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SPECIAL REPORT

Antagonism of striatal muscarinic receptors inhibiting dopamine D_1 receptor-stimulated adenylyl cyclase activity by cholinoceptor antagonists used to treat Parkinson's disease

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A number of cholinoceptor antagonists used in the treatment of Parkinson's disease were examined for their ability to antagonize either the muscarinic receptor-mediated inhibition of dopamine D_1 receptor-stimulated adenylyl cyclase or the muscarinic receptor-mediated stimulation of [^{3}H]-inositol phosphates ([^{3}H]-IPs) formation in rat striatal membranes. The drugs were found to block the receptors inhibiting adenylyl cyclase activation with high affinity and more potently than those stimulating [^{3}H]-IPs formation. Moreover, their rank order of potencies for the former effect showed good correlation with their clinical efficacies. These data suggest that the blockade of the muscarinic receptor-mediated inhibition of striatal dopamine D_1 receptor activation may be one of the mechanisms by which cholinoceptor blocking drugs exert their antiparkinsonian effect.

Keywords: Cholinoceptor blocking antiparkinson drugs; muscarinic receptors; dopamine D_1 receptors; adenylyl cyclase; inositol phosphate formation; rat striatum

Introduction Drugs capable of blocking central muscarinic acetylcholine receptors have long been used for the treatment of Parkinson's disease and extrapyramidal motor disturbances caused by neuroleptics. However, the sites of action and the mechanism underlying these therapeutic effects are not yet completely defined. In rat striatum, activation of muscarinic receptors inhibits the stimulation of adenylyl cyclase activity elicited by dopamine acting on D_1 receptors (Olianas *et al.*, 1983; Kelly & Nahorski, 1986). The relevance of dopamine D_1 receptors in the control of extrapyramidal functions is highlighted by the recent observations that full dopamine D_1 receptor agonists can improve the motor disturbances in animal models of Parkinson's disease (Taylor *et al.*, 1991; Blanchet *et al.*, 1993). Therefore, we considered the possibility that the blockade of the striatal muscarinic receptors inhibiting the dopamine D_1 receptor activity may participate in the antiparkinsonian action of the antimuscarinic drugs. In the present study, we have investigated the ability of various antimuscarinic drugs, currently used in the treatment of Parkinson's disease, to antagonize the carbachol (CCh)-induced inhibition of striatal dopamine D_1 receptor-stimulated adenylyl cyclase activity. For comparison, we also determined the affinities of the drugs in antagonizing the CCh stimulation of [^{3}H]-inositol phosphates ([^{3}H]-IPs) formation, another important mechanism of intracellular signalling regulated by muscarinic receptors in rat striatum.

Methods Adenylyl cyclase activity was determined as previously described (Olianas *et al.*, 1983). For stimulation of dopamine D_1 receptors, the selective agonist (±) 6-chloro-APB (SKF 82958) (Research Biochemicals Incorporated, Natick, MA, U.S.A.) was used at a concentration of 1 μM. The CCh stimulation of [^{3}H]-IPs formation was determined in striatal membranes prelabelled with [^{3}H]-myo-inositol essentially as described by Claro *et al.* (1992). Agonist concentration-response curves were analysed by a least squares curve-fitting computer program (Graph-Pad, San Diego, CA, U.S.A.). The antagonist inhibitory constant (K_i) was calculated from the equation:

$$EC_{50b} = EC_{50a}(1 + I/K_i)$$

where EC_{50a} and EC_{50b} are the concentrations of the agonist producing half-maximal effect in the absence and in the presence of the antagonist, respectively, and I is the antagonist concentration. Statistical significance between means was determined by unpaired Student's t test. Antagonists were obtained from the following sources: benztropine from Research Biochemical Inc. Natick, MA, U.S.A.; biperiden from Ravizza, Muggio, Italy; procyclidine, orphenadrine and trihexyphenidyl from Sigma Chemical Co., St. Louis, MO, U.S.A.

Results In rat striatal membranes, CCh inhibited the dopamine D_1 receptor-stimulated adenylyl cyclase activity in a concentration-dependent manner with an EC_{50} value of 1.3±0.1 μM. Maximal inhibition was obtained with 100 μM CCh and corresponded to a 45.1±2.9% reduction of the dopamine D_1 receptor-stimulated activity ($P<0.001$, $n=15$). The antiparkinson drugs, added at concentrations of 100-500 nM, antagonized the muscarinic inhibition by shifting to the right the agonist concentration-response curves. The estimated K_i values ranged from 2.4 nM for benztropine to 41.5 nM for orphenadrine (Table 1). None of the antiparkinson drugs significantly affected the dopamine D_1 receptor-stimulated adenylyl cyclase activity *per se*. In a similar cell-free tissue preparation, CCh caused a concentration-dependent stimulation of [^{3}H]-IPs formation with an EC_{50} of 7.83±2.0 μM. The maximal stimulation corresponded to a 55.1±5.6% increase of basal value ($P<0.001$, $n=18$). Addition of the antiparkinson drugs also caused a rightward shift of this agonist concentration-response curve. However, for each antagonist the K_i value was significantly higher than that displayed in counteracting the inhibition of dopamine D_1 receptor-stimulated adenylyl cyclase activity (Table 1). When the affinities of the antagonists in blocking either the adenylyl cyclase inhibition or the [^{3}H]-IPs formation were compared with the average daily doses of the drugs in the treatment of Parkinson's disease (Figure 1), the correlation was high for the former ($r=0.997$, $P=0.0002$) and low for the latter response ($r=0.868$, $P=0.056$).

