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Ciproxifan, a Histamine H₃-Receptor Antagonist/Inverse Agonist, Potentiates Neurochemical and Behavioral Effects of Haloperidol in the Rat

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By using double in situ hybridization performed with proenkephalin and H₃-receptor riboprobes on the same sections from rat brain, we show that histamine H₃ receptors are expressed within striatopallidal neurons of the indirect movement pathway. The majority (~70%) of striatal enkephalin neurons express H₃-receptor mRNAs. Similarly, whereas it was devoid of any motor effect when used alone, ciproxifan strongly potentiated haloperidol-induced locomotor hypoactivity and catalepsy, two behaviors in which striatal neurons are involved. The strong H₃-receptor mRNA expression in enkephalin neurons suggests that the synergistic neurochemical and motor effects of ciproxifan and haloperidol result from direct H₃/D₂-receptor interactions, leading to an enhanced activation of striatopallidal neurons of the indirect movement pathway. The potentiation of the effects of haloperidol by ciproxifan strengthens the potential interest of H₃-receptor antagonists/inverse agonists to improve the symptomatic treatment of schizophrenia.

Key words: histamine; H₃ receptor; ciproxifan; antagonist/inverse agonist; D₂ receptor; haloperidol; enkephalin; neurotensin; c-fos; in situ hybridization; catalepsy; locomotor activity

The histamine H₃ receptor (H₃R), a Gₛ/G₁₆-protein-coupled receptor, was identified as an autoreceptor controlling histamine neuron activity in the brain (Arrang et al., 1983, 1987). Thereafter, it was also shown to modulate the release of various neurotransmitters (Schlicker et al., 1994; Brown et al., 2001). It was recently cloned in human (Lovenberg et al., 1999), guinea pig (Tardivel-Lacombe et al., 2000), and rat (Lovenberg et al., 2000; Morisset et al., 2000; Drutel et al., 2001). Native H₃Rs display high constitutive activity, and H₂R antagonists/inverse agonists such as thioperamide and ciproxifan enhance histamine neuron activity in vivo (Ligneau et al., 1998; Morisset et al., 2000), a response primarily used to study the involvement of histaminergic neurons in various processes such as wakefulness and cognition (Onedera et al., 1994; Schwartz and Arrang, 2002).

Functional relationships between histamine and dopamine suggest that histaminergic systems could be involved in the pathophysiology of schizophrenia and/or the action of antipsychotics. In animals treated with methamphetamine (Ito et al., 1996; Morisset et al., 2002) as well as in patients with schizophrenia (Prell et al., 1995), hyperactivity of dopaminergic transmission is accompanied with an enhanced activity of histaminergic neurons. Typical neuroleptics decrease histamine neuron activity, whereas atypical antipsychotics stimulate histamine neurons, an effect that may underlie their pro-cognitive properties (Morisset et al., 1999). Thioperamide and ciproxifan attenuate the locomotor activation induced by dopaminergic agonists (Clapham and Kilpatrick, 1994; Morisset et al., 2002).

High densities of H₃Rs were found in the striatum where lesions indicated that most H₃Rs were present on projection neurons (Barbin et al., 1980; Cumming et al., 1991; Pollard et al., 1993; Ryu et al., 1994a,b, 1995; Anichtchik et al., 2000). In agreement, high densities of H₃R mRNAs were found in the striatum from rat (Lovenberg et al., 1999; Morisset et al., 2001; Drutel et al., 2001; Pillot et al., 2002), guinea pig (Tardivel-Lacombe et al., 2000), and human (Anichtchik et al., 2001).

These observations suggested the presence of H₃Rs on medium spiny neurons, which represent >90% of striatatal neurons (Gerfen, 1992; Parent and Harati, 1995). In agreement, various approaches indicated that H₃Rs are present on striatonigral neurons of the direct movement pathway. Striatal quinolinic acid lesions decreased, and 6-OHDA lesions increased, the number of H₃Rs in the striatum and substantia nigra, respectively (Ryu et al., 1994a, 1996). Moreover, activation of H₃Rs inhibited D₁-receptor
dependent GABA release in rat substantia nigra and striatum (Garcia et al., 1997; Arias-Montano et al., 2001).

