Ciproxifan, a Histamine H_3-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 H_3-receptor riboprobes on the same sections from rat brain, we show that histamine H_3 receptors are expressed within striatopallidal neurons of the indirect movement pathway. The majority (~70%) of striatal enkephalin neurons express H_3-receptor mRNAs.

This important degree of coexpression of proenkephalin and H_3-receptor mRNAs prompted us to explore the effect of H_3-receptor ligands on the regulation of enkephalin mRNA expression in the striatum. Acute administration of ciproxifan, a H_3-receptor antagonist/inverse agonist, did not modify the expression of the neuropeptide by itself but strongly increased the upregulation of its expression induced by haloperidol. This potentiation (1) was suppressed by the administration of (*R*)-α-methylhistamine, a H_3-receptor agonist, (2) occurred both in the caudate–putamen and nucleus accumbens, and (3) was also observed with a similar pattern on c-fos and neurotensin mRNA expression.

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 H_3-receptor mRNA expression in enkephalin neurons suggests that the synergistic neurochemical and motor effects of ciproxifan and haloperidol result from direct H_3/D_2-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 H_3-receptor antagonists/inverse agonists to improve the symptomatic treatment of schizophrenia.

Key words: histamine; H_3 receptor; ciproxifan; antagonist/inverse agonist; D_2 receptor; haloperidol; enkephalin; neurotensin; c-fos; in situ *hybridization; catalepsy; locomotor activity*

The histamine H_3 receptor (H_3R), a G_i/G_o-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 H_3Rs display high constitutive activity, and H_3R 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 (Onodera 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 H_3Rs were found in the striatum where lesions indicated that most H_3Rs 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 H_3R 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 H_3Rs on medium spiny neurons, which represent >90% of striatal neurons (Gerfen, 1992; Parent and Harati, 1995). In agreement, various approaches indicated that H_3Rs are present on striatonigral neurons of the direct movement pathway. Striatal quinolinic acid lesions decreased, and 6-OHDA lesions increased, the number of H_3Rs in the striatum and substantia nigra, respectively (Ryu et al., 1994a, 1996). Moreover, activation of H_3Rs inhibited D_1-receptor

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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 H_3Rs on GABAergic striatopallidal neurons of the indirect movement pathway, known to contain enkephalin. To this purpose we have (1) analyzed the expression of H_3R mRNAs in striatal enkephalin neurons by double *in situ* hybridization, (2) evaluated the effect of H_3R ligands on enkephalin and neurotensin expression in the striatum, and (3) assessed the effect of H_3R 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 (décret 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 (Iffa-Credo, L'Arbresle, France) were killed by decapitation, their brains were removed rapidly, immediately frozen (−40°C) by immersion in monochlorodifluoromethane, and stored at −70°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 proteinase K (1 μg/ml), acetylated for 10 min (in 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 4×10^6 dpm of ^{33}P-radiolabeled cRNA probes in hybridization buffer (50% formamide, 10% dextran sulfate, 2× SSC, 1× Denhardt's solution, 50 mM Tris-HCl buffer, 0.1% NaPPi, 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 (H_3R, proneurotensin, c-fos) to a β_{max} Hyperfilm (Amersham, UK). To avoid overexposure of the film caused by the inherently high striatal expression of proenkephalin (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 H_3R 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 H_3R, respectively (Lovenberg et al., 1999) (primer 1: 5′-AGTCGGATCCAGCTACGACCGCTTCCTGTC-3′ and primer 2: 5′-AGTCAAGCTTGGAGCCCCTCTTGAGTGAGC-3′). The amplified fragment was sequenced and corresponded to nucleotides 636 to 1243 of the rat H_3R sequence. It was previously shown to hybridize to the various H_3R mRNA isoforms expressed in the brain or peripheral tissues (Héron et al., 2001; Morisset et al., 2001). The probes for proenkephalin, proneurotensin, 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), ^{33}P-labeled antisense RNA probes were prepared by *in vitro* transcription using a Riboprobe kit (Promega).

For the study of the coexpression of H_3R mRNAs and proenkephalin mRNAs, sections were covered overnight with 50 μl of the hybridization buffer containing the ^{33}P-labeled cRNA probe for the H_3R 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-indolyl 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 ^{33}P-labeled riboprobe, the slides were dipped in Ilford K-5 liquid photographic emulsion for 2 weeks. Dipped sections were then observed with a photomicroscope (Axiophot 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 Starwise/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. Ciproxifan and (*R*)-α-methylhistamine were from Bioprojet (Paris, France). Haloperidol (HAL) was from Janssen Pharmaceutica (Beerse, Belgium).

