAT-induced hypothermia. Atipamezole is a selective α_2-antagonist which exhibits negligible affinity for the 5-HT_{1A} receptor (Newman-Tancredi et al., 1998). Our data (Fig. 2E) show that atipamezole does not inhibit 8-OH-DPAT induced hypothermia up to 10 mg/kg i.p., suggesting that the α_2 receptor is not involved in this effect.

Taken together the data show consistency between rats and mice suggesting a conservation of receptor function. The effects of 8-OH-DPAT observed were generally more pronounced in rats than in mice, but they are within the range of previously published data (Heisler et al., 1998; Li et al., 1999; McAllister-Williams et al., 1999).

In conclusion, the present study shows that both 5-HT_{1A} and 5-HT_7 receptors are involved in 5-HT-mediated thermoregulation. Such a dual involvement of 5-HT_{1A} and 5-HT_7 receptors has also been observed for the micturition reflex where central activation of both these receptors will inhibit the reflex (Read et al., 2003). A combined involvement of 5-HT_{1A} and 5-HT_7 receptors in multiple regulatory mechanisms is of particular interest since the two receptors have opposing actions on adenylyl cyclase. The 5-HT_{1A} receptor is negatively coupled, whereas the 5-HT_7 receptor is positively coupled to adenylyl cyclase (Hoyer et al., 2002). Under physiological conditions, the two receptors seem to be tuned to respond to different concentrations of 5-HT. The 5-HT_7 receptor appears to be more important at lower concentrations, thus being possibly involved in finer adjustments of temperature homeostasis. The 5-HT_{1A} receptor, on the other hand, becomes activated only at higher concentrations, possibly as a defense against hyperthermia.

Acknowledgements

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References

Assie, M.B., Koek, W., 1996. Effects of 5-HT1A receptor antagonists on hippocampal 5-hydroxytryptamine levels: (*S*)-WAY100135, but not WAY100635, has partial agonist properties. Eur. J. Pharmacol. 304, 15–21.

Baker, L.P., Nielsen, M.D., Impey, S., Metcalf, M.A., Poser, S.W., Chan, G., Obrietan, K., Hamblin, M.W., Storm, D.R., 1998. Stimulation of

type 1 and type 8 Ca2+/calmodulin-sensitive adenylyl cyclases by the Gs-coupled 5-hydroxytryptamine subtype 5-HT7A receptor. J. Biol. Chem. 273, 17469–17476.

Bard, J.A., Zgombick, J., Adham, N., Vaysse, P., Branchek, T.A., Weinshank, R.L., 1993. Cloning of a novel human serotonin receptor (5-HT7) positively linked to adenylate cyclase. J. Biol. Chem. 268, 23422–23426.

Bonaventure, P., Nepomuceno, D., Kwok, A., Chai, W., Langlois, X., Hen, R., Stark, K., Carruthers, N., Lovenberg, T.W., 2002. Reconsideration of 5-hydroxytryptamine (5-HT)(7) receptor distribution using [(3)H]5-carboxamidotryptamine and [(3)H]8-hydroxy-2-(di-*n*-propylamino)tetraline: analysis in brain of 5-HT(1A) knockout and 5-HT(1A/1B) double-knockout mice. J. Pharmacol. Exp. Ther. 302, 240–248.

Cliffe, I.A., Brightwell, C.I., Fletcher, A., Forster, E.A., Mansell, H.L., Reilly, Y., Routledge, C., White, A.C., 1993. (*S*)-*N*-*tert*-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamid e [(*S*)-WAY-100135]: a selective antagonist at presynaptic and postsynaptic 5-HT1A receptors. J. Med. Chem. 36, 1509–1510.

Eglen, R.M., Jasper, J.R., Chang, D.J., Martin, G.R., 1997. The 5-HT7 receptor: orphan found. Trends Pharmacol. Sci. 18, 104–107.

Guscott, M.R., Egan, E., Cook, G.P., Stanton, J.A., Beer, M.S., Rosahl, T.W., Hartmann, S., Kulagowski, J., McAllister, G., Fone, K.C., Hutson, P.H., 2003. The hypothermic effect of 5-CT in mice is mediated through the 5-HT7 receptor. Neuropharmacology 44, 1031–1037.

Gustafson, E.L., Durkin, M.M., Bard, J.A., Zgombick, J., Branchek, T.A., 1996. A receptor autoradiographic and in situ hybridization analysis of the distribution of the 5-HT7 receptor in rat brain. Br. J. Pharmacol. 117, 657–666.