In the present work, we have explored the presence and role of H₃Rs on GABAergic striatopallidal neurons of the indirect movement pathway, known to contain enkephalin. To this purpose we have (1) analyzed the expression of H₃R mRNAs in striatal enkephalin neurons by double in situ hybridization, (2) evaluated the effect of H₃R ligands on enkephalin and neurotensin expression in the striatum, and (3) assessed the effect of H₃R ligands on catalepsy and spontaneous locomotor activity.

MATERIALS AND METHODS

Tissue preparation. All animal experiments performed in the present study conformed to the National Institutes of Health guidelines (decreet number 2001–464, May 29, 2001, from the French Ministry of Agriculture). When required, drugs dissolved in saline solution (0.9% NaCl w/v) were administered intraperitoneally. After treatment, male Wistar rats (Ifca-Credo, L’Arbresle, France) were killed by decapitation, their brains were removed rapidly, immediately frozen (−40°C) by immersion in monochlorodifluoromethane, and stored at 0°C. Brain sections (10 μm) were prepared on a cryostat, thaw-mounted onto Superfrost slides, and immediately fixed for 40 min at 4°C in 4% paraformaldehyde made up in 0.1 M PBS, pH 7.4, and 0.1% diethylpyrocarbonate water. Sections were rinsed three times (5 min each) in 0.1 M PBS, pH 7.4, dehydrated through graded ethanol, and dried under a stream of cold air. All the sections were stored at −70°C until use.

In situ hybridization histochemistry. Sections were incubated at 37°C for 10 min with protease K (1 μg/ml), acetylated for 10 min (0.1 M triethanolamine, pH 8, and 0.25% acetic anhydride) at room temperature, and dehydrated in graded ethanol up to 100%. Hybridization was performed overnight at 55°C in the presence of 4 × 10⁶ dpm of 35P-radiolabeled cRNA probes in hybridization buffer (50% formamide, 10% dextran sulfate, 2× SSC, 1× Denhardt’s solution, 50 mM Tris-Cl buffer, 0.1% NaPSS, 0.1 mg/ml yeast tRNA, 0.1 mg/ml salmon sperm DNA, and 1 mM EDTA). Subsequently, the sections were rinsed with 2× SSC for 5 min and incubated for 40 min at 37°C with RNase A (200 μg/ml). The sections were then extensively washed in SSC, dehydrated in graded ethanol, dried, and exposed for 8–10 d (H₃R, pronenkephalin, c-fos) to a 35P-labeled antisense riboprobe revealed a high expression of H₃R and PE mRNAs (Fig. 1, B). For the study of the coexpression of H₃R mRNAs and proenkephalin mRNA expression (PE) mRNAs, sections hybridized with the proenkephalin cRNA probe were exposed only for 8–10 hr.

For the hybridization probes, a partial coding sequence of the rat H₃R was amplified from striatum cDNAs using primers 1 and 2 based on the sequence of the third transmembrane domain and the third intracellular loop of the human H₃R, respectively (Lovenberg et al., 1999) (primer 1: 5’-AGTCCGTATCACGTACCCGCTTCTGTC-3’ and primer 2: 5’-AGTCACGCCTCGTGTGGA-3’). The amplified fragment was sequenced and corresponded to nucleotides 636 to 1243 of the rat H₃R sequence. It was previously shown to hybridize to the various H₃R mRNA isoforms expressed in the brain or peripheral tissues (Héron et al., 2001; Morisset et al., 2001). The probes for proenkephalin, pronenkephalin, and c-fos were also obtained by PCR and corresponded to nucleotides 335–641, nucleotides 169–510, and nucleotides 583–790, respectively. After subcloning of the PCR products into pGEM-4Z (Promega, Charbonnières, France), 35P-labeled antisense RNA probes were prepared by in vitro transcription using a Riboprobe kit (Promega).

For the study of the coexpression of H₃R mRNAs and proenkephalin mRNAs, sections were covered overnight with 50 μl of the hybridization buffer containing the 35P-labeled cRNA probe for the H₃R and a digoxigenin-labeled cRNA probe for proenkephalin. After incubation overnight, the sections were rinsed in SSC and treated with RNase A as described above. The detection of the digoxigenin-labeled probe was performed as described (Bordet et al., 2000). Briefly, the sections were incubated overnight at 4°C with a phosphatase-conjugated antidigoxigenin antibody (Boehringer Mannheim, Mannheim, Germany). After washing, each slide was covered with 500 μl of a chromogen solution, containing nitroblue tetrazolium chloride, 5-bromo-4-chloro-3-indoly phosphate, and levamisole, to visualize the conjugated antibody. After completion of the reaction at room temperature and in the dark, the slides were washed and rinsed in distilled water. For the detection of the 35P-labeled riboprobe, the slides were dipped in Ilford K-5 liquid photographic emulsion for 2 weeks. Dipped sections were then observed with a photomicroscope (Axioskop Zeiss, Carl Zeiss, Germany).