RESULTS

Localization of H_3 receptor and proenkephalin gene transcripts in the striatum

Autoradiograms from frontal sections generated with selective antisense riboprobes revealed a high expression of H_3R 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 ^{33}P-labeled H_3R riboprobe and a digoxigenin-labeled PE riboprobe. The vast majority of striatal neurons expressed H_3R mRNAs, whereas PE mRNA expression was restricted to a smaller population of neurons, in which it occurred at an apparently variable level (Fig. 1*C*). Among PE mRNA-expressing neurons, a limited number did not express H_3R mRNAs (Fig. 1*D*), but the majority (~70%) coexpressed H_3R and PE mRNAs (Fig. 1*F*).

Effect of haloperidol and ciproxifan 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 ciproxifan, an H_3R 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 ± 8% of controls, respectively) (Fig. 2), but potentiated HAL-induced upregulation in both regions. Both in the caudate–putamen and nucleus accumbens, ciproxifan 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 H_3R agonist (10 mg/kg, i.p.)

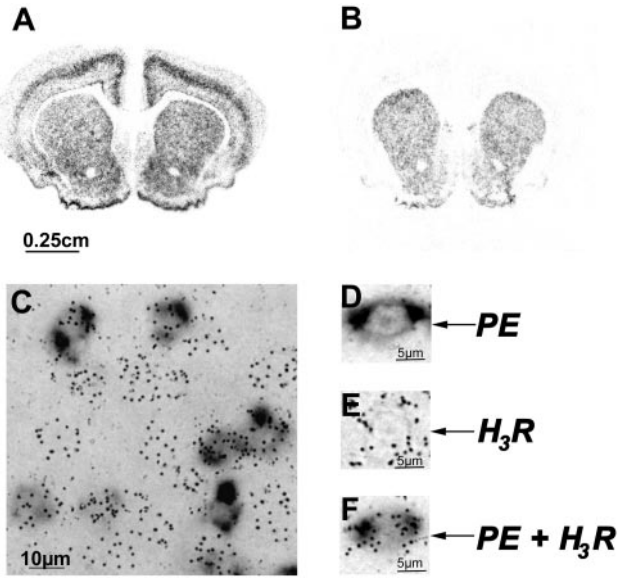

Figure 1. Colocalization of H_3R and PE mRNAs in rat striatum. *A, B,* Autoradiographic distribution of H_3R (*A*) and PE (*B*) gene transcripts in frontal sections of the rat brain (interaural distance: 10.2 mm), visualized using ^{33}P-labeled antisense riboprobes. *C–F,* Sections of the caudate–putamen were hybridized with a H_3R ^{33}P-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 H_3R 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 H_3R mRNAs (*C*) (neurons expressing PE mRNAs alone or together with H_3R mRNAs are shown at a higher magnification in *D* and *F,* respectively). Note that many striatal neurons did not express PE mRNAs but expressed H_3R mRNAs (as revealed by dark autoradiographic grains in *C* and *E*).

