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Muscarinic M_4 receptor inhibition of dopamine D1-like receptor signalling in rat nucleus accumbens

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Abstract

Several studies have indicated the occurrence of an antagonistic interaction between muscarinic and dopamine D1-like receptors in the ventral striatum, but the subtype(s) of muscarinic receptor involved has not been characterized. We show that in membranes of rat nucleus accumbens, carbachol inhibited the stimulation of adenylyl cyclase activity by dopamine and the dopamine D1-like receptor agonist (±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine without affecting the binding properties of dopamine to dopamine D1-like receptors. The carbachol inhibition was competitively counteracted by receptor antagonists with a rank order of potency typical of the involvement of the muscarinic M_4 receptor subtype. Moreover, muscarinic toxin 3, a selective muscarinic M_4 receptor antagonist, completely blocked the carbachol inhibition, whereas muscarinic toxin 7, a selective muscarinic M_1 receptor antagonist, had no effect. The muscarinic inhibition occurred to a similar extent in the core and shell regions. These data demonstrate that in nucleus accumbens, muscarinic M_4 receptors exert a direct inhibitory control on dopamine D1-like receptor signalling.

Keywords: Muscarinic receptor subtype; Dopamine D1-like receptor; cAMP; Nucleus accumbens

1. Introduction

The functional interaction between dopamine and acetylcholine constitutes a prominent feature of striatal neurotransmission and alterations of the normal balance between the two neurotransmitters have been proposed to play a role in the pathogenesis of psychomotor disfunctions (Carlsson and Carlsson, 1990). In the neostriatum, a biochemical correlate of the acetylcholine–dopamine interplay is the opposing effect exerted on the formation of cyclic AMP (Olianas et al., 1983; Kelly and Nahorski, 1986). Dopamine released from nigrostriatal terminals activates adenylyl cyclase activity via dopamine D1-like receptors, which comprise both D1 and D5 receptor subtypes (Lachowicz and Sibley, 1997), and activates the cyclic AMP/protein kinase A signalling pathway, which modulates striatal neuronal excitability, neurotransmitter release and gene expression (Greengard, 2001). On the other hand, acetylcholine by activating muscarinic M_4 receptors directly inhibits adenylyl cyclase activity and curtails dopamine D1-like receptor-mediated cyclic AMP formation (Olianas et al., 1996). Immunocytochemical studies have demonstrated that the majority of the spiny neurons projecting to the substantia nigra coexpress dopamine D1 and muscarinic M_4 receptors (Bernard et al., 1992), supporting the possibility that the receptors can functionally interact on a common adenylyl cyclase system. Moreover, it has recently been shown that in vivo blockade of striatal muscarinic M_4 receptors augments amphetamine-stimulated preprodynorphin and substance P mRNAs expression in striatonigral neurons, indicating that tonic muscarinic M_4 inhibition of dopamine D1-like receptor activity can affect striatal output in vivo (Wang et al., 1997).

The nucleus accumbens, a predominant part of the ventral striatum, is another brain area where the acetylcholine–dopamine interplay takes place and may have important implications with regard to reward and aversion (McGinty, 1999; Hajnal et al., 2000). Like the neostriatum, the nucleus accumbens contains a large population of output spiny neurons and a minor population of cholinergic interneurons and expresses a high density of dopamine D1-like and muscarinic M_4 receptors (Levey et al., 1991; Yung et al., 1995). However, there is no information as to whether

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these receptors can directly interact in the control of a common signalling pathway. In the present study, we provide evidence that in membranes of rat nucleus accumbens activation of muscarinic M_4 receptors inhibits dopamine D1-like receptor stimulated cyclic AMP formation.

