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Haloperidol-induced catalepsy is absent in dopamine D_2, but maintained in dopamine D_3 receptor knock-out mice

Denis Boulay [a,*], Ronan Depoortere [a], André Oblin [b], David J. Sanger [a], Hans Schoemaker [b], Ghislaine Perrault [a]

[a] *Department of Psychopharmacology, Sanofi-Synthélabo, 31 ave P. Vaillant-Couturier, 92220 Bagneux, France*
[b] *Department of Neurochemistry, Sanofi-Synthélabo, 31 ave P. Vaillant-Couturier, 92220 Bagneux, France*

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Abstract

We have previously found that mice homozygous for the deletion of the dopamine D_2 receptor gene ($D_2^{-/-}$ mice) do not present spontaneous catalepsy when tested in a ''bar test''. In the present study, we sought to analyse the reactivity of D_2 receptor mutant mice to the cataleptogenic effects of dopamine D_2-like or D_1-like receptor antagonists. In parallel, we assessed the cataleptogenic effects of these antagonists in dopamine D_3 receptor mutant mice. $D_2^{-/-}$ mice were totally unresponsive to the cataleptogenic effects of the dopamine D_2-like receptor antagonist haloperidol (0.125–2 mg/kg i.p.), while $D_2^{+/-}$ mice, at the highest haloperidol doses tested, showed a level of catalepsy about half that of wild-type controls. The degree of haloperidol-induced catalepsy was thus proportional to the level of striatal dopamine D_2 receptor expression (0.50, 0.30 and 0.08 pmol/mg protein as measured at 0.25 nM [^{3}H]spiperone for $D_2^{+/+}$, $D_2^{+/-}$ and $D_2^{-/-}$ mice, respectively). However, $D_2^{-/-}$ and $D_2^{+/-}$ mice were as sensitive as their wild-type counterparts to the cataleptogenic effects of the dopamine D_1-like receptor antagonist *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390: 0.03-0.6 mg/kg s.c.). Striatal dopamine D_1 receptor expression (as measured using [^{3}H]SCH 23390 binding) was not significantly affected by the genotype. The ability of SCH 23390 to induce catalepsy in $D_2^{-/-}$ mice suggests that their resistance to haloperidol-induced catalepsy is due to the absence of dopamine D_2 receptors, and not to the abnormal striatal synaptic plasticity that has been shown by others to occur in these mice. In agreement with the observation that dopamine D_2 and dopamine D_1 receptor expression was essentially identical in $D_3^{+/+}$, $D_3^{+/-}$ and $D_3^{-/-}$ mice, dopamine D_3 receptor homozygous and heterozygous mutant mice, on the whole, did not differ from their controls in the time spent in a cataleptic position following administration of either haloperidol (0.5–2 mg/kg i.p.) or SCH 23390 (0.03–0.6 mg/kg s.c.). Also, dopamine D_3 receptor mutant mice were no more responsive than wild-type controls when co-administered subthreshold doses of haloperidol (0.125 mg/kg) and SCH 23390 (0.03 mg/kg), suggesting that dopamine D_3 receptor knock-out mice are not more sensitive than wild-types to the synergistic effects of concurrent blockade of dopamine D_2 and dopamine D_1 receptors in this model. These results suggest that the dopamine D_2 receptor subtype is necessary for haloperidol to produce catalepsy, and that the dopamine D_3 receptor subtype appears to exert no observable control over the catalepsy produced by dopamine D_2-like, D_1-like and the combination of D_1-like and D_2-like receptor antagonists.

Keywords: Catalepsy; Dopamine D_2 receptor; Dopamine D_3 receptor; Haloperidol; Knock-out mice; SCH 23390

1. Introduction

The original classification of dopamine receptors in two families (the D1 and the D2; Kebabian and Calne, 1979) has given place to a newer subdivision into D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) receptors (Civelli et al., 1993). For each of these subtypes, the attribution of physiological roles and the elucidation of their implication in the various effects mediated by pharmacological manipulation of the dopaminergic system, is still the subject of sustained research. Progress in classical pharmacological studies has suffered from a lack of dopaminergic agents with clear in vivo selectivity for each subtype. Alternative approaches aimed at intervening at the level of the genes

* Corresponding author. Tel.: +33-1-45-36-24-90; fax: +33-1-45-36-20-70.
E-mail address: denis.boulay@sanofi.synthelabo.com (D. Boulay).

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coding for the various subtypes have more recently emerged.

Genetically modified mice lacking one of the subtypes of dopamine receptors (knock-out mice) have recently been engineered by Drago et al. (1994) and Xu et al. (1994) for the dopamine D_1 subtype. For the dopamine D_2 receptor family, D_2 receptor knock-out mice were generated by Baik et al. (1995), Kelly et al. (1997) and more recently, Jung et al. (1999). Dopamine D_3 receptor knock-out mice were described by Accili et al. (1996), Xu et al. (1997) and Jung et al. (1999), and D_4 receptor variants by Rubinstein et al. (1997). Among the five subtypes of dopamine receptors, two, the D_2 and the D_3, are of special interest, as they have been postulated to be involved in the pharmacological activity and therapeutic efficacy of antipsychotic (Seeman 1992; Malmberg et al., 1993) and anti-Parkinsonian (Piercey 1998) drugs. Dopamine D_2 receptor knock-out mice (homozygous: $D_2^{-/-}$, heterozygous: $D_2^{+/-}$) have been described as being akinetic/bradykinetic, and as presenting ''cataleptic-like'' behaviour using a ring test (Baik et al., 1995). However, Kelly et al. (1997, 1998) reported an absence of spontaneous catalepsy in their line of $D_2^{-/-}$ mice, and we have found that when tested in a bar test, our $D_2^{-/-}$ and $D_2^{+/-}$ mice (generated from individuals issued from the colony of mice analysed by Baik et al.) were not spontaneously cataleptic (Boulay et al., 1999a). The phenotype of the third line (Jung et al., 1999), on the whole, is reminiscent of the one described for mice generated by Baik et al. However, the severity of motor abnormalities of these mice was reported to vary with the age of the mice: motor dysfunction appeared 2 weeks postnatal, and significantly improved from day 45 onward.