(Fig. 2). 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

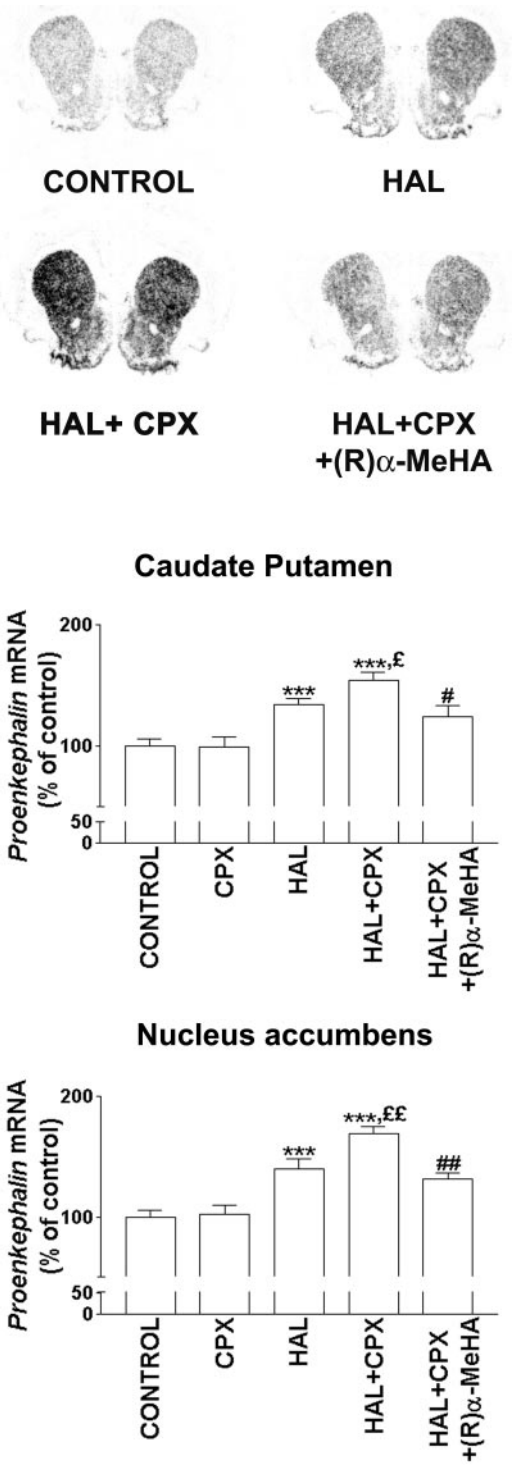

Figure 2. Potentiation by ciproxifan of the upregulation of proenkephalin mRNA expression elicited by haloperidol in the rat striatum. *Top,* The proenkephalin mRNAs were visualized by *in situ* hybridization on films 3 hr after intraperitoneal administration of saline solution (*CONTROL*), haloperidol (*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.

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

	Proenkephalin mRNA (% of control)	
Treatment	Caudate–putamen	Nucleus accumbens
Control	100 ± 6	100 ± 6
HAL 1 mg/kg	134 ± 5***	140 ± 8***
HAL 1 mg/kg + CPX	154 ± 7***,£	169 ± 6***,££
HAL 2 mg/kg	141 ± 18*	125 ± 19
HAL 2 mg/kg + CPX	171 ± 20***	162 ± 37
HAL 20 mg/kg	142 ± 12*	187 ± 59
HAL 20 mg/kg + CPX	164 ± 10**	172 ± 32

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.

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. 5*A*). 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. 5*A*). The cumulative measurement for 60 min showed that haloperidol decreased the overall spontaneous activity by 30% (Fig. 5*B*). 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. 5*B*). 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 ($p < 0.001$) 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. 5*A*). 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. 5*B*). 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. 5*B*).

