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A comparative study of the anticholinesterase activity of several antipsychotic agents

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Abstract

Drug-induced inhibition of plasma and tissue cholinesterase activity was evaluated in rats. The dopamine receptor antagonists haloperidol (HALO), chlorpromazine (CPZ), thioridazine (THIO), fluphenazine (FLU), clozapine (CLO) and sulpiride (SULP), used as neuroleptics, were tested. Two biochemical parameters were measured in vitro: the minimal effective concentration (MEC) for cholinesterase inhibition and the 50% inhibitory concentration (IC50). In addition, animals were tested for rotational activity after a unilateral intrastriatal injection of the drugs. The doses used for each drug were previously determined IC50s. After unilateral striatal drug injection, rats were challenged with intraperitoneal amphetamine injection in order to stimulate rotation. All drugs tested induced decreases in cholinesterase activity. Plasma MEC for THIO, FLU, HALO and CPZ were significantly lower than for CLO and SULP. In striatum, the MEC for TIO, CPZ and FLU was significantly lower than for HAL. According to plasma IC50, THIO, CPZ and CLO are potent cholinesterase inhibitors. CLO showed the lowest potency of cholinesterase inhibition in the striatum and THIO showed the highest potency in plasma and striatum. In conclusion, anticholinesterase activity is not related to D_2 receptor blockade or antipsychotic potency; and therefore the antipsychotic effects are not related to an increase in acetylcholine. All drugs induced similar contralateral rotation, except for CLO that was different from SULP and was not different from its control. Since equivalent cholinesterase inhibitory concentrations were used for all drugs and no differences in nigrostriatal behavioral effects were observed, these data suggest the participation of an important cholinergic component in this behavior. Therapeutically, the stronger the cholinesterase inhibition is, the more potent the cholinergic effects are and, consequently, the induction of extrapyramidal symptoms becomes more feasible.

Keywords: Dopamine; Caudate–putamen; Rotational behavior; Haloperidol; Clozapine

1. Introduction

The relation between the dopaminergic and cholinergic systems has been widely studied. The effects of dopamine-blocking agents are predominantly a consequence of their binding to central and peripheral dopamine receptors but many of them also result from other actions such as direct inhibition of cholinesterase activity (Iwanaga et al., 1990). Chlorpromazine is able to inhibit acetylcholinesterase in microsomal membranes of different organs both in vitro and in vivo (Mazumder et al., 1990). In humans, metoclopramide induces reversible inhibition of cholinesterase in the central nervous system and in blood. All isoenzymes studied were inhibited in a concentration-dependent manner. This inhibition of cholinesterase in vivo may contribute both to the prokinetic and antiemetic actions of metoclopramide and to its extrapyramidal side effects (Chemnitius et al., 1996). The effect of D2 receptor antagonists may be mediated by an increase in prolactin levels. Prolactin also inhibits cholinesterase activity (Drago et al., 1982; Ramaswamy et al., 1988). The rise of acetylcholine has been associated with stimulation of catecholamine release (Giacobini et al., 1996). Acetylcholinesterase inhibition has a critical role in the dopaminergic regulation of acetylcholine release (Acquas and Fibiger, 1998). Muscarinic receptor agonists can decrease avoidance responding in a manner similar to dopamine-receptor antagonist antipsychotic drugs (Shannon et al., 1999). Mesopontine cholinergic neurons influence midbrain dopaminergic neurons (German et al., 1999) and

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multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release (Zhang et al., 2002). It has also been described that activation of D2 dopamine receptors reduces synaptic inputs to striatal cholinergic neurons (Pisani et al., 2000). On the other hand, acetylcholinesterase inhibitors block acetylcholine release of dopamine in rat striatum, in vivo (Dajas-Bailador et al., 1996). In this respect, a differential sensitivity of central nervous system regions to acetylcholinesterase inhibition has been pointed out (Howerton et al., 1991). Moreover, it should be remembered that cholinesterases are widespread in the brain, including regions not related to cholinergic synaptic activity. Many other functions besides acetylcholine hydrolysis have been described (For a review, see Soreq and Seidman, 2001). Recent studies have shown a possible participation of different molecular and physiological nicotinic acetylcholine receptors in midbrain dopaminergic nuclei which may modulate reinforcement and motor behavior in different manners and may be involved in drug addiction, schizophrenia and Parkinson's disease (Klink et al., 2001; Zhou et al., 2001).