The absence of spontaneous catalepsy in $D_2^{-/-}$ mice (Kelly et al., 1997, 1998; Boulay et al., 1999a) seems at odds with the well known cataleptogenic properties of dopamine D_2-like receptor antagonists such as haloperidol (Janssen et al., 1968), and the catalepsy produced by treatment with antisense oligonucleotides directed against the mRNA coding for the dopamine D_2 receptor (Zhang and Creese, 1993; Quin et al., 1995). However, important compensatory mechanisms may counteract the effects of the dopamine D_2 receptor gene deletion. Jung et al. (1999) showed that their $D_2^{-/-}$ mice have an increased expression of dopamine D_3 receptors: they suggested that this augmented level of dopamine D_3 receptors might partially compensate for the absence of dopamine D_2 receptors. This hypothesis was based on the observation that $D_2^{-/-}$/ $D_3^{-/-}$ double mutants showed more severe impairment of locomotor activity than single $D_2^{-/-}$ mutants. Other subtypes of dopamine receptors, and in particular the D_1 subtype, are potential candidates to mediate these compensatory processes. However, biochemical data indicate that there is no detectable modification of the dopamine D_1 receptor system following deletion of the dopamine D_2 gene (as illustrated by the absence of dopamine D_1 receptor upregulation in dopamine D_2 receptor knock-out mice (Baik et al., 1995; Kelly et al., 1998).

To the best of our knowledge, there are currently no published studies that have assessed the sensitivity of D_2 receptor mutant mice to catalepsy produced by dopamine D_1-like or D_2-like receptor antagonists. We hypothesised that, if as suggested by Jung et al., dopamine D_3 receptors partially compensate for the absence of dopamine D_2 receptors for levels of locomotor activity, such functional compensation might also underlie the absence of spontaneous catalepsy in our $D_2^{-/-}$ mice. To test this hypothesis, we compared the sensitivity of $D_2^{-/-}$, $D_2^{+/-}$ and $D_2^{+/+}$ mice to the cataleptogenic effects of the prototypical dopamine D_2-like receptor antagonist haloperidol. If dopamine D_3 receptors can indeed partially compensate for the absence of dopamine D_2 receptors, then blockade of the former by haloperidol might produce catalepsy in $D_2^{-/-}$and $D_2^{+/-}$ mice. Furthermore, based on reports that there is synergism between dopamine D_1-like and dopamine D_2-like receptor antagonists in producing catalepsy in rodents (Klemm and Block, 1988, Parashos et al., 1989; Wanibuchi and Usuda, 1990), we also assessed the sensitivity of these knock-out mice to the cataleptogenic effects of *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390), a prototypical dopamine D_1-like receptor antagonist (Hyttel 1983; Iorio et al., 1983).

Based on the observation that the putative dopamine D_3 receptor antagonist (+)-S 14297 ((+)-7-*N*,*N*-dipropylamino-5 ,6 ,7 ,8-tetrahydro-naphtho(2,3*b*)dihydro,2,3-furane) reversed catalepsy induced by haloperidol, Millan et al. (1997) suggested that blockade of dopamine D_3 receptors was able to oppose dopamine D_2 receptor-mediated catalepsy. Therefore, we tested the hypothesis that dopamine D_3 receptor knock-out mice would be less sensitive than their wild-type counterparts to the cataleptogenic effects of haloperidol. As a control experiment, we also investigated the cataleptogenic effects of SCH 23390 in these mice. Finally, because it has been suggested that there could be a positive interaction between dopamine D_1 and dopamine D_2 receptors in rats (Schwartz et al., 1998) and in dopamine D_3 receptor knock-out mice (Xu et al., 1997), we tested the possibility that $D_3^{-/-}$ mice might show differential sensitivity to concurrent blockade of dopamine D_1 and dopamine D_2 receptors, by combining subthreshold doses of haloperidol and of SCH 23390. To complement these behavioural studies, dopamine D_1 and dopamine D_2 receptor expression levels in the striatum — a brain structure thought to be intimately involved in catalepsy produced by dopamine receptor antagonists (Costall et al., 1972) — were assessed by means of radioligand membrane binding. Dopamine D_3 receptor expression was evaluated by [^{125}I]iodosulpiride autoradiography, given the relatively restricted and low level of distribution of these receptors (Sokoloff et al., 1990; Booze and Wallace 1995).

2. Materials and method

2.1. Animals

The $D_2^{+/-}$ mice (129.SvJae/C57BL.6J hybrids) used for the generation of the colony of D_2 mutants ($D_2^{-/-}$, $D_2^{+/-}$ and $D_2^{+/+}$ individuals) were obtained from Dr. E. Borrelli (I.G.B.C.M., Strasbourg, France). The founder $D_3^{-/-}$ male mouse used for the generation of the colony of D_3 mutants ($D_3^{-/-}$, $D_3^{+/-}$ and $D_3^{+/+}$ individuals) was generously supplied by Dr. Accili (NIH, Bethesda, USA). This 129.SvJae/C57BL.6J male was mated with C57BL.6J females in the laboratory of Dr. W. Rostene (Inserm U.139, Hôpital St. Antoine, Paris) to generate heterozygous mutants. $D_2^{+/-}$ and $D_3^{+/-}$ mutant mice were bred with C57BL.6J mice by Transgenic Alliance (L'Arbresle, France). This heterozygous mutant generation was intermated to produce either $D_3^{-/-}$, $D_3^{+/-}$ and $D_3^{+/+}$ or $D_2^{-/-}$, $D_2^{+/-}$ and $D_2^{+/+}$ animals used for experimentation. The genotyping of mice was determined by reverse transcription-PCR analysis of products derived from tail mRNA. Mice of both sexes were used in the studies. The age of the individuals ranged from 3 to 4 months, and their weights varied between 25 and 30 g at time of testing. For each colony, mice were randomly (with respect to the genotype) housed in groups of six individuals of the same sex in Plexiglas cages ($30 \times 20 \times 14$ cm high). Food and water were available ad lib. Animals were kept in conditions of constant temperature (23°C), humidity (50%) and light–dark cycle (light on from 8:00 am to 8:00 pm). Animals were housed and tested in accordance with current French legislation on animal experimentation.

2.2. Apparatus and behavioural procedures

Following a 60-min period of isolation in a Plexiglas box, each mouse was injected with haloperidol, SCH 23390 or the haloperidol/SCH 23390 combined treatment, and returned to the Plexiglas box. Recording of the time spent in a cataleptic position (see below) was done 1 and 2 h following haloperidol injection, 15 and 30 min following SCH 23390. For the haloperidol/SCH 23390 combined treatment, mice were injected 60 min before testing with haloperidol (0.125 mg/kg i.p.), followed 45 min later by SCH 23390 (0.03 mg/kg s.c.). SCH 23390 was injected later because of its short duration of action; assessment of catalepsy was done 15 and 30 min post-SCH 23390 injection. Reading of the time spent in a cataleptic position was done by positioning the mouse so that both front paws rested on a 0.4 cm diameter steel rod (covered with non-slippery tape) that was 3.5 cm above the surface of the bench. The time during which each mouse maintained this position was recorded up to a maximum of 2 min.