Spontaneous locomotor activity. After saline or drug administration, rats were immediately introduced into an actimeter (Imétronic, Pessac, France), consisting in individuals boxes placed in a quiet room. Spontaneous locomotor activity of the animals was evaluated for 60 min by numbering infrared crossed beams.

Assessment of catalepsy. Catalepsy was assessed in an all-or-none manner 2 hr after intraperitoneal administration of the drugs. Each rat was placed gently so that both front limbs rested on top of an horizontal rod placed at a height of 10 cm above the floor. An animal was considered to be in catalepsy if it remained with its hind legs on the floor and its front limbs on the rod for >5 sec.

Data analysis. For in situ hybridization, mRNA signals generated in the caudate–putamen and nucleus accumbens were quantified on two to three sections per animal using a camera and an image analyzer with Starwse/Autorad 210 program (Imstar, Paris, France). Results were means ± SEM of values from 4–10 rats and were expressed as percentages of mRNA levels in control (saline) rats. Statistical evaluation of the results was performed using one-way ANOVA followed by Student–Newman–Keuls test.

For spontaneous locomotor activity, cumulative results were analyzed using one-way ANOVA followed by Student–Newman–Keuls test. When repeated measures were performed at each 10 min interval, between-group differences were analyzed with the Statistica software using two-way ANOVA followed by least significance difference (LSD) post hoc tests.

Radiochemicals and drugs. Ciprofloxin and (R)-α-methylhistamine were from Bioprejet (Paris, France). Haloperidol (HAL) was from Janssen Pharmaceutica (Beerse, Belgium).

RESULTS

Localization of H₃ receptor and proenkephalin gene transcripts in the striatum

Autoradiograms from frontal sections generated with selective antisense riboprobes revealed a high expression of H₃R and PE mRNAs both in the caudate–putamen and nucleus accumbens (Fig. 1, A, B).

The cellular location of both transcripts was analyzed in the caudate–putamen using a 35P-labeled H₃R riboprobe and a digoxigenin-labeled PE riboprobe. The vast majority of striatal neurons expressed H₃R mRNAs, whereas PE mRNA expression was restricted to a smaller population of neurons, in which it occurred at an apparently variable level (Fig. 1C). Among PE mRNA-expressing neurons, a limited number did not express H₃R mRNAs (Fig. 1D), but the majority (~70%) coexpressed H₃R and PE mRNAs (Fig. 1F).

Effect of haloperidol and ciprofloxin on striatal proenkephalin mRNA expression

HAL moderately but significantly increased proenkephalin mRNA expression in the striatum as compared with saline administration (controls) (Fig. 2). In the caudate–putamen, a significant HAL-induced upregulation (by 30–40%) was found at 1, 2, and 20 mg/kg. In the nucleus accumbens, the increase in PE mRNA expression induced by HAL occurred to a similar extent (~40%; p < 0.001) at 1 mg/kg but did not reach statistical significance at 2 and 20 mg/kg (Table 1, Fig. 2). The administration of ciprofloxin, an H₃R antagonist/inverse agonist (1.5 mg/kg, i.p.), did not modify by itself PE mRNA expression in the caudate–putamen and nucleus accumbens (99 ± 8% and 102 ± 5% of controls, respectively) (Fig. 2), but potentiated HAL-induced upregulation in both regions. Both in the caudate–putamen and nucleus accumbens, ciprofloxin significantly potentiated (by 60–70%) the upregulation evoked by 1 mg/kg of haloperidol (Fig. 2, Table 1), an effect that was completely blocked after coadministration of (R)-α-methylhistamine, an H₃R agonist (10 mg/kg, i.p.)
The ciproxifan-evoked potentiation observed in both regions was not observed or did not reach statistical significance when the same dose of ciproxifan was coadministered with 2 or 20 mg/kg of haloperidol (Table 1).