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Table 1 Affinities of various antiparkinson cholinoceptor blocking drugs in antagonizing the muscarinic inhibition of dopamine D_1 receptor-stimulated adenylyl cyclase and stimulation of [^{3}H]-inositol phosphates formation in rat striatal membranes

	K_i (nM)	
	Adenylyl cyclase activation	*Inositol phosphate formation*
Benztropine	2.4±0.4	48.8±3.8**
Biperiden	3.1±0.1	9.6±0.7*
Trihexyphenidyl	5.0±0.2	30.9±0.4**
Procyclidine	8.2±1.0	97.9±7.6**
Orphenadrine	41.5±3.7	608.2±98.3*

Values are the mean±s.e.mean of three determinations for each antagonist. *$P<0.01$, **$P<0.001$ when compared to the corresponding K_i value in antagonizing the carbachol inhibition of adenylyl cyclase activity.

Discussion The present study shows that the cholinoceptor blocking drugs used to treat Parkinson's disease are potent antagonists of the striatal muscarinic receptors that inhibit the dopamine D_1 receptor-mediated stimulation of adenylyl cyclase. Previous radioligand binding studies with the cloned muscarinic receptor subtypes have shown that antiparkinson drugs, such as biperiden, procyclidine and trihexyphenidyl, possess high affinity and some selectivity for the m1 and m4 muscarinic receptor subtypes (Bolden *et al.*, 1992). The binding to striatal m4 receptors may explain the high potencies in counteracting the muscarinic inhibition of dopamine D_1 receptor activity, as recent data indicate that this response is also potently blocked by the selective m4 ligand muscarinic toxin 3 (Olianas *et al.*, unpublished data). Conversely, the drugs display lower affinities in blocking [^{3}H]-IPs formation, indicating that the striatal muscarinic receptors coupled to phospholipase C are unlikely to be a major site of their action. Although antiparkinson cholinoceptor antagonist drugs may also act in brain areas other than the striatum (Mavridis *et al.*, 1995), the finding that these drugs are potent antagonists of the adenylyl cyclase-coupled muscarinic receptors and that their potencies are highly correlated with their clinical efficacies suggests that blockade of the muscarinic-mediated inhibition of striatal dopamine D_1 receptor activity may be an important component of their therapeutic effect.

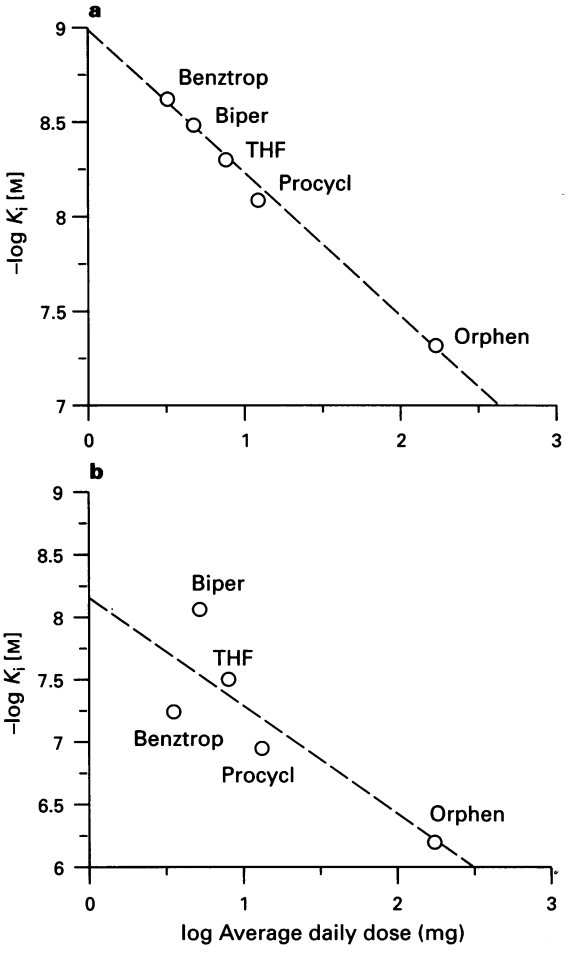

Figure 1 Comparison of the log of the average daily doses of cholinoceptor blocking drugs for the treatment of Parkinson's disease and the −log of their affinities in antagonizing the muscarinic receptor-mediated inhibition of dopamine D_1 receptor-stimulated adenylyl cyclase activity (a) and the stimulation of [^{3}H]-inositol phosphates formation (b) in rat striatum. Benztrop, benztropine; Biper, biperiden; THF, trihexyphenidyl; procycl, procyclidine; orphen, orphenadrine.

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