2.3. Drugs and injection protocols

Haloperidol (Sigma, St. Louis, MO, USA) was prepared in distilled water (10% w/w of haloperidol, 90% w/w ascorbate) and administered i.p. in a volume of 20 ml/kg. SCH 23390 (RBI, Natick, MA, USA) was dissolved in saline and administered s.c. in a volume of 10 ml/kg. Doses are expressed as the weights of the free base. For the haloperidol and SCH 23390 treatments, mice were injected four times (vehicle and three doses of the drug). For the haloperidol/SCH 23390 combined treatment, each mouse was injected with vehicle/vehicle, haloperidol/vehicle, vehicle/SCH 23390 and haloperidol/SCH 23390. Injections were administered at the appropriate time and route of administration. For all experiments, injections were administered in a counterbalanced order; two consecutive test sessions were separated by at least 72 h. Three batches of $D_2^{-/-}$, $D_2^{+/-}$ and $D_2^{+/+}$ mice, and three batches of $D_3^{-/-}$, $D_3^{+/-}$ and $D_3^{+/+}$ mice were used to assess the effects of haloperidol, SCH 23390 and haloperidol/SCH 23390 (one treatment per batch).

2.4. Radioligand binding studies

Following completion of behavioural studies, mice were killed by cervical dislocation and decapitated. For radioligand binding studies, their brains were rapidly removed and the striata dissected out. The tissue was homogenised (5 mg tissue/ml) in ice-cold 10 mM Tris–HCl buffer (pH = 7.4). Homogenates were centrifuged (20 000 g) for 10 min at 4°C, and the membrane pellets were washed by homogenisation in the original volume of buffer and centrifuged. Pellets were resuspended in 50 mM Tris–HCl buffer (pH = 7.4) to a final concentration of 150 to 200 μg of protein/ml.

Dopamine D_1 receptor saturation studies were performed on 10–20 μg of striatal protein, using 0.1 to 5 nM of [^{3}H]SCH 23390 (specific activity: 70 Ci/mmol; NEN, Boston, MA) in 50 mM Tris–HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$, essentially as described in Billard et al. (1984). Nonspecific binding was determined in the presence of 1 μM SCH 23390. For the colony of dopamine D_3 knock-out mice, dopamine D_2 receptor saturation studies were performed using 0.01 to 0.25 nM of [^{3}H]spiperone (specific activity: 98 Ci/mmol, Amersham, France) in 50 mM Tris–HCl buffer (pH = 7.4) containing 120 mM NaCl and 5 mM KCl as described by Briley and Langer (1978). Nonspecific binding was determined in the presence of 10 μM haloperidol. Expression studies of dopamine D_2 receptor in the colony of dopamine D_2 mutant mice, using a single, near-saturating (0.25 nM) concentration of [^{3}H] spiperone, were performed under otherwise identical conditions in 50 mM Tris–HCl.

For the quantitative autoradiography, the brains were rapidly removed and frozen in isopentane (−48°C). Serial 10 μm thick cryostat sections were thaw-mounted onto gelatin-coated slides. Autoradiography was performed on coronal brain sections (+0.98 mm) with respect to the bregma (Franklin and Paxinos, 1996), essentially as described by Booze and Wallace (1995). Sections were incubated for 1 h at room temperature in 50 mM Tris–HCl (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mM ascorbic acid with 0.3 nM [^{125}I]iodosulpride (specific activity 2000 Ci/mmol, Amersham) in the absence and presence of 1 μM (−)-sulpiride or 50 nM 7-OH-DPAT (7-hydroxy-*N*,*N*-di-*n*-propylaminotetralin) to determine specific $D_2 + D_3$ binding and D_2 binding, respectively. Sections were then rinsed twice for 2 min in ice-cold Tris–HCl buffer, briefly dipped in ice-cold deionised water, dried rapidly and apposed to Hyperfilm-^{3}H (Amersham) in an X-ray cassette for 4 days. Autoradiograms were analysed using a computer-assisted image analyser (Biocom, Paris, France). Optical densities were transformed into nCi/mg tissue equivalent, using standard curves generated with ^{3}H-microscales (Amersham) and converted to fmol/mg tissue.

2.5. Data analysis

Behavioural data: the mean time spent in a cataleptic position (the two front paws resting on the bar) was calculated by averaging the two measures. For the haloperidol and SCH 23390 experiments, these mean times were subjected to two-way analyses of variance (ANOVAs), with the genotype as the between factor and the dose of the drug as the within factor, followed when appropriate by one-way ANOVAs for each genotype, and complementary post-hoc (Dunnett's) tests. For the haloperidol/SCH 23390 combined treatment, data were analysed by three-way ANOVAs, with the genotype as the between factor, and the drug treatments (haloperidol and SCH 23390) as the within factors.

Radioligand saturation data were analysed by nonlinear regression (Munson and Rodbard, 1980). Radioligand binding parameters were subjected to one-way ANOVAs with the genotype as the between factor. For the autoradiography experiment, results were expressed as a percentage of the mean value of the corresponding wild-type group for each structure. All statistical analyses were performed using the SAS software (SAS Institute, Cary, NC, USA).

3. Results

In both the dopamine D_2 and D_3 receptor knock-out mice colonies, neither mutant nor wild-type mice showed spontaneous catalepsy under control (i.e., after vehicle injection) conditions: all scores were below 5 s (see for example symbols above Vehicle [Veh] labels in (Figs. 2 and 3).

3.1. Effects of haloperidol on the time spent in a cataleptic position in the colony of dopamine D_2 receptor knock-out mice

In dopamine D_2 receptor knock-out mice (Fig. 1, top panel), there was a marked genotype effect [$F(2,20) = 14.14$, $P < 0.0001$], a dose effect [$F(3,60) = 7.74$, $P < 0.001$] and a genotype x dose interaction [$F(6,60) = 2.46$, $P < 0.05$]. Most notably, homozygous mice were totally insensitive to the cataleptogenic effects of 0.5 to 2 mg/kg of haloperidol, whereas heterozygous mice were about half as responsive as wild-types at the two highest doses tested.