**Effect of haloperidol and ciproxifan on striatal proneurotensin mRNA expression**

The level of proneurotensin mRNA expression observed in the striatum after intraperitoneal administration of saline (control) was very low (Fig. 3). It was dramatically increased in the caudate–putamen and nucleus accumbens 3 hr after intraperitoneal administration of haloperidol. HAL-evoked upregulation was much higher in the caudate–putamen (particularly in its dorsolateral part) than in the nucleus accumbens with 100-fold and sevenfold increases, respectively. In both regions, the effect of haloperidol was dose-dependent with a subthreshold increase observed at 1 mg/kg and the maximal change reached at 2 mg/kg and 20 mg/kg (Table 2, Fig. 3). Ciproxifan used alone (1.5 mg/kg, i.p.), did not modify striatal proneurotensin mRNA expression, which represented 102 ± 5% and 86 ± 14% in the caudate–putamen and nucleus accumbens, respectively, but potentiated HAL-induced upregulation (Fig. 3). In both regions, ciproxifan potentiated by 70% the upregulation evoked by 1 mg/kg of haloperidol (Fig. 3, Table 2). This effect was reduced by 80% in the caudate–putamen and was completely blocked in the nucleus accumbens, after coadministration of (R)-α-methylhistamine (10 mg/kg, i.p.) (Fig. 3). The potentiation evoked by ciproxifan was strongly dependent on the dose of haloperidol and was no more

![Figure 1. Colocalization of H3R and PE mRNAs in rat striatum. A, B. Autoradiographic distribution of H3R (A) and PE (B) gene transcripts in frontal sections of the rat brain (interaural distance: 10.2 mm), visualized using 32P-labeled antisense riboprobes. C–F. Sections of the caudate–putamen were hybridized with a H3R 32P-labeled- and a PE digoxigenin-labeled antisense riboprobe. The cellular localization of PE mRNAs was revealed in a first step using a anti-digoxigenin antibody, and the colocalization of H3R mRNAs with the latter was revealed in a second step using a photographic emulsion (bright-field photomicrographs). Among neurons expressing PE mRNAs (in dark), ~70% coexpressed H3R mRNAs (C) (neurons expressing PE mRNAs alone or together with H3R mRNAs are shown at a higher magnification in D and E, respectively). Note that many striatal neurons did not express PE mRNAs but expressed H3R mRNAs (as revealed by dark autoradiographic grains in C and E).](image)

![Figure 2. Potentiation by ciproxifan of the upregulation of proenkephalin mRNA expression elicited by haloperidol in the rat striatum. Top. The proenkephalin mRNAs were visualized by in situ hybridization on films 3 hr after intraperitoneal administration of saline solution (CONTROL), haloperidol (HAL, 1 mg/kg) alone or together with ciproxifan (CPX, 1.5 mg/kg), and, when required, (R)-α-methylhistamine [(R)α-MeHA, 10 mg/kg]. Bottom. Quantification of mRNA signals observed in the caudate–putamen and nucleus accumbens. Results are means ± SEM of values from 5–10 animals, expressed as percentage of proenkephalin mRNA level in control rats. **p < 0.001 versus control; °p < 0.05, **p < 0.01 versus HAL; °°p < 0.05, **°p < 0.01 versus HAL + CPX.](image)
observed when ciproxifan was coadministered with 2 or 20 mg/kg of haloperidol (Table 2).

Effect of haloperidol and ciproxifan on striatal c-fos mRNA expression

A threefold to fourfold increase of c-fos mRNA expression was found in the caudate–putamen and nucleus accumbens 1 hr after administration of haloperidol (1 mg/kg, i.p.) (Fig. 4). Ciproxifan (1.5 mg/kg, i.p.) did not change by itself c-fos mRNA expression (data not shown), but significantly potentiated (by 60 and 80%, respectively) the upregulation induced by haloperidol in both regions (Fig. 4).