In a previous paper (Nasello et al., 1995) we showed that two different D2 dopamine receptor antagonists used in clinical gastroenterology, i.e., bromopride and domperidone, were able to inhibit cholinesterase in plasma and in intestinal and brain tissues.

The objective of the present study was to assess in a comparative manner the anticholinesterase activity of dopaminergic antagonist agents used in clinical psychiatry. For this purpose, one drug from each different chemical group was chosen, i.e., chlorpromazine (CPZ) from aliphatic phenothiazines, thioridazine (THIO) from piperidine phenothiazines, fluphenazine (FLU) from piperazine phenothiazines, haloperidol (HALO) from butyrophenones, clozapine (CLO) from atypical neuroleptic dibenzodiazepine-derived compounds, and sulpiride (SULP) from the substituted benzamide group. The rationale to study a drug from each chemical group was based on the fact that these groups are clinically different both in terms of their therapeutic properties and of their undesirable side effects. For this purpose, biochemical determinations of cholinesterase inhibitory activity were performed on plasma and

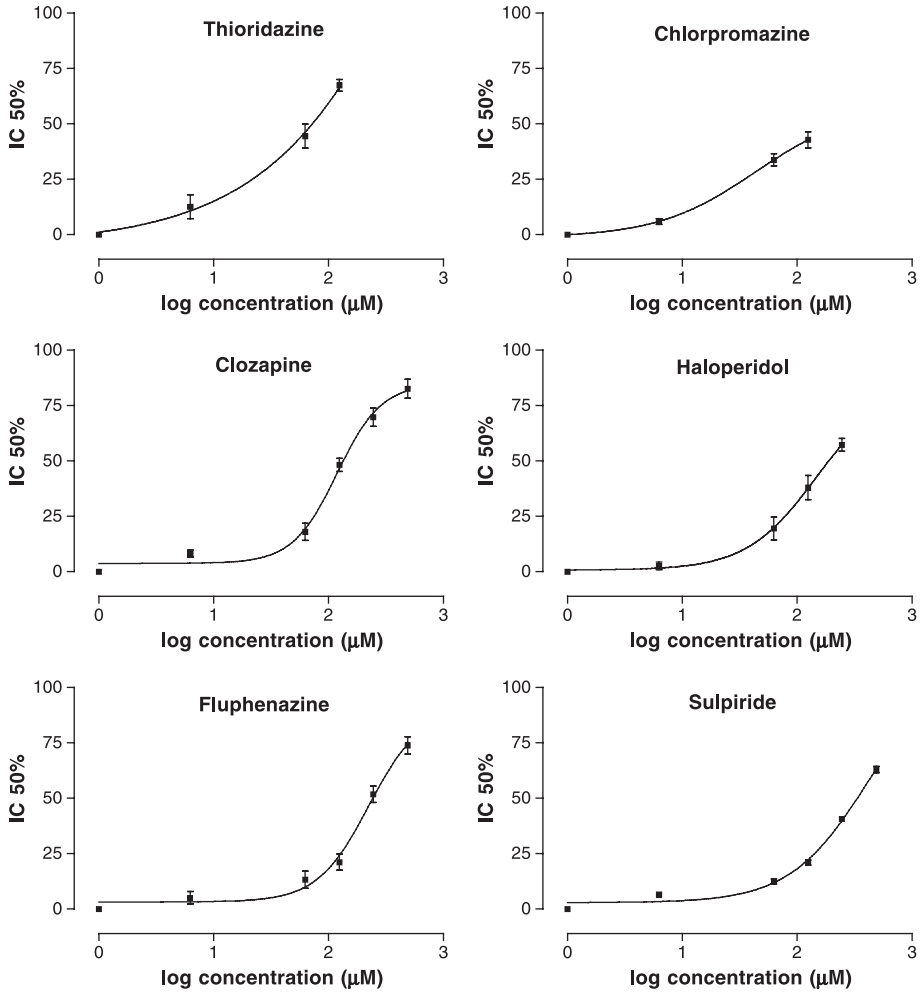

Fig. 1. Concentration–response curves for plasmatic inhibitory cholinesterase activity.

striatum. Also, since rotational behavior is traditionally used to assess the dopaminergic activity of striatal neurons (Gagnon et al., 1991), we tested the effects of the drugs on this behavior in order to elucidate some possible cholinergic participation.