Because dopamine D_2 receptor heterozygous mice presented catalepsy times that reached a maximum value (around 25 s) from the lowest dose of haloperidol (0.5

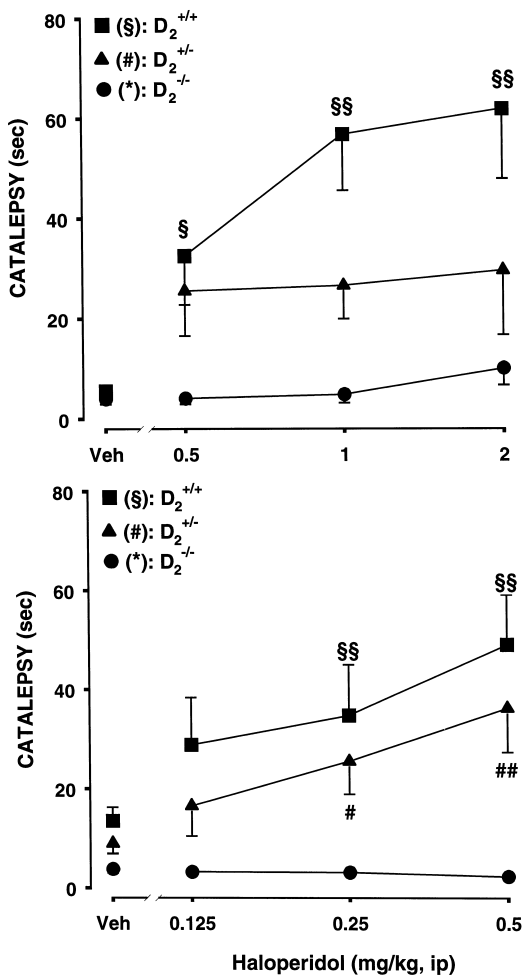

Fig. 1. Effects of the dopamine D_2-like receptor antagonist haloperidol (high doses range: top panel; low doses range: bottom panel) on the time spent in a cataleptic position in the colony of dopamine D_2 receptor knock-out mice. Each symbol represents the mean time (±S.E.M.). $^{\S\#}P < 0.05$, $^{\S\S\#\#}P < 0.01$ compared to vehicle injection (Veh), Dunnett's post-hoc tests following a one-way ANOVA for each genotype. $N = 7$–10 mice per genotype.

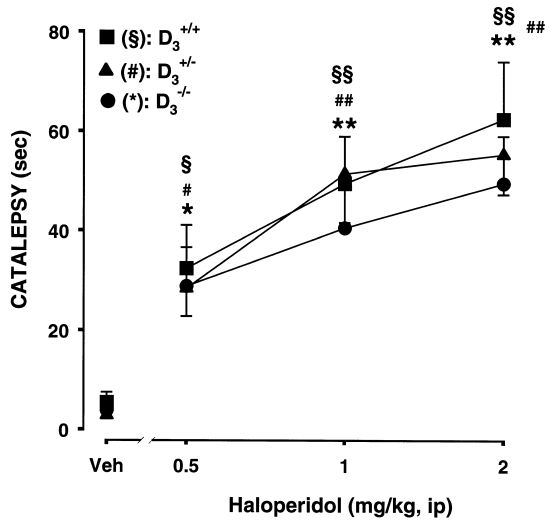

Fig. 2. Effects of the dopamine D_2-like receptor antagonist haloperidol on the time spent in a cataleptic position in the colony of dopamine D_3 receptor knock-out mice. Each symbol represents the mean time (±S.E.M.). $^{\S\#*}P < 0.05$, $^{\S\S\#\#**}P < 0.01$ compared to vehicle injection (Veh), Dunnett's post-hoc tests following a one-way ANOVA for each genotype. $N = 11–12$ mice per genotype.

mg/kg: see above), this colony was additionally tested with 0.125, 0.25 and 0.5 mg/kg of the drug (Fig. 1, bottom panel)). This was done to investigate whether these heterozygous mice were more sensitive than wild-type mice to the cataleptogenic effects of low doses of haloperidol. This complementary study yielded results basically similar to those obtained with the three higher doses of haloperidol in the preceding experiment. There were highly significant genotype $[F(2,27) = 9.23,\ P < 0.001]$ and dose $[F(3,81) = 9.93,\ P < 0.0001]$ effects, as well as a significant interaction $[F(6,81) = 3.0,\ P = 0.01]$. In particular, the dose–response curve for D_2 receptor heterozygotes was below and parallel to that of wild-type mice, showing that these mutant mice were less responsive than their control homologues to the cataleptogenic effects of haloperidol.

3.2. Effects of haloperidol on the time spent in a cataleptic position in the colony of dopamine D_3 receptor knock-out mice

In contrast, for the colony of dopamine D_3 receptor knock-out mice (Fig. 2), there was no genotype effect $[F(2,32) = 0.53,\ P > 0.05]$, and no genotype × dose interaction $[F(6,96) = 0.24,\ P > 0.05]$. Haloperidol induced catalepsy [dose effect: $F(3,96) = 29.99,\ P < 0.0001$] to a similar extent in the three genotypes, with significant effects (compared to vehicle controls) at all three doses.

3.3. Effects of SCH 23390 on the time spent in a cataleptic position

There was no genotype effect $[F(2,32) = 0.50,\ P > 0.05]$ and no genotype × dose interaction ($[F(8,128) = 0.58,\ P > 0.05]$ in the colony of dopamine D_2 receptor knock-out mice (Fig. 3, upper panel). SCH 23390 induced catalepsy to a very similar extent in the three genotypes. The dose factor was found to be highly significant $[F(4,128) = 55.54,\ P < 0.0001]$. Post-hoc analysis showed that, for all three genotypes, the time spent in a cataleptic position was significantly different from vehicle values at the three highest doses tested.