The same low dose of haloperidol (0.1 mg/kg) induced cata-

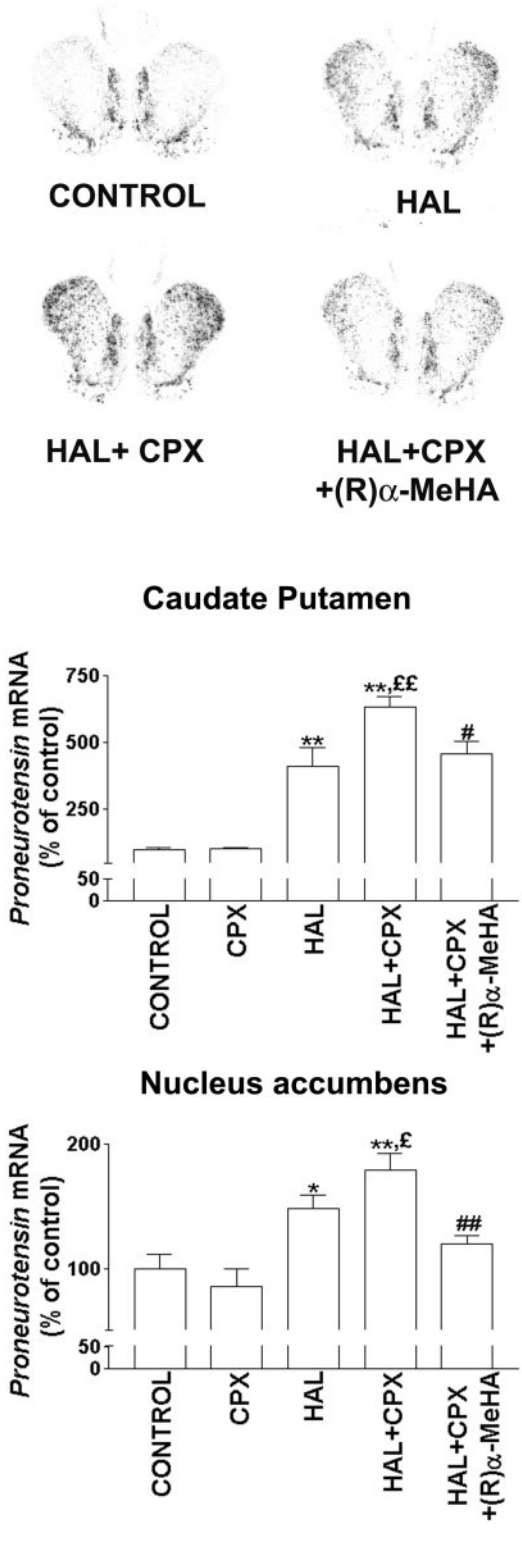

Figure 3. Potentiation by ciproxifan of the upregulation of proneurotensin mRNA expression elicited by haloperidol in the rat striatum. *Top,* The proneurotensin mRNAs were visualized by *in situ* hybridization on films 3 hr after intraperitoneal administration of saline solution (*CONTROL*), haloperidol (*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 4–10 animals, expressed as percentage of proneurotensin mRNA level in control rats. $^{*}p < 0.05$, $^{**}p < 0.001$ versus control; $^{£}p < 0.05$, $^{££}p < 0.01$ versus HAL; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus HAL + CPX.

Table 2. Effect of ciproxifan on the upregulation of proneurotensin mRNA expression elicited by administration of haloperidol in increasing dosages

	Proneurotensin mRNA (% of control)	
Treatment	Caudate–putamen	Nucleus accumbens
Control	100 ± 6	100 ± 11
HAL 1 mg/kg	409 ± 70**	148 ± 11*
HAL 1 mg/kg + CPX	632 ± 38**,£££	179 ± 13**,£
HAL 2 mg/kg	13127 ± 1759**	697 ± 92**
HAL 2 mg/kg + CPX	9790 ± 1319**	524 ± 34**
HAL 20 mg/kg	10710 ± 2335**	696 ± 135**
HAL 20 mg/kg + CPX	8774 ± 1324**	599 ± 105**

The proneurotensin mRNA hybridization signals were quantified 3 hr after intraperitoneal administration of saline (control) or drugs. Results are means ± SEM of values from 4–10 animals, expressed as percentage of proneurotensin mRNA level in control rats. *$p < 0.05$; **$p < 0.001$ versus control; £$p < 0.05$, ££$p < 0.01$ versus HAL.

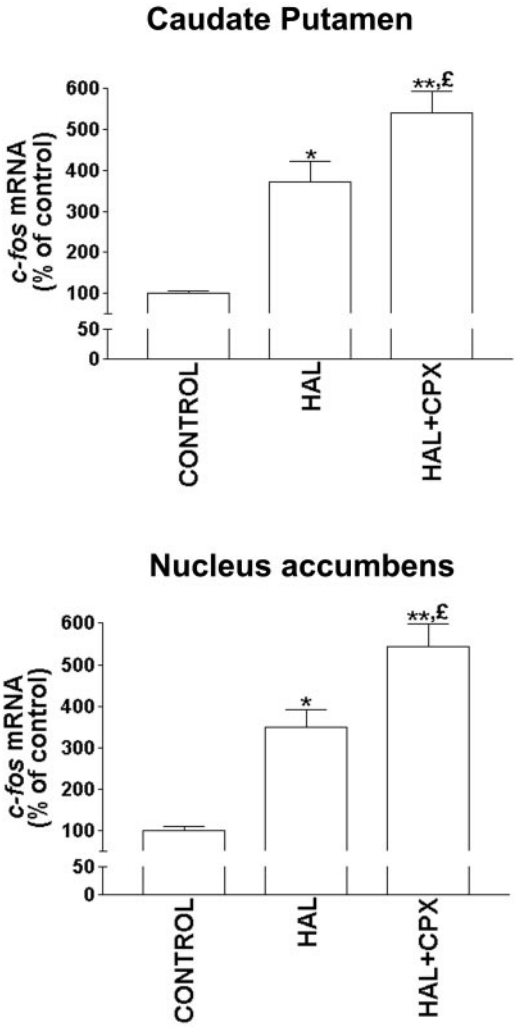

Figure 4. Potentiation by ciproxifan of the upregulation of c-fos mRNA expression elicited by haloperidol in the rat striatum. c-fos hybridization signals were quantified on films 1 hr after intraperitoneal administration of saline solution (*CONTROL*), haloperidol (*HAL*, 1 mg/kg) alone or together with ciproxifan (*CPX*, 1.5 mg/kg). Results are means ± SEM of values from 7–10 animals, expressed as percentage of c-fos mRNA level in control rats. *$p < 0.05$, **$p < 0.001$ versus control; £$p < 0.05$ versus HAL.