Effect of haloperidol and ciproxifan on spontaneous locomotor activity and catalepsy

Spontaneous locomotor activity of rats was measured for 60 min after intraperitoneal administration of the drugs, and two-way ANOVA indicated that it decreased with time ($F_{(5,450)} = 106.65; p < 0.0001$) (Fig. 5A). A low dose of haloperidol (0.1 mg/kg, i.p.) induced a significant hypolocomotor effect, as compared with saline (controls) ($F_{(1,66)} = 10.93; p = 0.001$) (Fig. 5A). The cumulative measurement for 60 min showed that haloperidol decreased the overall spontaneous activity by 30% (Fig. 5B). Neither ciproxifan (1.5 mg/kg, i.p.) nor ($R$)-α-methylhistamine (10 mg/kg, i.p.) did modify spontaneous locomotor activity, as indicated by the cumulative values for 60 min, which represented 112 ± 11% and 100 ± 11% of controls, respectively (Fig. 5B). However, ciproxifan used at the same dose significantly potentiated (by 70%) the hypolocomotor effect of haloperidol ($F_{(1,59)} = 9.56; p = 0.003$). Post hoc analysis revealed a significant potentiation by ciproxifan after 10 min ( $p < 0.0001$) and at set times 20, 40, and 50 min, the hypolocomotion induced by the coadministration of haloperidol and ciproxifan reached a higher degree of significance compared with controls than that induced by haloperidol alone (Fig. 5A). In addition, the cumulative locomotor activity for 60 min represented 66 ± 5% and 42 ± 4% of controls after administration of haloperidol alone or in combination with ciproxifan, respectively, leading to a 70% potentiation by ciproxifan ($p < 0.01$) (Fig. 5B). This potentiating effect was completely blocked by the coadministration of ($R$)-α-methylhistamine because the spontaneous locomotor activity then represented 77 ± 12% of controls (Fig. 5B).

The same low dose of haloperidol (0.1 mg/kg) induced catalepsy by 70% potentiation by ciproxifan ( $p < 0.01$) (Fig. 5B).

### Table 1. Effect of ciproxifan on the upregulation of proenkephalin mRNA expression elicited by administration of haloperidol in increasing dosages

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proenkephalin mRNA (% of control)</th>
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<tr>
<td></td>
<td>Caudate–putamen</td>
</tr>
<tr>
<td>Control</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>HAL 1 mg/kg</td>
<td>134 ± 5***</td>
</tr>
<tr>
<td>HAL 1 mg/kg + CPX</td>
<td>154 ± 7***</td>
</tr>
<tr>
<td>HAL 2 mg/kg</td>
<td>141 ± 18*</td>
</tr>
<tr>
<td>HAL 2 mg/kg + CPX</td>
<td>171 ± 20***</td>
</tr>
<tr>
<td>HAL 20 mg/kg</td>
<td>142 ± 12*</td>
</tr>
<tr>
<td>HAL 20 mg/kg + CPX</td>
<td>164 ± 10**</td>
</tr>
</tbody>
</table>

The proenkephalin mRNAs visualized on films 3 hr after intraperitoneal administration of saline (control), haloperidol (HAL, 1, 2, or 20 mg/kg) alone or in combination with ciproxifan (CPX, 1.5 mg/kg), were quantified in the caudate–putamen and nucleus accumbens. Results are means ± SEM of values from 4–10 animals, expressed as percentage of proenkephalin mRNA level in control rats. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus control; ¥ $p < 0.05$, ¥¥ $p < 0.01$ versus HAL.

Figure 3. Potentiation by ciproxifan of the upregulation of pronorotensin mRNA expression elicited by haloperidol in the rat striatum. Top, The pronorotensin mRNAs were visualized in situ hybridization on films 3 hr after intraperitoneal administration of saline solution (CONTROL), haloperidol (HAL, 1 mg/kg) alone or together with ciproxifan (CPX, 1.5 mg/kg), and, when required, ($R$)-α-methylhistamine [(R)-α-MeHA, 10 mg/kg]. Bottom, Quantification of mRNA signals observed in the caudate–putamen and nucleus accumbens. Results are means ± SEM of values from 4–10 animals, expressed as percentage of pronorotensin mRNA level in control rats. * $p < 0.05$, ** $p < 0.001$ versus control; ¥ $p < 0.05$, ¥¥ $p < 0.01$ versus HAL.
Hybridization. H3R mRNA expression itself may be dependent on that we observed within neurons positively labeled by may account for the variable density of proenkephalin mRNAs activity (Gerfen et al., 1990; Angulo and McEwen, 1994), which parallels their expression is selectively found in striatopallidal neurons. Enkephalin mRNAs that we evidence here shows that they are also present on projection neurons of the indirect pathway. Enkephalin expression is inhibited by D2 receptors, leading to its increase by haloperidol in the nucleus accumbens. Moreover, the two systems responded differently to haloperidol because the dose of 1 mg/kg induced subthreshold neurotensin and submaximal enkephalin upregulations, respectively.