In the colony of dopamine D_3 receptor knock-out mice, SCH 23390, on the whole, induced catalepsy to a similar extent in the three genotypes (Fig. 3, bottom panel). The dose factor was found to be highly significant $[F(4,132) = 63.15,\ P < 0.0001]$; the genotype effect was found to be significant $[F(2,33) = 4.23,\ P = 0.02]$, but not the genotype × dose interaction $[F(8,132) = 0.78,\ P > 0.05]$. For each genotype, catalepsy time was significantly above vehicle values at the three highest doses tested. In addition, at the dose of 0.1 mg/kg only, the time that heterozygotes

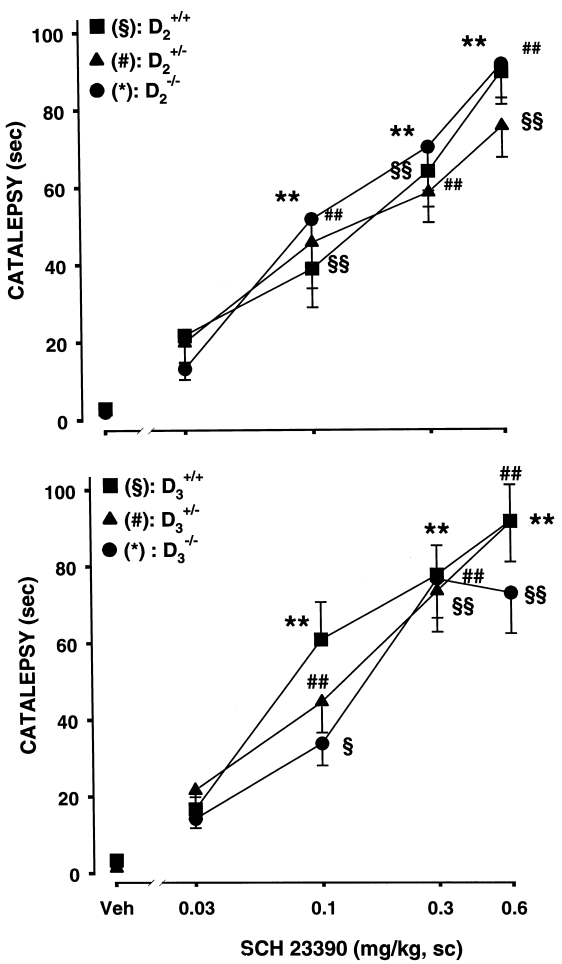

Fig. 3. Effects of the dopamine D_1-like receptor antagonist SCH 23390 on the time spent in a cataleptic position in the colonies of dopamine D_2 and dopamine D_3 receptor knock-out mice. Each symbol represents the mean time (±S.E.M.). $^{\S\#*}P < 0.05$, $^{\S\S\#\#**}P < 0.01$ compared to vehicle injection (Veh), Dunnett's post-hoc tests following a one-way ANOVA for each genotype. $N = 11–12$ mice per genotype, for both the colonies of dopamine D_2 and D_3 receptor knock-out mice.

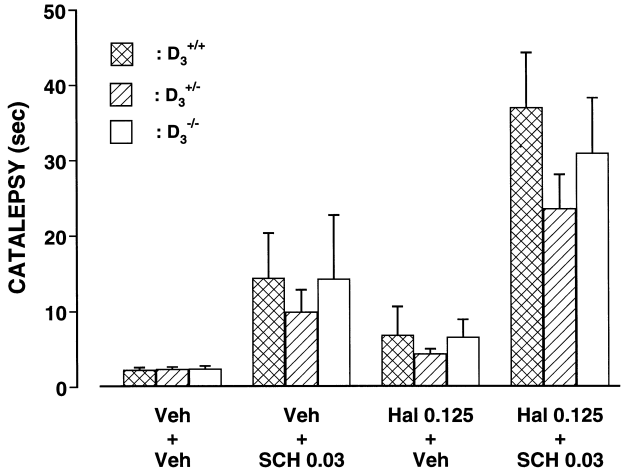

Fig. 4. Effects of the dopamine D_1-like receptor antagonist SCH 23390 (0.03 mg/kg s.c.) and of the dopamine D_2-like receptor antagonist haloperidol (0.125 mg/kg i.p.), alone and in combination, on the time spent in a cataleptic position in the colony of dopamine D_3 receptor knock-out mice. Each bar represents the mean time (+S.E.M.). $N = 12$ mice per genotype.

spent in a cataleptic position was significantly different from that of wild-types. At all other doses, there was no difference between the three genotypes.

3.4. Effects of combined treatment with subthreshold doses of haloperidol and SCH 23390 on the time spent in a cataleptic position in dopamine D_3 receptor knock-out mice

When given alone, SCH 23390 (0.03 mg/kg s.c.) and haloperidol (0.125 mg/kg i.p.) produced minimal levels of catalepsy (less than 15 s, see Fig. 4). When combined, these two pharmacological agents acted synergistically to produce a level of catalepsy that differed from that produced by each treatment separately [$F(1,33) = 8.58$, $P < 0.01$, for the haloperidol × SCH 23390 interaction effect]. There was no indication of a difference between the three genotypes [$F(2,33) = 0.64$, $P > 0.05$, and $F(2,33) = 0.18$, $P > 0.05$, for the genotype factor and the genotype × haloperidol × SCH 23390 interaction effects, respectively].

3.5. Receptor binding studies

In the colony of dopamine D_2 receptor knock-out mice, [^{3}H]spiperone binding to striatal membranes — which is thought to largely represent the dopamine D_2 receptor (Briley and Langer 1978; Palacios et al., 1981; Schoemaker et al., 1997) — showed a highly significant genotype dependence [$F(2,11) = 27.86$, $P < 0.0001$]. $D_2^{+/-}$ mice showed a 40% reduction of specific [^{3}H]spiperone binding, while $D_2^{-/-}$ mice presented an almost total (84% reduction) disappearance of binding (Table 1). [^{3}H]SCH 23390 saturation analyses revealed that neither the K_d [$F(2,11) = 0.94$, $P > 0.42$] nor the B_{max} [$F(2,11) = 0.01$, $P > 0.99$] were affected by the genotypic status.

In the colony of dopamine D_3 receptor knock-out mice (Table 2), saturation analyses of [^{3}H]SCH 23390 and [^{3}H]spiperone binding did not reveal any differences for the B_{max} or K_d between $D_3^{-/-}$, $D_3^{+/-}$ and $D_3^{+/+}$ mice [$0.06 < F$s < 3.15; $0.08 < P$s < 0.95].

[^{125}I]iodosulpride autoradiography confirmed that in the colony of dopamine D_2 receptor knock-out mice, there was a significant decrease of specific binding representing D_2 and D_3 receptors in the caudate [$F(2,15) = 49.48$, $P < 0.0001$], as well as in the islands of Calleja major [$F(2,15) = 20.75$, $P < 0.0001$]. More specifically, in $D_2^{-/-}$ mice, there was a near total disappearance of [^{125}I]iodosulpride binding in the caudate–putamen, and a 37% reduction in the islands of Calleja (Fig. 5, upper left panel). In $D_2^{+/-}$ mice, $D_2 + D_3$ binding in the caudate was diminished by 34%, whereas that in the islands of Calleja was unaffected.