2. Methods

Adult male Wistar rats weighing 200–300 g were anesthetized with sodium pentobarbital (30 mg/kg ip). Blood was collected from the portal hepatic vein with a heparinized syringe and plasma was obtained by immediate centrifugation at 11,000 g for 10 min. Brain tissue (striatum) was collected and homogenized in saline (80-mg tissue in 1 ml). Cholinesterase activity was measured as described by Ellman et al. (1961). Briefly, the enzyme activity is measured using acetylthiocholine as substrate and recording the increase of yellow color produced from thiocholine when it reacts with the dithiobisnitrobenzoate ion (Beckman spectrophotometer DU80). The amount of micromoles of acetylthiocholine degraded min^{-1} ml^{-1} of plasma or mg of tissue was recorded as change in absorbance per minute (Δ). After tissue collection, drugs were added to the solutions in vitro at concentrations ranging from 0.627 to 125 μM for all drugs studied, i.e., CPZ, HALO, THIO, FLU, CLO and SULP, and the minimal effective inhibitory concentration was determined for each drug. CPZ, HALO, THIO and FLU were dissolved in saline, and CLO and SULP were dissolved in DMSO. There were no differences between saline and DMSO controls, and neither had any effect on cholinesterase activity.

The percentage of enzyme activity inhibition for each drug concentration was used to construct a concentration–response curve to obtain an individual IC50 (the concentration of drug that produces an inhibition of the enzyme activity which is 50% of control). At least five different and increasing drug concentration–response curves were used to obtain one individual IC50 for each tissue (Figs. 1 and 2). In each group, the individual IC50 were used to calculate the mean IC50 with 95% confidence. ANOVA followed by the Student–Newman–Keuls test was used to

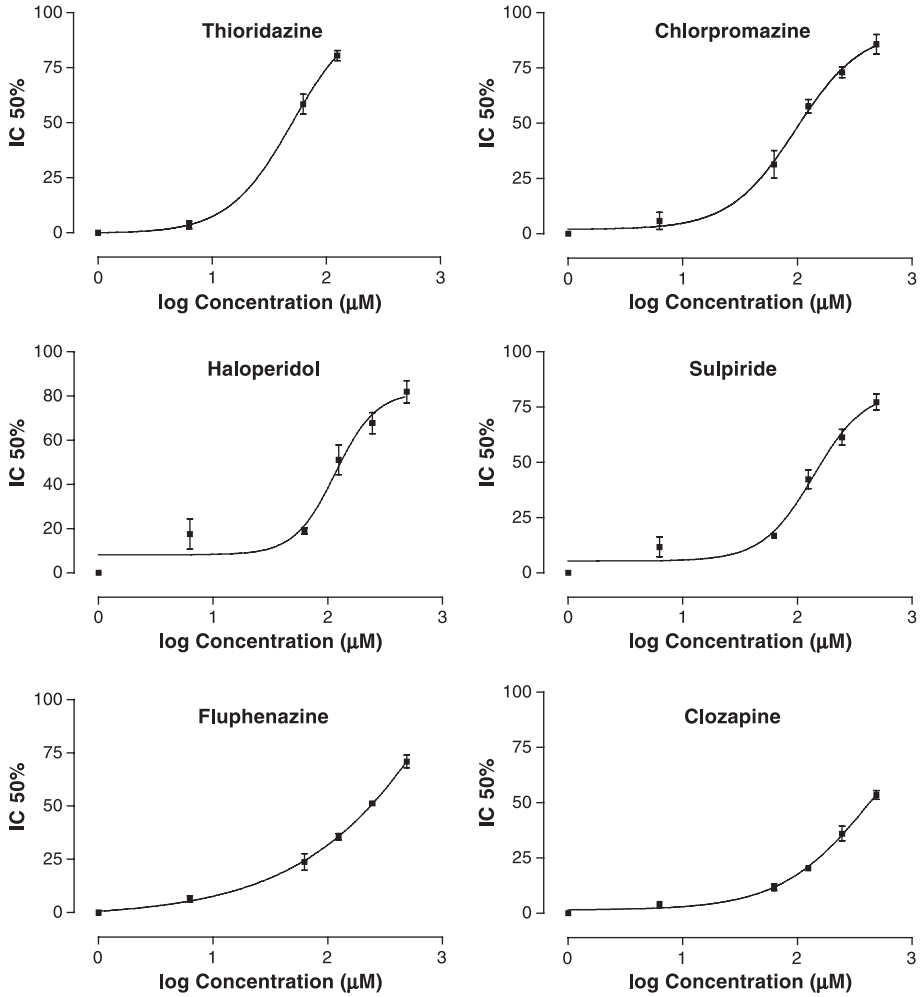

Fig. 2. Concentration–response curves for striatal inhibitory cholinesterase activity.