2. Materials and methods

2.1. Materials

[α-^{32}P]ATP (30–40 Ci/mmol) and [2,8-^{3}H]cyclic AMP (25 Ci/mmol) were obtained from NEN Life Science Products (Boston, MA, USA). [*R*-(+)-8-Chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol] maleate (SCH 23390) was obtained from Schering-Plough (Bloomfield, NJ, USA). [*N*-methyl-^{3}H]SCH 23390 ([^{3}H]SCH 23390) (75.5 Ci/mmol) was purchased from NEN Life Science Products. (±)-6-Chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide ((±)-APB) and *p*-fluoro-hexahydro-sila-difenidol (pFHHSiD) were purchased from Research Biochemical (Natick, MA, USA). Pirenzepine and AF-DX 116 (11[[2-[(diethylamino)methyl]-1-piperidynyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3*b*] [1,4]benzodiazepine-6-one) were obtained from Dr. Karl Thomae (Biberach an der Riss, Germany), whereas PD 102807 (9-methoxy-2-methyl-11,12-dihydro-3*H*,6*aH*,13*H*-6-oxa-3,12*a*-diaza-benzo[*a*]cyclopenta[*h*]anthracene-1-carboxylic acid ethyl ester) was from Parke-Davis (Ann Arbor, MI, USA). Muscarinic toxin 3 (MT-3), isolated from the venom of *Dendroaspis angusticeps*, was kindly provided by Profs. E. Karlsson and A. Adem (Karolinska Institute, Stockholm, Sweeden). Muscarinic toxin 7 (MT-7) was obtained from Peptide International (Louisville, KY, USA). Dopamine hydrochloride, carbachol hydrochloride and the other reagents were from Sigma (St. Louis, MO, USA).

2.2. Tissue microdissection and membrane preparation

Male Sprague–Dawley rats (200–350 g) were used. Animals were maintained in a 12 h light/dark cycle with food and water ad libitum. Experiments were performed according to the principles of laboratory animal care (Law on animal experiments in Italy, D.L. 116/92). The animals were killed by decapitation. The brain was rapidly removed from the skull, placed in ice-cold phosphate buffered saline and the meninges and major blood vessels were peeled off. The brain was then placed on its dorsal surface, exposing the ventral surface of the forebrain. By using a razor blade, a first transverse cut was made just anterior to the olfactory tubercle and a second transverse cut was made through the midbrain. The tissue block was placed on a tissue chopper with the dorsal surface down. Three hundred micron-thick sections were cut, one at a time. Five to six sections were collected from one brain. Each section was immediately placed in ice-cold buffered saline until it was dissected. After cutting a sufficient number of brains, individual sections were transferred to a glass slide and, by using a dissecting microscope with a diascopic illuminator base, the nucleus accumbens was dissected with small knifes. Landmarks used for dissections were the anterior commissure, the olfactory and lateral ventricles, the corpus striatum, the septal nuclei and the olfactory tubercle. Particular caution was taken to avoid contamination by septum and striatum. In some experiments, the inner core and the peripheral shell regions of the nucleus were dissected from selected brain slices comprising the median part of the nucleus (approximate coordinates A11–A10 mm anterior to the interaural line in the atlas of Paxinos and Watson, 1986), according to the procedure described by Deutch and Cameron (1991), with slight modification. The core region was punch-dissected using a punch with an inside diameter of 800 μm positioned eccentrically around the anterior limb of the anterior commessure. The remaining portion of the nucleus accumbens was taken as the shell region.

The tissue fragments from individual slices were pooled and homogenized in an ice-cold buffer containing 10 mM HEPES/NaOH, 1 mM EGTA, 1 mM $MgCl_2$,1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 50 kallikrein inhibitor units (KIU)/ml of aprotinin and 10 μg/ml of soyabean trypsin inhibitor (pH 7.4), using a Teflon-glass tissue grinder. The homogenate was centrifuged at 27,000×*g* for 20 min at 4 °C. The pellet was resuspended in the same buffer at a protein concentration of 0.8–1.0 mg/ml and used immediately for adenylyl cyclase and radioligand binding assays.

2.3. Adenylyl cyclase assay

The adenylyl cyclase activity was assayed in a reaction mixture (final volume 100 μl) containing 50 mM HEPES/NaOH (pH 7.4), 0.5 mM $MgCl_2$, 0.3 mM EGTA, 0.1 mM [α-^{32}P]ATP (100 cpm/pmol), 0.5 mM [^{3}H] cyclic AMP (80 cpm/nmol), 1 mM 3-isobutyl-1-methylxanthine, 5 mM phosphocreatine, 50 U/ml creatine phosphokinase, 10 μM GTP, 50 μg of bovine serum albumin, 10 μg of bacitracin and 10 KIU of aprotinin. The reaction was started by adding the tissue preparation (20–25 μg of protein) and was carried out at 30 °C for 10 min. The reaction was stopped by adding 200 μl of a solution containing 2% (w/v) sodium dodecyl sulfate, 45 mM ATP, 1.3 mM cyclic AMP (pH 7.5). Cyclic AMP was isolated by sequential chromatography on Dowex and alumina columns as described by Salomon et al. (1974). The recovery of [^{32}P] cyclic AMP from each sample was calculated on the basis of the recovery of [^{3}H] cyclic AMP. Assays were carried out in duplicate.