An important finding is that ciproxifan, a H3R antagonist/ inverse agonist (Ligneau et al., 1998; Morisset et al., 2000), strongly potentiated the upregulation of proenkephalin and neurotensin mRNAs elicited by haloperidol. This potentiation...
HAL in two-way ANOVA followed by LSD post hoc 
p
cumulative photocell counts were measured for 60 min after administra-
followed by Newman–Keuls test. (A) Each point represents the cumulative photocell counts measured for each 10 min interval during 1 hr. *p < 0.05, **p < 0.01, ***p < 0.001 versus control; ‡p < 0.001 versus HAL in two-way ANOVA followed by LSD post hoc tests. B, The cumulative photocell counts were measured for 60 min after administration of the compounds. Results are means ± SEM expressed as percentage of the value obtained for control rats. *p < 0.001 versus control; ‡p < 0.01 versus HAL; #p < 0.05 versus HAL+CPX in one-way ANOVA followed by Newman–Keuls test.

occurred in the caudate–putamen and nucleus accumbens and was H3R-mediated, being suppressed by (R)-α-methylhistamine, a prototypical H3R agonist (Arrang et al., 1987). However, the mechanisms involved remain unclear. H3Rs modulate striatal dopamine and glutamate release (Schlicker et al., 1994; Molina-Hernandez et al., 2001) but their in vivo contribution remains doubtful (Blandina et al., 1998). Similarly, it remains unclear if D2-receptors upregulating enkephalin are activated by endoge-
ous dopamine under basal conditions (Wang and McGinty, 1997; Alburges et al., 2001). The absence of modulation by ciproxifan used alone and in situ hybridization data rather suggest that the effect of ciproxifan results from synergistic interactions between H3 and D2 receptors located within the same striatal neurons. Most enkephalin neurons express H3 and D2 receptors (Le Moine and Bloch, 1995), indicating that both receptors are coexpressed in striatopallidal neurons. Therefore, the potentia-
tion of haloperidol by ciproxifan may result from direct synergis-
tic interactions between H3 and D2 receptors via their transduc-
tion pathway or pathways, inasmuch as both receptors couple to G(1,5)/G(3)-proteins in the brain (Vallar and Meldolesi, 1989; Clark and Hill, 1996; Takeshita et al., 1998). Consistent with such synergistic interactions, ciproxifan potentiated a subthreshold dose of haloperidol (1 mg/kg) but not maximally effective doses (2–20 mg/kg) on neurotensin upregulation.

Previous studies suggested that proenkephalin and neurotensin genes were physiological targets for Fos (Sonnenberg et al., 1990; Merchant and Dorsa, 1993; Merchant, 1994). Haloperidol up-
regulates Fos expression in the caudate–putamen and nucleus accumbens (Dragunow et al., 1990; Deutch et al., 1992; Nguyen et al., 1992; Merchant and Miller, 1994) and predominantly within striatopallidal neurons (Robertson et al., 1992). Interestingly, ciproxifan potentiated the haloperidol-induced upregulation of c-fos mRNAs, but had no effect when used alone, suggesting the involvement of Fos in the potentiation of neuropeptide expression.

The effect of ciproxifan on c-fos, a marker of neuronal activation (Morgan and Curran, 1991), further suggests that H3R antagonists/inverse agonists potentiate the activation of striato-
pallidal neurons induced by neuroleptics. The synergistic motor effects of ciproxifan and haloperidol are also consistent with this proposal. Blockade of D2 receptors, by activating striatopallidal 
neurons of the indirect pathway, leads to inhibition of motor functions, e.g., catalepsy and locomotor hypoactivity. Therefore, the enhanced activation of striatopallidal neurons by ciproxifan was expected to potentiate haloperidol-induced motor effects. Indeed, ciproxifan dramatically potentiated haloperidol-induced catalepsy. This effect was suppressed by (R)-α-methylhistamine, confirming the involvement of H3Rs. Although a functional distinc-
tion between the dorsal striatum and nucleus accumbens is not entirely well founded (Carlsson, 1993; Carlsson et al., 1997) and additional structures might contribute to catalepsy (Hauber, 1998), the crucial role of the dorsal striatum in voluntary move-
ments (Albin et al., 1995) suggests that the potentiation of catalepsy results from H3/D2-receptor interactions in this structure. Recently (Morisset et al., 1999), we failed to detect in mice the poten-
tiation of haloperidol-induced catalepsy that we evidence here in rats. However, besides species differences, we used a higher dose of haloperidol in mice. This may suggest that the potentiation of catalepsy is also dependent on the dose of halo-
peridol and supports synergistic interactions between D2 and H3 receptors. The absence of catalepsy after administration of cip-
roxifan alone in mice (Morisset et., 1999) or rats suggests that H3Rs are not involved in this behavior under basal conditions. Interestingly, H3Rs do not regulate dopamine neuron activity in