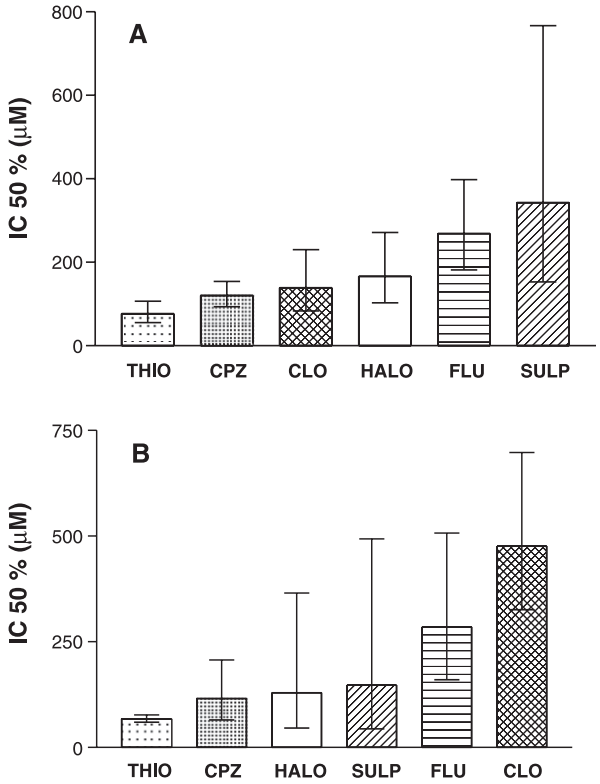

Fig. 3. Effects of different antipsychotic drugs on cholinesterase activity. Values are expressed as IC50 (concentration of the drug that produces an inhibition of the enzyme activity that is 50% of control). Data are means ± 95% confidence intervals. Data were compared by ANOVA ($F=2.18$) followed by the Student–Newman–Keuls test. (A) IC50 of plasma cholinesterase activity. $P<.05$ for CPZ, HALO, FLU and SULP compared with THIO; FLU and SULP compared with CPZ; FLU compared with CLO; FLU and SULP compared with HALO. (B) IC50 of striatum cholinesterase activity. $P<.05$ for CPZ, HALO, SULP, FLU and CLO compared with THIO; FLU and SULP compared with CPZ; FLU compared with CLO; CLO compared with SULP.

analyze the differences among the IC50s obtained. Differences were considered significant when $P<.05$.

To evaluate rotational activity, adult male rats were implanted with a unilateral guide cannula directed at the left caudate–putamen area. One week later they received injections of 1 μl of vehicle or of one of the antipsychotic drugs in equivalent doses (striatum cholinesterase IC50 determined previously) at the following coordinates: A +5.8 (from the IA0); L +3.5, V +5.5 (according to system A of the Atlas of Pellegrino et al., 1986). Intrastriatal injections were made using a Harward infusion pump. All animals received dl-amphetamine (5 mg kg^{-1} ip) after the striatum injection to stimulate rotation since amphetamine increases dopaminergic activity and therefore stimulates motor activity. The increases in motor activity permit the clearest expression of the effects of the drugs under study on rotational behavior. Immediately after amphetamine injection animals were held in an appropriate harness connected to a rotation sensor, placed in the Plexiglas cylinder of a rotameter (Columbus) and the number of rotations were automatically recorded. The ipsi- and contralateral rotations were measured for 1 h, and their percentages were calculated (Gagnon et al., 1991). The differences in the ipsi- and contralateral rotations for each drug and in the percentage of contralateral rotation between each drug and its control and between drugs were evaluated by the Kruskall–Wallis test followed by the Mann–Whitney test. Differences were considered significant when $P<.05$. Striatum slides were analyzed 3 weeks after the experiments to check cannula placement and the striatal cannula placement was confirmed in all animals. The animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

3. Results

All neuroleptic drugs used (HALO, THIO, CLO, CPZ, FLU and SULP) significantly decreased cholinesterase activity both in plasma and striatum in vitro. The plasma minimal effective concentration was 65 μM for THIO, FLU, HALO and CPZ and 125 μM for CLO and SULP. On the other hand, in the striatum the minimal effective concentration was 65 μM for THIO, CPZ and FLU and 125 μM for HALO. With respect to IC50, THIO showed the highest inhibitory action both in plasma and striatum (Fig. 3A and B). THIO, CPZ, CLO, and HALO had similar inhibitory effects on plasma (Fig. 3A). Besides THIO, CPZ, CLO and HALO were the most potent inhibitors in plasma (Fig. 3A); however, CLO was the least efficient in striatum (Fig. 3B).