2.4. [^{3}H]SCH 23390 binding assay

The binding of [^{3}H]SCH 23390 to membranes of nucleus accumbens was performed in an incubation mixture (final

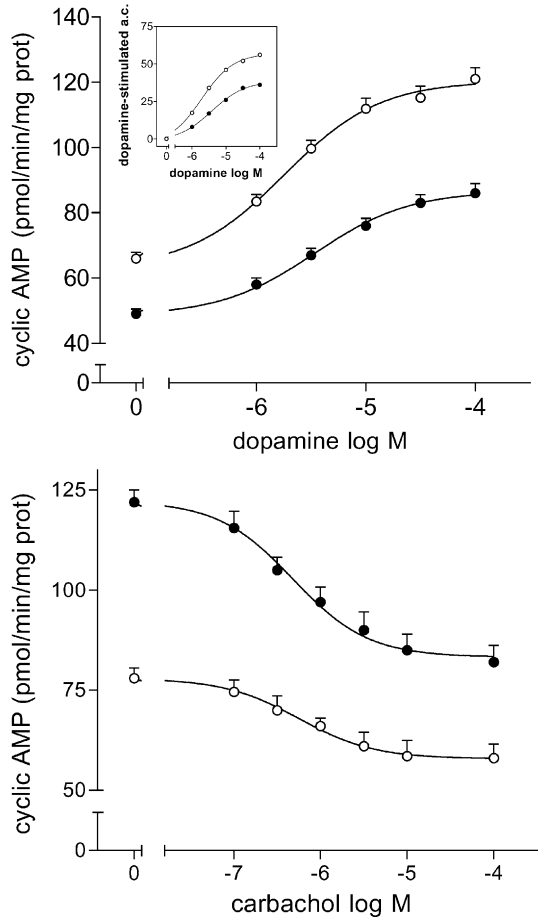

Fig. 1. Upper panel: Concentration-dependent stimulation of adenylyl cyclase activity by dopamine in the absence and in the presence of carbachol. The enzyme activity was assayed at the indicated concentrations of dopamine in the absence (control) (○) and in the presence of 100 μM carbachol (●). The inset shows the net stimulation of adenylyl cyclase (a.c.) elicited by each concentration of dopamine above control values. Data represent the means±S.E. of four experiments. $P<0.05$ for the difference between control and carbachol curves by analysis of variance. Lower panel: Concentration-dependent inhibition of basal and dopamine-stimulated adenylyl cyclase activities by carbachol. The enzyme activity was assayed at the indicated concentrations of carbachol in the absence (basal) (○) and in the presence of 10 μM dopamine (●). Data represent the means±S.E. of three experiments.

volume 1.0 ml) containing 50 mM HEPES/NaOH (pH 7.4), 1 mM EGTA, 1 mM $MgCl_2$, 1 mM dithiothreitol and 60–70 μg of membrane protein. The concentration of [^{3}H]SCH 23390 was 0.4 nM. The incubation was carried out at 37 °C for 30 min and was terminated by adding 5 ml of an ice-cold buffer containing 10 mM HEPES/NaOH (pH 7.4) and 1 mM $MgCl_2$, immediately followed by rapid filtration through glass-fiber filters (Whatman GF/C) presoaked in 0.1% polyethylenimine. The filters were washed three times with 5 ml of buffer and the radioactivity trapped was determined by liquid scintillation spectrometry. Nonspecific binding was determined in the presence of 10 μM unlabeled SCH 23390 and corresponded to 3% of the total binding. Total binding was less than 10% of the radioactivity added. Assays were carried out in duplicate.

Protein content was determined by the method of Bradford (1976), using bovine serum albumin as a standard.