lepsy in 2 of 12 rats. Whereas no catalepsy was observed during 2 hr after intraperitoneal administration of ciproxifan alone (1.5 mg/kg), the coadministration of ciproxifan (1.5 mg/kg) with haloperidol (0.1 mg/kg) induced catalepsy in all the animals tested, i.e., in 12 of 12 rats. The number of cataleptic animals was reduced to three of eight when (*R*)-α-methylhistamine (10 mg/kg, i.p.) was administered together with the two compounds (Table 3).

DISCUSSION

The first finding of this study is that striatal enkephalin neurons express H_3Rs. H_3Rs were already shown on striatonigral neurons of the direct pathway (Ryu et al., 1994a, 1996; Garcia et al., 1997; Arias-Montano et al., 2001). The coexpression of H_3R and proenkephalin mRNAs that we evidence here shows that they are also present on projection neurons of the indirect pathway. Enkephalin expression is selectively found in striatopallidal neurons (Reiner and Anderson, 1990; Gerfen, 1992) and parallels their activity (Gerfen et al., 1990; Angulo and McEwen, 1994), which may account for the variable density of proenkephalin mRNAs that we observed within neurons positively labeled by *in situ* hybridization. H_3R mRNA expression itself may be dependent on enkephalin neuron activity because it was not observed in all these neurons and was increased in the external pallidum of patients with Parkinson's disease (Anichtchik et al., 2001). H_3R mRNAs within enkephalin neurons probably account for the dense H_3R binding in the external pallidum in rat (Pillot et al., 2002) and human (Martinez-Mir et al., 1990), inasmuch as the latter is dramatically reduced in Huntington's disease (Goodchild et al., 1999), which is characterized by degeneration of projection neurons (Albin et al., 1989).

The coexpression of proenkephalin and H_3R mRNAs prompted us to explore the effect of H_3R ligands on striatal enkephalin expression. Enkephalin expression is known to be inhibited by D_2 receptors, leading to its increase by haloperidol in the caudate–putamen and nucleus accumbens, two structures with similar organizations (Heimer et al., 1985; Svensson et al., 1995). Although upregulation of proenkephalin mRNAs was mainly reported after chronic treatments (Tang et al., 1983; Romano et al., 1987; Morris et al., 1988; Angulo et al., 1990), our data confirm that it is also observed after acute administration of haloperidol (Angulo, 1992). As expected (Deutch et al., 1992; Merchant and Dorsa, 1993), the same treatment also upregulated proneurotensin mRNAs. It remains unclear whether neurotensin and enkephalin were upregulated within the same cells. Neurotensin striatopallidal neurons have been described (Sugimoto and Mizuno, 1987; Fuxe et al., 1992), and neurotensin cells regulated by D_2 receptors may represent striatopallidal neurons (Castel et al., 1994). However, as already described (Angulo, 1992; Merchant et al., 1992), we observed that enkephalin was moderately and homogeneously upregulated within the striatum, whereas the neurotensin upregulation was much higher in the caudate–putamen, particularly in its dorsolateral part, than 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 H_3R antagonist/inverse agonist (Ligneau et al., 1998; Morisset et al., 2000), strongly potentiates the upregulation of proenkephalin and proneurotensin mRNAs elicited by haloperidol. This potentiation