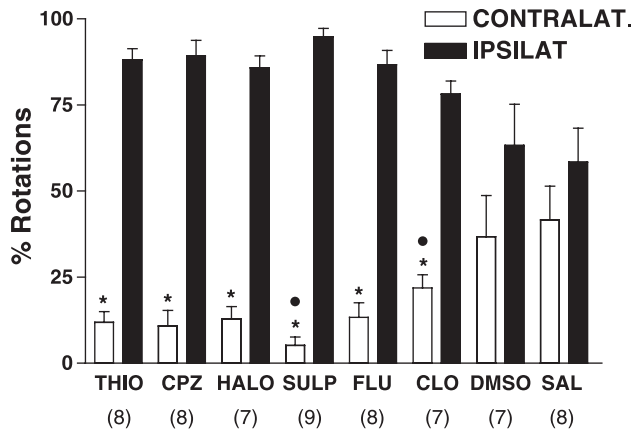

Fig. 4. Percentage of animals showing ipsi- and contralateral rotation. The different D2 antagonists were injected into the striatum at the IC50 dose for cholinesterase inhibition or vehicle (DMSO and saline). The number of animals is given in parentheses. * $P<.05$ for ipsi- vs. contralateral values. The differences between the ipsi- and contralateral rotations and the percentage of contralateral rotation were evaluated for each drug. The Kruskall–Wallis test (KW = 94.9, corrected for ties) followed by the Mann–Whitney U test was applied to compare each drug with its control and with the other drugs. All drug effects differed from control but did not differ from one another except for CLO that was different from SULP (● $P<.05$) and was not different from DMSO.

Concerning rotational activity, the percentages of ipsi- and contralateral rotations were significantly different for all drugs. No differences were observed between saline and DMSO, used as controls. There were no differences between drugs in terms of contralateral rotations but all drugs differed from their respective controls, except for CLO that differed from SULP but not from DMSO (Fig. 4).

4. Discussion

The present results show that all the dopamine receptor antagonists studied are able to inhibit cholinesterase activity in plasma and striatum in vitro in a dose dependent manner. Thioridazine was the most potent inhibitor both in plasma and in striatum, as can be seen by comparing the IC50 (Fig. 3). However, clinically, THIO has few extrapyramidal side effects because it is also a potent anticholinergic drug. Thus, we may suggest that the anticholinergic effects of THIO overlap its anticholinesterase properties. The results obtained with THIO suggest a direct relation between enzyme affinity (anticholinesterase effects) and muscarinic receptor affinity (anticholinergic effects) both in plasma and in striatum (Bekpinar et al., 1994; Roman et al., 2002).

The minimal effective concentrations (μM) of all drugs studied were similar (see Results) but their IC50 were quite different (Fig. 3), showing that the efficacy in inhibiting the enzyme depends mainly on the affinity and not on the number of molecules of the drugs, as is classically known for enzymes.

In striatum, both FLU and CLO have a very high IC50 (Fig. 3). FLU is much more potent than CLO as an antipsychotic drug (Seeman, 1987). CLO has virtually no extrapyramidal effects (Peacock et al., 1996). Antipsychotic ability is primarily related to D2 dopamine receptor affinity (Seeman, 1987). On this basis, our results show that the ability to inhibit cholinesterase activity is not directly related to antipsychotic properties. Some drugs selective for D2 receptors that are potent antipsychotic agents such as SULP and HALO have lower cholinesterase inhibitory actions than THIO and CPZ, that are not so potent as antipsychotics (Seeman, 1987). Although CLO and FLU have very different effects on cholinesterase activity, neither one is able to antagonize amphetamine-induced stereotyped locomotion (Moore and Kenyon, 1994; Mueller, 1993).

Our data show that these antidopaminergic drugs also have cholinergic effects. These effects are important for some therapeutic actions but also account for neurological and endocrine side effects. As an example of their neurological actions, we may mention that striatum cholinesterase inhibition induces catalepsy (Castello et al., 1992) and the cataleptic response is a result of dopaminergic and cholinergic interaction (Ushijima et al., 1997). It has been proposed that dopamine cells possess functional muscarinic receptors both in the A9 and A10 regions. These receptors may be able to greatly affect the activity of midbrain dopamine and may play a role in, and/or be a therapeutic target for, brain disorders in which dopamine is involved such as Parkinson's disease, drug addiction and schizophrenia (Gronier and Rasmussen, 1998). Muscarinic receptor agonists can decrease avoidance responding, similarly to antipsychotic drugs (Shannon et al., 1999). Muscarinic cholinergic processes may be involved in tolerance to caffeine-induced contralateral turning in a test used to assess rotational behavior (Casas et al., 1999).