2.5. Statistical analysis

Results are reported as means±S.E. Data from concentration–response curves were analysed by a least-squares curve fitting computer programme (Graph Pad Prism, USA). Antagonist pA_2 values were calculated from Arunlakshana–Schild regressions in which the dose ratio −1 is plotted as a function of the antagonist concentration. For each antagonist, three concentrations ranging 50–100-fold were used. The pA_2 values were calculated by using the PHARM/PCS programme of Tallarida and Murray (1987). When the antagonist was examined for the ability to completely reverse the effect elicited by a fixed concentration of the agonist, the data were analysed as competition curves by

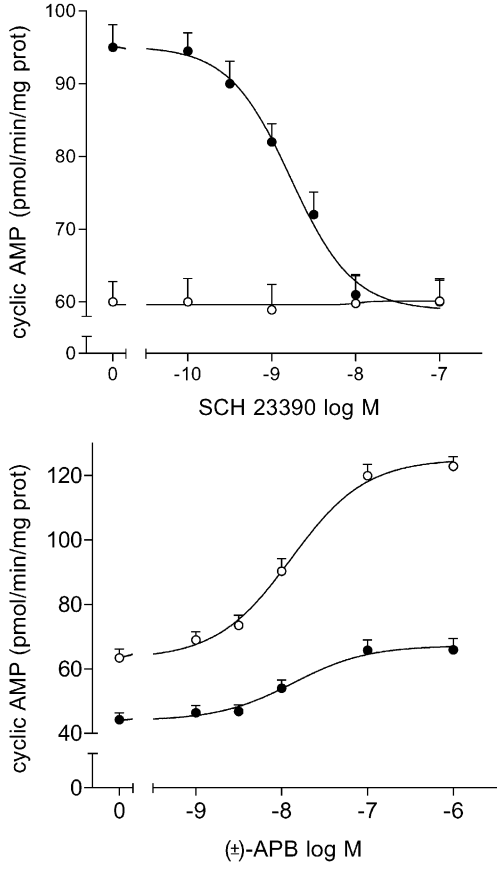

Fig. 2. Upper panel: Antagonism of dopamine stimulation of adenylyl cyclase activity by SCH 23390 in membranes of rat nucleus accumbens. The enzyme activity was assayed at the indicated concentrations of SCH 23390 in the absence (○) and in the presence of 10 μM dopamine (●). Data are the means±S.E. of three experiments. Lower panel: Inhibition by carbachol of the enzyme activity stimulated by (±)-APB. The enzyme activity was assayed at the indicated concentrations of (±)-APB in the absence (○) and in the presence of 100 μM carbachol (●). Data are the means±S.E. of three experiments.

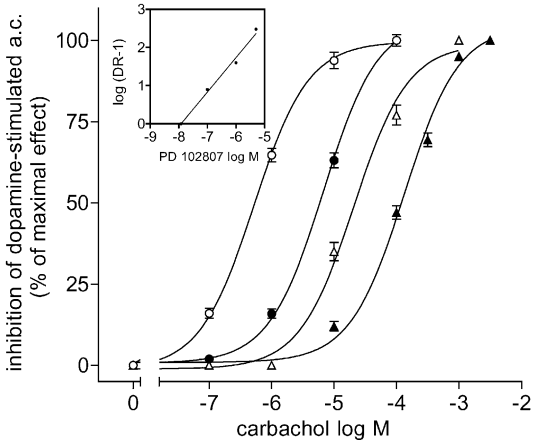

Fig. 3. Antagonism of carbachol inhibition of dopamine-stimulated adenylyl cyclase (a.c.) activity by PD 102807 in membranes of rat nucleus accumbens. The carbachol inhibition of the enzyme activity stimulated by 30 μM dopamine was determined in the absence (○) and in the presence of 0.1 (●), 1.0 (△) and 5.0 μM (▲) PD 102807. Data are expressed as percent of the maximal effect observed in the absence of antagonist and represent the means±S.E. of three experiments. Inset: Schild plot of PD 102807 antagonism.

nonlinear regression analysis for models of one or two noninteracting sites. The K_i value was calculated according to Cheng and Prusoff (1973). Radioligand binding data were analysed by the computer programme LIGAND (Biosoft, UK). The statistical significance between concentration–response curves was determined by analysis of variance (ANOVA). The statistical significance between means was determined by Student's *t*-test.

3. Results

3.1. Effects of carbachol on dopamine D1-like receptor-induced stimulation of cyclic AMP formation

In membranes of rat nucleus accumbens, dopamine stimulated adenylyl cyclase activity with an EC_{50} value of 1.8±0.4 μM and a maximal effect corresponding to 96±3% increase of the basal rate (Fig. 1). The addition of carbachol (1 mM) inhibited the maximal stimulation of cyclic AMP formation elicited by dopamine by 42.2±2.8% (n=4, P<0.05). Carbachol (1 mM) also inhibited basal adenylyl cyclase activity by 30±2% (P<0.05). The effects of carbachol were concentration-dependent, with EC_{50} values of 0.65±0.03 and 0.74±0.06 μM for the inhibition of basal and dopamine-stimulated adenylyl cyclase activities, respectively (Fig. 1).