When incubated in the presence of 50 nM 7-OH-DPAT to mask the dopamine D_3 receptor (Fig. 5, lower left panel), [^{125}I]iodosulpride binding was reduced in both the caudate–putamen [$F(2,15) = 590.64$, $P < 0.0001$] and the islands of Calleja [$F(2,15) = 57.62$, $P < 0.0001$]. For both structures dopamine D_2 binding in $D_2^{-/-}$ mice was quasi-absent, while that of $D_2^{+/-}$ mice was reduced by 24% and 47% in the caudate–putamen and island of Calleja, respectively.

For the colony of dopamine D_3 receptor knock-out mice, total specific [^{125}I]iodosulpride binding (Fig. 5, up-

Table 1
Dopamine D_2 and D_1 receptor binding parameters in the striatum for the colony of dopamine D_2 receptor knock-out mice
Membranes (10–20 μg protein) from the mouse striatum were incubated with a near-saturating (0.25 nM) concentration of [^{3}H]spiperone to assess expression of dopamine D_2 receptors, or with 0.1–5 nM of [^{3}H]SCH 23390 to establish the saturation characteristics of dopamine D_1 receptors. Data shown are the mean ± S.E.M.; for each genotype, *n*s are indicated in parentheses in the first column.

Genotype	D_2 binding (pmol/mg protein)	D_1 binding	
		K_d (nM)	B_{max} (pmol/mg protein)
$D_2^{+/+}$ ($n = 5$)	0.50 ± 0.06	0.43 ± 0.04	1.82 ± 0.18
$D_2^{+/-}$ ($n = 5$)	0.30 ± 0.01[a]	0.40 ± 0.02	1.80 ± 0.09
$D_2^{-/-}$ ($n = 4$)	0.08 ± 0.02[a]	0.38 ± 0.03	1.80 ± 0.15

[a] $P < 0.01$ vs. $D_2^{+/+}$, Dunnett's post-hoc test.

Table 2
Dopamine D_2 and D_1 receptor binding parameters in the striatum for the colony of dopamine D_3 receptor knock-out mice
Membranes (10–20 μg protein) from the mouse striatum were incubated with 0.01–0.25 nM of [^{3}H]spiperone or with 0.1–5 nM of [^{3}H]SCH 23390 to establish the saturation characteristics of dopamine D_2 and D_1 receptors, respectively. Data shown are the mean ± S.E.M.; for each genotype, *n*s are indicated in parentheses in the first column.

Genotype	D_2 binding		D_1 binding	
	K_d (nM)	B_{max} (pmol/mg protein)	K_d (nM)	B_{max} (pmol/mg protein)
$D_3^{+/+}$ ($n = 5–6$)	0.078 ± 0.016	0.85 ± 0.06	0.44 ± 0.05	2.06 ± 0.12
$D_3^{+/-}$ ($n = 5–6$)	0.088 ± 0.011	0.99 ± 0.06	0.44 ± 0.03	2.30 ± 0.20
$D_3^{-/-}$ ($n = 6$)	0.075 ± 0.014	0.81 ± 0.04	0.43 ± 0.03	2.17 ± 0.12

per right panel) was not affected in the caudate–putamen [$F(2,15) = 0.17$, $P > 0.85$], but reduced by about 40 and 45% in $D_3^{+/-}$ and $D_3^{-/-}$ mice, respectively, in the islands of Calleja [$F(2,15) = 6.0$, $P = 0.01$]. Dopamine D_2 bind-