The relations between some neuroleptic drugs and anticholinesterase activity have been previously described in different experimental situations (Bekpinar et al., 1994; Mazumder et al., 1990; Spinedi et al., 1991).

The differences between plasma and brain cholinesterase activity may be related to the presence of different isoenzymes (Korenovsky et al., 1990). These differences lead us to suggest that sometimes there is no correlation between plasma and brain effective drug concentrations. In this case it is not valid to deduce the brain effect of substances, principally on enzymatic activity, based on data from plasma or cerebrospinal fluid. In this respect, pseudocholinesterase studies on psychiatric patients are inconclusive (Modai et al., 1987). Schizophrenic patients treated with HALO did not show differences in acetylcholinesterase levels in cerebrospinal fluid. However, differences were observed in striatum of rats that received equivalent doses of the same drug (Huff et al., 1988).

Striatal tissue lesions or striatal administration of antidopaminergic drugs allows the expression of the activity of the contralateral nigrostriatal system and indirectly reveals the degree of damage or the dopaminergic blocking effects in the ipsilateral nigrostriatal system (Ungerstedt and Arbuthnott, 1970). In our case, we administered the IC50 for cholinesterase activity of all drugs and found that the participation of cholinergic effects was the same for all the drugs assayed. All of them decreased the percentage of contralateral rotation compared to control. There were no differences among them, except for CLO that was different from SULP and was not different from DMSO (Fig. 4). CLO is the least potent inhibitor of cholinesterase activity and therefore presents the lowest cholinergic effects. This may be at least one of the reasons why this drug practically does not have extrapyramidal effects (Peacock et al., 1996). Our results suggest that the cholinergic effects of all drugs tested are relevant for rotational behavior. This hypothesis is supported by the fact that the effect on cholinesterase activity is the only action that all these drugs have in common. The differences among them concern both to the pharmacological characteristics and the doses used (King, 1998; Kane et al., 1998; Lidow et al., 1998).

It has been proposed that muscarinic receptor agonists may provide an alternative approach to the treatment of psychosis (Shannon et al., 1999); these cholinoceptors may be involved in brain disorders in which dopamine is implicated, such as schizophrenia, Parkinson's disease and others (Gronier and Rasmussen, 1998). On the other hand,

as previously reported (German et al., 1999), there is no difference in mesopontine cholinergic neurons in schizophrenia. However, it has been described that antagonists of dopamine D2 receptors work though a common mechanism in the treatment of mania. In this kind of disorder muscarinic agonists and cholinesterase inhibitors do not seem to play a key role (Bymaster and Felder, 2002). Our results agree with this last hypothesis. We also show there is no correlation between antipsychotic potencies and inhibitory effects on cholinesterase activity (i.e., increase of acetylcholine and overstimulation of muscarinic receptors). These results disagree with a cholinergic hypothesis of schizophrenia (Gronier and Rasmussen, 1998; German et al., 1999; Shannon et al., 1999). On the other hand, a participation of nicotinic receptors cannot be ruled out (Klink et al., 2001; Zhou et al., 2001). Other acetylcholinesterase actions not related to acetylcholine hydrolysis have also been described (Soreq and Seidman, 2001).

Extrapyramidal symptoms are related to an increase of cholinergic activity in nigrostriatal dopaminergic system. Cholinesterase inhibition is directly correlated with cholinergic effects and, consequently, the induction of extrapyramidal symptoms is more likely (Hsieh et al., 2001). From the present results, we may conclude that all the dopaminergic antagonists evaluated showed cholinesterase inhibition activity. This activity is not related to a clinical antipsychotic effect or to D2 dopaminergic blocker efficacy. Therefore, antipsychotic effects are not related to an increase in acetylcholine. When equivalent doses were used, i.e., IC50, all drugs induced similar contralateral rotation. Our data suggest the presence of an important cholinergic participation in rotational behavior. Therapeutically, the more potent cholinesterase inhibition, the more potent the cholinergic effects and consequently the more likely the induction of extrapyramidal symptoms.

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