The stimulation of adenylyl cyclase by dopamine was completely blocked by the selective dopamine D1-like receptor antagonist SCH 23390 with a K_i value of 0.24±0.05 nM (n=3) (Fig. 2). Moreover, the selective dopamine D1-like receptor agonist (±)-APB maximally stimulated cyclic AMP formation by 98.2±6% with an EC_{50} value of 10±1.0 nM (Fig. 2). Carbachol (1 mM) decreased maximal cyclic AMP stimulation by 57±2% without significantly changing the potency of the dopamine receptor agonist (EC_{50}=11.2±2.0 nM).

3.2. Effects of carbachol on dopamine inhibition of [^{3}H]SCH 23390 binding

Increasing concentrations of dopamine inhibited the specific binding of [^{3}H]SCH 23390 with a curve which fitted better to a two-site model, indicating the presence of a high- and a low-affinity component with IC_{50} values of 0.44±0.09 and 23±4 μM, respectively (n=3; results not shown). The percentage of the high-affinity component was 25.1±3% (n=3). The addition of carbachol (1 mM) affected neither the specific binding of [^{3}H]SCH 23390, the potencies of dopamine in competing with the radioligand at the high- and the low-affinity sites (IC_{50} values of dopamine were: 0.33±0.1 and 22±5 μM, respectively, n=3), nor the percentage of the high-affinity component (22.8±2.1%, n=3, P>0.05) (results not shown).

3.3. Antagonism of carbachol inhibition

The addition of increasing concentrations of PD 102807, a muscarinic M_4 receptor-preferring antagonist (Schwarz et al., 1997), progressively shifted to the right the concentration–response curve of carbachol in inhibiting dopamine-stimulated adenylyl cyclase activity (Fig. 3). The pA_2 value of PD 102807 was 7.92 and the slope value of the Schild regression was 0.93. AF-DX 116, a muscarinic M_2 receptor-

Table 1
Affinity constants of muscarinic receptor antagonists in counteracting carbachol inhibition of dopamine-stimulated adenylyl cyclase activity in rat nucleus accumbens and in blocking the distinct muscarinic receptor subtypes

Antagonist	Nucleus accumbens[a]		Muscarinic receptor subtypes[b]				
	pA_2	Slope	M_1	M_2	M_3	M_4	M_5
PD 102807	7.92±0.05	0.92±0.03	5.3	5.7	6.2	7.3	5.2
pFHHSiD	7.41±0.03	0.95±0.04	7.2–7.5	6.0–6.9	7.8–7.9	7.5	7.0
Pirenzepine	6.92±0.02	0.93±0.02	7.9–8.2	6.3–6.7	6.7–7.1	7.1–7.6	6.2–7.1
AF-DX 116	6.40±0.05	0.96±0.03	6.4–6.7	7.1–7.2	5.9–6.6	6.6–7.0	6.6

[a] Values are the means±S.E. of three determinations for each antagonist.
[b] pA_2 values are from Caufield (1993) and Caufield and Birdsall (1998).

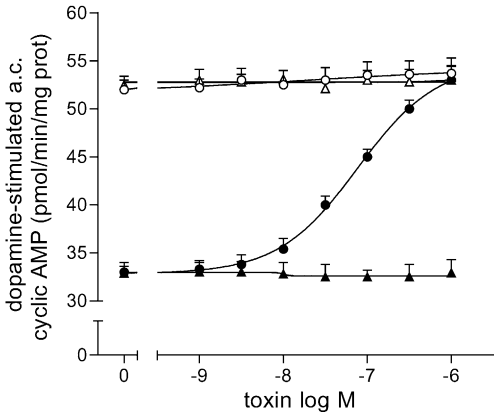

Fig. 4. Effects of MT-3 and MT-7 on carbachol inhibition of dopamine-stimulated adenylyl cyclase (a.c.) in membranes of rat nucleus accumbens. The carbachol (10 μM) inhibition of the enzyme activity stimulated by dopamine (30 μM) was determined in the presence of the indicated concentrations of either MT-3 (●) or MT-7 (▲). In the absence of carbachol, both toxins failed to affect dopamine-stimulated enzyme activity (open symbols). Data are the means±S.E. of three experiments.

preferring antagonist, pirenzepine, a muscarinic M_1 receptor-preferring antagonist, and pFHHSiD, a muscarinic M_3 receptor-preferring antagonist, were less potent. The rank order of potency was: PD 102807>pFHHSiD>pirenzepine>AFDX 116 (Table 1).