Hagan, J.J., Price, G.W., Jeffrey, P., Deeks, N.J., Stean, T., Piper, D., Smith, M.I., Upton, N., Medhurst, A.D., Middlemiss, D.N., Riley, G.J., Lovell, P.J., Bromidge, S.M., Thomas, D.R., 2000. Characterization of SB-269970-A, a selective 5-HT7 receptor antagonist. Br. J. Pharmacol. 130, 539–548.

Hedlund, P.B., Carson, M.J., Sutcliffe, J.G., Thomas, E.A., 1999. Allosteric regulation by oleamide on the binding properties of 5-hydroxytryptamine7 receptors. Biochem. Pharmacol. 58, 1807–1813.

Hedlund, P.B., Danielson, P.E., Thomas, E.A., Slanina, K., Carson, M.J., Sutcliffe, J.G., 2003. No hypothermic response to serotonin in 5-HT7 receptor knockout mice. Proc. Natl. Acad. Sci. 100, 1375–1380.

Heidmann, D.E., Metcalf, M.A., Kohen, R., Hamblin, M.W., 1997. Four 5-hydroxytryptamine7 (5-HT7) receptor isoforms in human and rat produced by alternative splicing: species differences due to altered intron–exon organization. J. Neurochem. 68, 1372–1381.

Heisler, L.K., Chu, H.M., Brennan, T.J., Danao, J.A., Bajwa, P., Parsons, L.H., Tecott, L.H., 1998. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. Proc. Natl. Acad. Sci. 95, 15049–15054.

Hjorth, S., 1985. Hypothermia in the rat induced by the potent serotonin-ergic agent 8-OH-DPAT. J. Neural Transm. 61, 131–135.

Hoyer, D., Hannon, J.P., Martin, G.R., 2002. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol. Biochem. Behav. 71, 533–554.

Jasper, J.R., Kosaka, A., To, Z.P., Chang, D.J., Eglen, R.M., 1997. Cloning, expression and pharmacology of a truncated splice variant of the human 5-HT7 receptor (h5-HT7b). Br. J. Pharmacol. 122, 126–132.

Kikuchi, C., Nagaso, H., Hiranuma, T., Koyama, M., 1999. Tetrahydrobenzindoles: selective antagonists of the 5-HT7 receptor. J. Med. Chem. 42, 533–535.

Krobert, K.A., Levy, F.O., 2002. The human 5-HT7 serotonin receptor splice variants: constitutive activity and inverse agonist effects. Br. J. Pharmacol. 135, 1563–1571.

Li, Q., Wichems, C., Heils, A., Van De Kar, L.D., Lesch, K.P., Murphy, D.L., McAllister-Williams, R.H., Man, M.S., Young, A.H., 1999. Reduction of 5-hydroxytryptamine (5-HT)(1A)-mediated temperature and neuroendocrine responses and 5-HT(1A) binding sites in 5-HT transporter knockout mice. J. Pharmacol. Exp. Ther. 291, 999–1007.

Lovenberg, T.W., Baron, B.M., de Lecea, L., Miller, J.D., Prosser, R.A., Rea, M.A., Foye, P.E., Racke, M., Slone, A.L., Siegel, B.W., Danielson, P.E., Sutcliffe, J.G., Erlander, M.G., 1993. A novel adenylyl cyclase-activating serotonin receptor (5-HT7) implicated in the regulation of mammalian circadian rhythms. Neuron 11, 449–458.

McAllister-Williams, R.H., Man, M.S., Young, A.H., 1999. Effects of adrenalectomy on 8-OH-DPAT induced hypothermia in mice. Psychopharmacology (Berl.) 142, 73–77.

Mengod, G., Vilaro, M.T., Raurich, A., Lopez-Gimenez, J.F., Cortes, R., Palacios, J.M., 1996. 5-HT receptors in mammalian brain: receptor autoradiography and in situ hybridization studies of new ligands and newly identified receptors. Histochem. J. 28, 747–758.

Millan, M.J., Colpaert, F.C., 1991. Alpha 2 receptors mediate the antinociceptive action of 8-OH-DPAT in the hot-plate test in mice. Brain Res. 539, 342–346.

Morishima, Y., Shibano, T., 1995. Evidence that 5-HT2A receptors are not involved in 5-HT-mediated thermoregulation in mice. Pharmacol. Biochem. Behav. 52, 755–758.