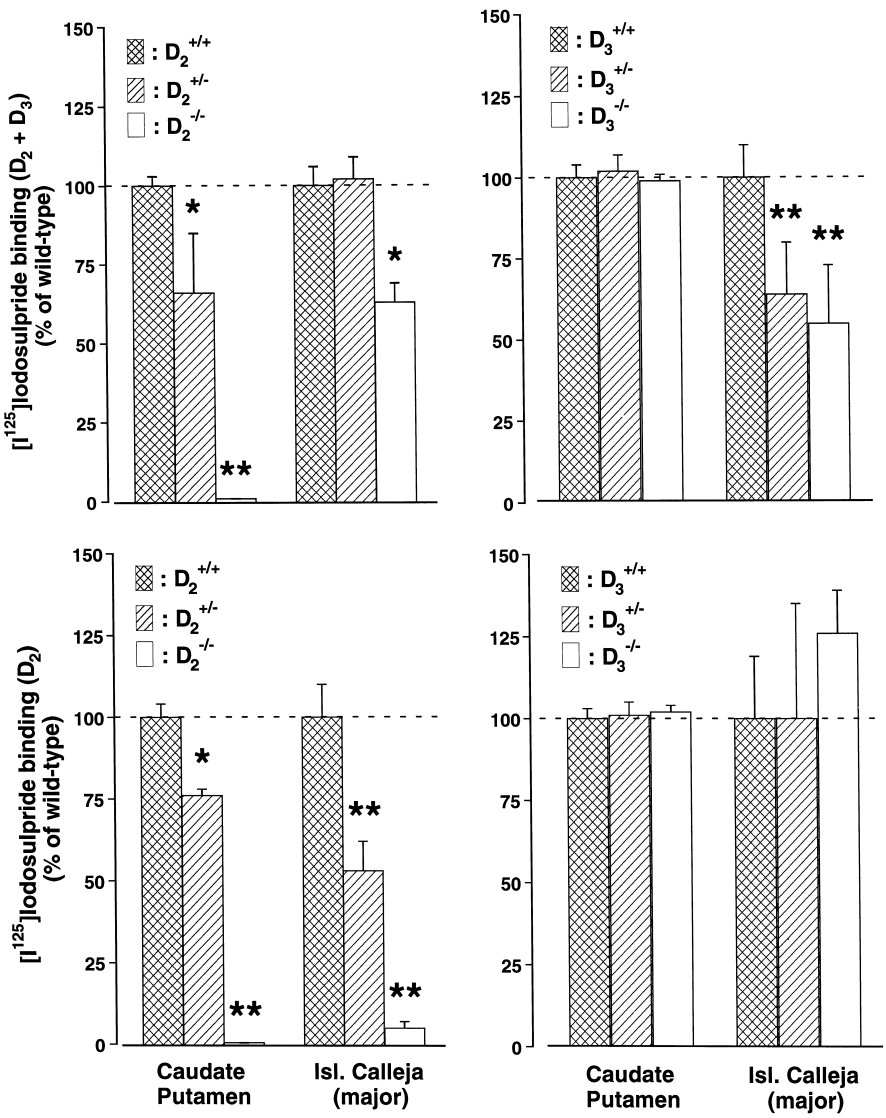

Fig. 5. Quantitative analysis of [^{125}I]iodosulpride binding in the caudate–putamen and islands of Calleja major by autoradiography for the colony of dopamine D_2 receptor knock-out mice (left panels) and for the colony of dopamine D_3 receptor knock-out mice (right panels). Upper panels: specific radioligand binding to dopamine D_2 and D_3 ($D_2 + D_3$) receptors; lower panels: dopamine D_2 receptor binding density is represented, D_3 binding sites having been masked by the addition of 50 nM 7-OH-DPAT. Each bar represents the mean density (+S.E.M.) expressed as percent of wild-type (+/+) control group. $^*P < 0.05$, $^{**}P < 0.01$ compared to wild-type group, Dunnett's post-hoc tests following a one-way ANOVA for each structure. $N = 6$ mice per genotype.

ing studied in the presence of 50 nM 7-OH-DPAT (Fig. 5, lower right panel) was not significantly affected in both structures and for either mutant genotype [Fs < 0.82, Ps > 0.46], although there was a tendency for an increase of dopamine D_2 binding in $D_3^{-/-}$ mutant mice.

4. Discussion

The main findings can be summarised as: (a) dopamine D_2 receptor homozygotes were totally unresponsive to the cataleptogenic effects of the dopamine D_2-like receptor antagonist haloperidol, while heterozygotes showed a level of catalepsy about half that of wild-types; (b) however, these dopamine D_2 receptor homozygotes and heterozygotes were as responsive as their wild-type counterparts to the cataleptogenic effects of the dopamine D_1-like receptors antagonist SCH 23390; (c) dopamine D_3 receptor mutant mice, on the whole, did not differ from their controls in the time spent in a cataleptic position following administration of either haloperidol or SCH 23390.

Acute pharmacological blockade of dopamine D_2-like receptors results in catalepsy in experimental animals, an effect thought to be primarily mediated by the dopamine D_2 receptors in the striatum (Ellenbroek et al., 1985). This hypothesis was reinforced by the finding that i.c.v. administration of antisense oligonucleotides directed against the mRNA coding for the dopamine D_2 receptor also elicits catalepsy in rodents (Zhang and Creese 1993; Quin et al., 1995). Likewise, the main finding of the present study strongly suggests that the dopamine D_2 receptor subtype is necessary for the induction of catalepsy by haloperidol. Dopamine $D_2^{-/-}$ receptor mice failed to display catalepsy following administration of doses of haloperidol that produced marked catalepsy in wild-type controls. The most parsimonious interpretation of these data is that the dopamine D_2 receptor is indeed the primary mediator of haloperidol-induced catalepsy, the absence of catalepsy in $D_2^{-/-}$ mice being due to the absence of the dopamine D_2 receptor subtype (as reflected by a near total absence of [^{3}H]spiperone and [^{125}I]iodosulpride binding in the striatum of these mice). Of equal significance was our finding that $D_2^{+/-}$ mice showed the same sensitivity to the cataleptogenic effects of haloperidol, even though the maximal cataleptic response seen at the highest doses tested was about half of that present in controls. As previously reported (Baik et al., 1995; Kelly et al., 1997), these heterozygous mice were shown to have levels of expression of striatal dopamine D_2 receptors reduced by about 50%, as indicated by [^{3}H]spiperone and [^{125}I]iodosulpride binding studies. This could mean that the magnitude of this pharmacologically induced catalepsy is proportional to the absolute number of dopamine D_2 receptors blocked. In other words, the biochemical events that ultimately result in catalepsy and that are triggered by the occupation of dopamine D_2 receptors by haloperidol, would be of lesser magnitude in these heterozygotes. It also suggests that in these mice, dopamine D_2 receptors do not become supersensitive.

In the present study, neither homozygous nor heterozygous dopamine D_2 receptor mutant mice presented spontaneous cataleptic behaviour. This is in accordance with others' (Kelly et al., 1997, 1998; but see Baik et al., 1995 for observation of a ''cataleptic-like'' behaviour using a ''ring-test'') as well as with our previous observations (Boulay et al., 1999a). Considering that it has been clearly established that blockade of dopamine D_2-like receptors results in catalepsy, and that this effect is thought to be primarily mediated by the D_2 subtype (see above), it appears rather surprising that D_2 receptor homozygous mice do not present spontaneous catalepsy. This would suggest that substantial compensatory mechanisms occur in these $D_2^{-/-}$ mice. Compensation could be taking place at the level of dopaminergic neurotransmission. For instance, augmented dopamine tone could counteract the absence of dopamine D_2 receptors: this increased tone might permit the activation of other subtypes of dopamine receptors to a greater extent than is seen under normal conditions. However, it appears that the level of tissue (Kelly et al., 1998; L'hirondel et al., 1998; Jung et al., 1999) or extracellular (Dickinson et al., 1999) dopamine is not elevated in $D_2^{-/-}$ mice. Alternatively, other subtypes of dopamine receptors might be expressed at a higher level, and/or become supersensitive to the effects of dopamine. Jung et al. (1999) have suggested that increased expression of dopamine D_3 receptors might partially substitute for the lack of dopamine D_2 receptors. Although a D_3 receptor mediated functional compensation seems to apply for the control of spontaneous locomotor activity (Jung et al., 1999), it appears not to be the case for some aspects of postural control (i.e., absence/presence of spontaneous catalepsy). Had D_3 receptors partially compensated, their blockade by haloperidol would have been expected to produce more or less severe catalepsy. Alternatively, it might be that in our $D_2^{-/-}$ mice, there is no, or a more limited, increase of the level of expression of D_3 receptors.