The inhibitory effect elicited by carbachol (10 μM) was completely antagonized by MT-3 with a Hill slope of 0.91 and a pK_i value of 8.50±0.05 (Fig. 4). On the other hand, MT-7 failed to affect the carbachol inhibition at concentrations up to 1 μM.

3.4. Effects of carbachol in core and shell regions

In membranes prepared from microdissected core and shell regions, dopamine maximally stimulated adenylyl cyclase activity by 150±5.0% and 105±2.0% with EC_{50} values of 1.9±0.2 and 2.2±0.3 μM, respectively. The addition of 1 mM carbachol inhibited the dopamine response by 44±2.1% and 45±2.4% in the core and the shell, respectively (n=3) (results not shown).

4. Discussion

The present study demonstrates for the first time that in rat nucleus accumbens muscarinic M_4 receptors and dopamine D1-like receptors interact antagonistically in the control of adenylyl cyclase activity. The interaction occurs in a broken cell preparation, a condition which limits the possibility of indirect mechanisms, such as those induced through the release of endogenous neurotransmitters. Previous studies have shown that in rat striatal membranes the binding properties of dopamine D1-like receptors were modulated by adenosine A_1 receptor activation, possibly through a mechanism of intramembrane receptor–receptor interaction (Ferre' et al., 1994). To investigate whether the activation of muscarinic receptors could exert a similar action in membranes of nucleus accumbens, we examined the effect of carbachol on dopamine inhibition of [^{3}H]SCH 23390 binding. In agreement with previous radioligand binding studies (Reader et al., 1988; Ferre' et al., 1994), dopamine competition curves display a high and a low affinity binding component. The addition of carbachol fails to significantly affect the dopamine binding properties, suggesting that a negative modulation of D1-like receptor affinity for dopamine is unlikely to be responsible for the muscarinic inhibition of dopamine D1-like receptor signalling.

The involvement of dopamine D1-like receptors is demonstrated by the complete blockade of the dopamine-stimulated adenylyl cyclase activity by the selective dopamine D1-like receptor antagonist SCH 23390. The potency of SCH 23390 (K_i=0.24 nM) is close to that previously obtained in the antagonism of dopamine-stimulated adenylyl cyclase activity in rat striatal membranes (Iorio et al., 1983). It is unlikely that β-adrenergic receptors, which are expressed in the nucleus accumbens (Summers and McMartin, 1993), participate to the overall stimulation of adenylyl cyclase by dopamine, as SCH 23390 displays negligible affinity for these receptors (Cross et al., 1983). Moreover, the dopamine D1-like receptor agonist (±)-APB stimulates cyclic AMP formation as effectively as dopamine and this effect is similarly inhibited by carbachol. In addition to dopamine D1 receptors, dopamine D5 receptors are expressed in the rat nucleus accumbens (Sunahara et al., 1991) and may contribute to the dopamine stimulation of adenylyl cyclase. As available ligands do not distinguish between dopamine D1 and D5 receptors, it remains to be characterized which receptor subtype is under muscarinic negative control.

Like other brain areas, the nucleus accumbens expresses multiple subtypes of muscarinic receptors, particularly the M_1, M_4 and M_2 (Levey et al., 1991). In neostriatum, each of these receptor subtypes has been found to be colocalized with dopamine D1 receptors in phenotypically different neuronal populations (Bernard et al., 1992) and therefore has the potential of establishing a negative interaction. Both muscarinic M_2 and M_4 receptor subtypes preferentially couple to G proteins of the G_i/G_o family and have been shown to inhibit adenylyl cyclase activity in different brain regions (McKinney et al., 1989). Although activation of the muscarinic M_1 receptor subtype has generally been found to be either not coupled or weakly coupled to adenylyl cyclase, there is evidence that in some cellular systems muscarinic M_1 receptors can directly modulate cyclic AMP formation both in a positive and in a negative manner (Onali and Olianas, 1998; Stein et al., 1988). In the present study, we have used different muscarinic receptor subtype-preferring antagonists to characterize the pharmacological nature of the receptor mediating the inhibition of dopamine D1-like receptor signalling in nucleus accumbens. Schild plot anal-