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Catalepsy</th>
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<tr>
<td>HAL (0.1 mg/kg)</td>
<td>2/12</td>
</tr>
<tr>
<td>CPX (1.5 mg/kg)</td>
<td>0/8</td>
</tr>
<tr>
<td>HAL + CPX</td>
<td>12/12</td>
</tr>
<tr>
<td>HAL + CPX + (R)α-MeHA (10 mg/kg)</td>
<td>3/8</td>
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Catalepsy was assessed in an all-or-none manner 2 hr after intraperitoneal admin-
istration of the drugs. The number of animals in catalepsy out of the total number of animals (8–12) is indicated.
vivo (Oishi et al., 1990; Imaizumi and Onodera, 1993; Miyazaki et al., 1997). H3R antagonists/inverse agonists are proposed to improve cognitive deficits (Ligneau et al., 1998; Bacciottini et al., 2001). Our data predict that no extrapyramidal side effects should result from their therapeutic use.

As also expected from an enhanced activation of striatopallidal neurons, ciproxifan potentiated haloperidol-induced locomotor hypoactivity. This effect may result from an enhanced activation of neurons from the nucleus accumens, known to play a crucial role in the regulation of locomotor function (Svensson et al., 1995). Like thioperamide (Imaizumi and Onodera, 1993; Clapham and Kilpatrick, 1994), ciproxifan did not change spontaneous locomotor activity when used alone. We also confirmed that activation of H3R by (R)-α-methylhistamine had no effect (Clapham and Kilpatrick, 1994). These data on catalepsy and locomotor activity suggest that H3R do not play an important role in motor functions under basal conditions, a proposal consistent with our neurochemical findings.

The potentiation of haloperidol by ciproxifan suggests that endogenous histamine and dopamine cooperate to modulate the activity of the indirect pathway. However, native H3R in brain display high constitutive activity that is abrogated by ciproxifan acting as an inverse agonist (Morisset et al., 2000; Rouleau et al., 2002). H3Rs mediating the present effects may therefore be spontaneously active in the absence of histamine. No other data are available on the effect of endogenous histamine on neuropeptide expression and catalepsy. Central administration of histamine modulated spontaneous locomotor activity (Nisticò et al., 1980; Tuonisto and Erikkson, 1980; Kalivas, 1982; Bristow and Bennett, 1988; Chiavegatto et al., 1998). However, the role of endogenous histamine remained unclear (Sakai et al., 1992, Inoue et al., 1996; Yanai et al., 1998), and our data do not support such a role under basal conditions because ciproxifan, which potently enhances histamine release in vivo (Ligneau et al., 1998; Morisset et al., 2000), did not modify locomotor activity when used alone.

The locomotor hypoactivity induced by ciproxifan was revealed when the dopaminergic transmission was reduced by haloperidol. Interestingly, ciproxifan and thioperamide also decreased locomotion induced by dopaminergic agonists (Clapham and Kilpatrick, 1994; Morisset et al., 2002). Whether these hypoactivities result from the same mechanisms remains unknown. The involvement of H3Rs coexpressed with D2 receptors in striatopallidal neurons would suggest that histamine cooperates with dopamine to induce motor hyperactivity. However, previous studies suggested that endogenous histamine inhibits motor hyperactivity induced by methamphetamine (Itoh et al., 1984; Clapham and Kilpatrick, 1994; Ito et al., 1997; Morisset et al., 2002).

In summary, the H3R mRNA expression in enkephalin neurons and the synergistic neurochemical and motor effects of ciproxifan and haloperidol support the existence of direct functional H3/D2-receptor interactions in striatopallidal neurons of the indirect pathway. In addition to their procognitive properties against the negative symptomatology of the disease (Morisset et al., 1999), the potentiation of the effects of haloperidol by ciproxifan suggests that H3R antagonists/inverse agonists might be helpful to improve the symptomatic treatment of schizophrenia.

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