Based on the well known synergistic effects between dopamine D_1 and dopamine D_2 receptor antagonists on catalepsy in rodents (Klemm and Block, 1988; Parashos et al., 1989; Wanibuchi and Usuda, 1990), the dopamine D_1 receptor subtype appears to be another potential candidate for this compensation. However, if that were the case, $D_2^{-/-}$ and $D_2^{+/-}$ mice should have been differentially responsive to the cataleptogenic effects of the dopamine D_1-like receptors antagonist SCH 23390, but that was clearly not the case (see below). Of course, this absence of spontaneous catalepsy in dopamine D_2 receptor homozygotes could result from compensation mechanisms taking place at the level of one or more other neurotransmission systems such as the γ-amino butyric acid, acetylcholine, serotonin, etc... systems.

It was shown that the dopamine D_1-like receptor antagonist SCH 23390, known to induce catalepsy in rodents (Christensen et al., 1985), produced catalepsy in $D_2^{-/-}$ mice to an extent similar to that obtained in heterozygotes or wild-types. This result demonstrates that these mutant mice are able to display pharmacologically induced catalepsy. It also suggests that their resistance to the cataleptogenic effects of haloperidol most likely results from the absence of dopamine D_2 receptors, and not from the abnormal striatal synaptic plasticity that has been shown to occur in $D_2^{-/-}$ mice (Calabresi et al., 1997). The finding that D_2 mutants were no more responsive than controls to the cataleptogenic effects of SCH 23390 also tends to indicate that there is no major modification of the dopamine D_1 receptor system following mutation of the dopamine D_2 receptor gene. This is in line with our results and with data from the literature (Baik et al., 1995; Kelly et al., 1998), indicating that there is no detectable change in the dopamine D_1 receptor system (absence of dopamine D_1 receptor upregulation) as a result of the deletion of the dopamine D_2 receptor gene in these mice. Furthermore, in monoamine-depleted D_2 knock-out mice, there was only a small locomotor response to either a dopamine D_1 receptor agonist or to combined D_1 and D_2 receptor agonist treatment (Kelly et al., 1998). These authors suggested that the primary compensatory mechanisms that might account for the relative good locomotor function of D_2 knock-out mice do not result from dopamine D_1 receptor upregulation and/or supersensitivity.

Based on the observation that the putative dopamine D_3 receptor antagonist (+)-S 14297 could reverse catalepsy induced by haloperidol, Millan et al. (1997) proposed that blockade of dopamine D_3 receptors was able to oppose dopamine D_2 receptor-mediated catalepsy. From this hypothesis, one could have expected D_3 knock-out mice to have been less responsive to the cataleptogenic effects of haloperidol. However, the present data show that there was no clear difference in the responsivity of the three genotypes to haloperidol, though we found that D_3 receptor homozygous mice were somewhat less responsive than wild-types to the cataleptogenic effects of SCH 23390, this differential sensitivity being limited to a single dose (0.1 mg/kg). Nonetheless, based on the emerging evidence that the D_3 receptor might interact preferentially with the dopamine D_1 receptor (Xu et al., 1997; Schwartz et al., 1998), this preliminary result warranted further investigation. A combination of subthreshold doses of haloperidol (0.125 mg/kg) and SCH 23390 (0.03 mg/kg) produced, as expected, a synergistic effect, that was of a similar magnitude in the three genotypes. Although a more detailed investigation using several doses of haloperidol and SCH 23390, used alone and in combination, would have been necessary to more thoroughly test this hypothesis of preferential interaction, the present result does not support the possibility that dopamine D_3 receptor knock-out and wild-type mice would show differential sensitivity to the synergistic effects of concurrent blockade of dopamine D_2 and dopamine D_1 receptors in this model of catalepsy. This is in contrast with the greater synergistic effect in dopamine D_3 receptor homozygotes on locomotor activity for combined treatment with dopamine D_1-like (SKF 81297: (±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide) and dopamine D_2-like (PD 128907: (±)-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]-benzopyrano[4,3-*b*]-1,4-oxazin-9-ol hydrochloride) receptor agonists (Xu et al., 1997). In any case, radioligand binding studies reported here, in accordance with previous literature findings (Accili et al., 1996; Xu et al., 1997), failed to demonstrate a modification of dopamine D_1 or dopamine D_2 receptor expression in $D_3^{-/-}$ and $D_3^{+/-}$ mice, even though, as expected, D_3 receptor expression was reduced in these animals.

Under control conditions (after injection of vehicle), dopamine D_3 receptor homozygous and heterozygous mice did not present spontaneous cataleptic behaviour. This is in line with our previous findings (Boulay et al., 1999b), and with the absence of cataleptic behaviour in $D_3^{-/-}$ mice analysed in other laboratories (Accili et al., 1996; Xu et al., 1998). It is also consistent with the lack of catalepsy following treatment with antisense oligonucleotides directed against the mRNA coding for the dopamine D_3 receptor (Sun et al., 1996), or antagonists considered selective for dopamine D_3 receptors (Millan et al., 1995).

Based on correlational studies that compared the potency of a range of dopamine D_2/D_3 receptor agonists to induce a behavioural effect and their in vitro potency in a mitogenesis test, the dopamine D_3 receptor has been linked to the control of several functions, including locomotor activity and core temperature (see Boulay et al., 1999b for details). However, dopamine D_2 receptor homozygotes have been found to be totally unresponsive to the locomotor and core temperature decreasing effects of dopamine D_2/D_3 receptor agonists such as 7-OH-DPAT and PD 128907, whereas heterozygotes were about half as responsive as wild-types (Boulay et al., 1999a). By contrast, dopamine D_3 homozygotes and heterozygotes did not differ from their controls for these dopamine receptor agonist-induced effects (Boulay et al., 1999b; Xu et al., 1999). The present data extend these previous findings with regard to the absence/decrease of responsivity of dopamine $D_2^{-/-}$ and $D_2^{+/-}$ mice, and the normal responsivity of $D_3^{-/-}$ and $D_3^{+/-}$ mice, to the cataleptogenic effects of the dopamine D_2-like receptor antagonist haloperidol. Whether or not this is also the case for other effects of haloperidol (interference with active avoidance, anhedonic effects, etc...) or for the behavioural effects of other dopamine D_2-like receptor antagonists will need to be established. These pharmacological studies with dopamine D_2 and D_3 receptor knock-out mice (Kelly et al., 1998; Boulay et al., 1999a,b; Xu et al., 1999; present data) highlight the primary role that the D_2 subtype appears to play among the D_2-like family of dopamine receptors in the mediation of

several in-vivo effects induced by dopamine receptor ligands. Additional efforts will need to be deployed to assess the role of the other members (D_3 and D_4) of this family of receptors.

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