Mullins, U.L., Gianutsos, G., Eison, A.S., 1999. Effects of antidepressants on 5-HT7 receptor regulation in the rat hypothalamus. Neuropsychopharmacology 21, 352–367.

Newman-Tancredi, A., Nicolas, J.P., Audinot, V., Gavaudan, S., Verriele, L., Touzard, M., Chaput, C., Richard, N., Millan, M.J., 1998. Actions of alpha2 adrenoceptor ligands at alpha2A and 5-HT1A receptors: the antagonist, atipamezole, and the agonist, dexmedetomidine, are highly selective for alpha2A adrenoceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 358, 197–206.

Plassat, J.L., Amlaiky, N., Hen, R., 1993. Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. Mol. Pharmacol. 44, 229–236.

Prow, M.R., Martin, K.F., Heal, D.J., 1996. 8-OH-DPAT-induced mydriasis in mice: a pharmacological characterisation. Eur. J. Pharmacol. 317, 21–28.

Read, K.E., Sanger, G.J., Ramage, A.G., 2003. Evidence for the involvement of central 5-HT7 receptors in the micturition reflex in anaesthetized female rats. Br. J. Pharmacol. 140, 53–60.

Ruat, M., Traiffort, E., Leurs, R., Tardivel-Lacombe, J., Diaz, J., Arrang, J.M., Schwartz, J.C., 1993. Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT7) activating cAMP formation. Proc. Natl. Acad. Sci. U. S. A. 90, 8547–8551.

Schoeffter, P., Ullmer, C., Bobirnac, I., Gabbiani, G., Lübbert, H., 1996. Functional, endogenously expressed 5-hydroxytryptamine 5-HT7 receptors in human vascular smooth muscle cells. Br. J. Pharmacol. 117, 993–994.

Shen, Y., Monsma Jr., F.J., Metcalf, M.A., Jose, P.A., Hamblin, M.W., Sibley, D.R., 1993. Molecular cloning and expression of a 5-hydroxytryptamine7 serotonin receptor subtype. J. Biol. Chem. 268, 18200–18204.

Stam, N.J., Roesink, C., Dijcks, F., Garritsen, A., van Herpen, A., Olijve, W., 1997. Human serotonin 5-HT7 receptor: cloning and pharmacological characterisation of two receptor variants. FEBS Lett. 413, 489–494.

Sugimoto, Y., Yamada, J., Horisaka, K., 1991. Activation of peripheral serotonin2 receptors induces hypothermia in mice. Life Sci. 48, 419–423.

Terron, J.A., Falcon-Neri, A., 1999. Pharmacological evidence for the 5-HT7 receptor mediating smooth muscle relaxation in canine cerebral artery. Br. J. Pharmacol. 127, 609–616.

Thomas, E.A., Carson, M.J., Neal, M.J., Sutcliffe, J.G., 1997. Unique allosteric regulation of 5-hydroxytryptamine receptor-mediated signal transduction by oleamide. Proc. Natl. Acad. Sci. U. S. A. 94, 14115–14119.

Tsou, A.P., Kosaka, A., Bach, C., Zuppan, P., Yee, C., Tom, L., Alvarez, R., Ramsey, S., Bonhaus, D.W., Stefanich, E., et al., 1994. Cloning and expression of a 5-hydroxytryptamine7 receptor positively coupled to adenylyl cyclase. J. Neurochem. 63, 456–464.

Tuladhar, B.R., Ge, L., Naylor, R.J., 2003. 5-HT(7) receptors mediate the inhibitory effect of 5-HT on peristalsis in the isolated guinea-pig ileum. Br. J. Pharmacol. 138, 1210–1214.

Vanhoenacker, P., Haegeman, G., Leysen, J.E., 2000. 5-HT7 receptors: current knowledge and future prospects. Trends Pharmacol. Sci. 21, 70–77.

Winter, J.C., 1988. Generalization of the discriminative stimulus properties of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and ipsapirone to yohimbine. Pharmacol. Biochem. Behav. 29, 193–195.

Won, S.J., Lin, M.T., 1988. 5-Hydroxytryptamine receptors in the hypothalamus mediate thermoregulatory responses in rabbits. Naunyn-Schmiedeberg's Arch. Pharmacol. 338, 256–261.

Yamada, J., Sugimoto, Y., Wakita, H., Horisaka, K., 1988. The involvement of serotonergic and dopaminergic systems in hypothermia induced in mice by intracerebroventricular injection of serotonin. Jpn. J. Pharmacol. 48, 145–148.