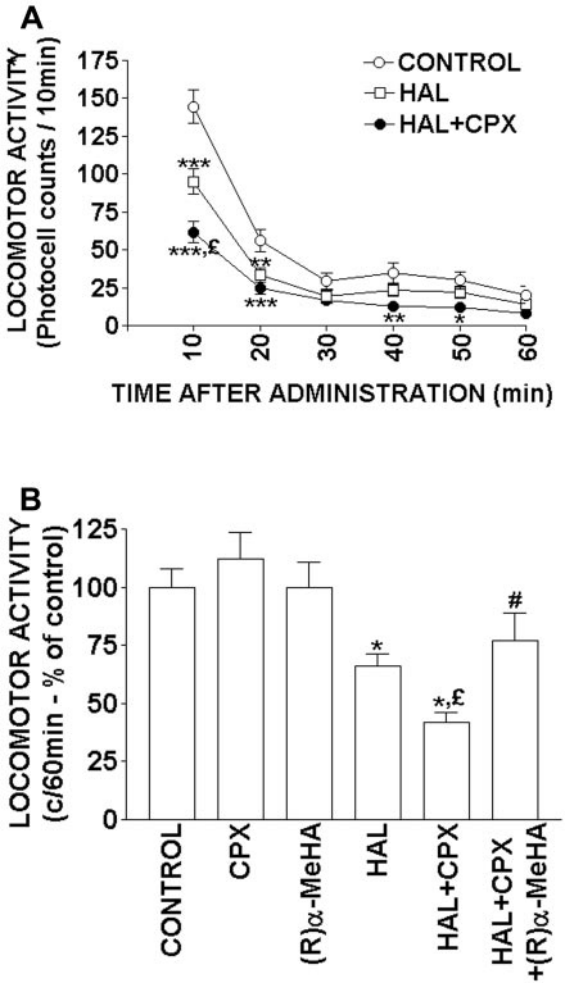

Figure 5. Potentiation by ciproxifan of the decrease in spontaneous locomotor activity elicited by haloperidol in the rat. The spontaneous locomotor activity of 8–36 animals was evaluated after intraperitoneal administration of saline solution (*CONTROL*), haloperidol (*HAL*, 0.1 mg/kg), ciproxifan (*CPX*, 1.5 mg/kg), or (*R*)-α-methylhistamine [(*R*)α-*MeHA*,10 mg/kg], alone or in combination. *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 H_3R-mediated, being suppressed by (*R*)-α-methylhistamine, a prototypical H_3R agonist (Arrang et al., 1987). However, the mechanisms involved remain unclear. H_3Rs 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 D_1-receptors upregulating enkephalin are activated by endogenous 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 H_3 and D_2 receptors located within the same striatal neurons. Most enkephalin neurons express H_3 and D_2 receptors

Table 3. Effect of ciproxifan on haloperidol-induced catalepsy in the rat

Drugs	Catalepsy
HAL (0.1 mg/kg)	2/12
CPX (1.5 mg/kg)	0/8
HAL + CPX	12/12
HAL + CPX + (*R*)α-MeHA (10 mg/kg)	3/8

Catalepsy was assessed in an all-or-none manner 2 hr after intraperitoneal administration of the drugs. The number of animals in catalepsy out of the total number of animals (8–12) is indicated.

(Le Moine and Bloch, 1995), indicating that both receptors are coexpressed in striatopallidal neurons. Therefore, the potentiation of haloperidol by ciproxifan may result from direct synergistic interactions between H_3 and D_2 receptors via their transduction pathway or pathways, inasmuch as both receptors couple to G_i/G_o-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 upregulates 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 H_3R antagonists/inverse agonists potentiate the activation of striatopallidal neurons induced by neuroleptics. The synergistic motor effects of ciproxifan and haloperidol are also consistent with this proposal. Blockade of D_2 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 H_3Rs. Although a functional distinction 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 movements (Albin et al., 1995) suggests that the potentiation of catalepsy results from H_3/D_2-receptor interactions in this structure. Recently (Morisset et al., 1999), we failed to detect in mice the potentiation 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 haloperidol and supports synergistic interactions between D_2 and H_3 receptors. The absence of catalepsy after administration of ciproxifan alone in mice (Morisset et., 1999) or rats suggests that H_3Rs are not involved in this behavior under basal conditions. Interestingly, H_3Rs do not regulate dopamine neuron activity *in*

vivo (Oishi et al., 1990; Imaizumi and Onodera, 1993; Miyazaki et al., 1997). H_3R 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 accumbens, 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 H_3Rs by (*R*)-α-methylhistamine had no effect (Clapham and Kilpatrick, 1994). These data on catalepsy and locomotor activity suggest that H_3Rs 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 H_3Rs in brain display high constitutive activity that is abrogated by ciproxifan acting as an inverse agonist (Morisset et al., 2000; Rouleau et al., 2002). H_3Rs 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 (Nistico et al., 1980; Tuomisto and Eriksson, 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 H_3Rs coexpressed with D_2 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 H_3R mRNA expression in enkephalin neurons and the synergistic neurochemical and motor effects of ciproxifan and haloperidol support the existence of direct functional H_3/D_2-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 H_3R antagonists/inverse agonists might be helpful to improve the symptomatic treatment of schizophrenia.

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