ysis indicate that the muscarinic M_4 receptor antagonist PD 102807 is highly potent, displaying a pA_2 value (7.92) which correlates well with the reported drug affinity for the cloned human muscarinic M_4 receptor subtype (Schwarz et al., 1997). On the other hand, the muscarinic M_3 receptor antagonist pFHHSiD (pA_2=7.41), the muscarinic M_1 receptor antagonist pirenzepine (pA_2=6.92) and the muscarinic M_2 receptor blocker AF-DX 116 (pA_2=6.40) were weaker and each displayed a potency lower than its affinity for the preferred receptor subtype (Caufield, 1993). In addition, the inhibitory effect of carbachol is completely antagonized by the selective muscarinic M_4 receptor antagonist MT-3 with a potency (pK_i=8.50) consistent with its affinity for the muscarinic M_4 receptor (Adem and Karlsson, 1997). Conversely, MT-7, which selectively blocks the muscarinic M_1 receptor subtype (Adem and Karlsson, 1997), is completely inactive. Collectively, these data demonstrate that the muscarinic M_4 receptor subtype mediates the muscarinic inhibition of dopamine D1-like receptor signalling.

Two regions of the rat nucleus accumbens, a centrally located core and a peripherally distributed shell, have been shown to differ with respect to anatomical connections and some neurochemical properties (Groenewegen et al., 1999). Functionally, the core region is considered to be a part of the extrapyramidal motor system, whereas the shell region has close relationships with the limbic system governing visceral and motivational mechanisms. The present study shows that muscarinic M_4 receptor activation inhibits dopamine D1-receptor-induced stimulation of adenylyl cyclase by the same extent in membranes of microdissected core and shell, indicating that this type of functional interaction can be operative in both neuronal circuits.

The negative control of dopamine D1-like receptor activity by muscarinic M_4 receptors may constitute an important molecular background for different behavioural, neurochemical and pharmacological observations. Gomeza et al. (1999) recently reported that the administration of the selective dopamine D1-like receptor agonist SKF 38393 elicited an enhanced locomotor response in muscarinic M_4-receptor-deficient mice as compared with their wild-type littermate. As dopamine D1-like receptor agonist-induced hypermotility is largely mediated by dopamine D1-like receptors of nucleus accumbens (Dreher and Jackson, 1989), it is possible that the altered behavioural response observed in muscarinic M_4 receptor-deficient mice is, at least in part, due to the lack of muscarinic inhibition of dopamine D1-like receptor activity in this brain region. In vivo studies have previously shown that the administration of the muscarinic antagonist scopolamine to rats increased dopamine D1-like receptor-induced immediate early gene and neuropeptide gene expression in dorsal and ventral striatum (McGinty, 1999). The present findings support the possibility that in both brain regions the scopolamine stimulatory effects may result from the blockade of muscarinic M_4 receptors inhibiting adenylyl cyclase-coupled dopamine D1-like receptors. Furthermore, a number of studies have demonstrated an important role of dopamine D1-like receptors in the stimulatory effects of cocaine in both humans (Romach et al., 1999) and rodents (Caine and Koob, 1994) and have indicated that these receptors are localized in the nucleus accumbens (McGregor and Roberts, 1993). In addition, it has been shown that in this brain area cholinergic cell ablation by immunotoxin-mediated cell targeting results in an enhanced responses to the locomotor-activating and rewarding effects of cocaine (Hikida et al., 2001), indicating that the accumbal cholinergic tone is critical in reducing the sensitivity to cocaine. Thus, the present demonstration that in nucleus accumbens activation of muscarinic M_4 receptors curtails dopamine D1-like receptor signalling suggests new therapeutic approaches to cocaine addiction through the development of drugs acting as selective muscarinic M_4 receptor agonists.

References

Adem, A., Karlsson, E., 1997. Muscarinic receptor subtype selective toxins. Life Sci. 60, 1069–1076.

Bernard, V., Normand, E., Bloch, B., 1992. Phenotypical characterization of the rat striatal neurons expressing muscarinic receptor genes. J. Neurosci. 12, 3591–3600.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Caine, S.B., Koob, G.F., 1994. Effects of dopamine D-1 and D-2 antagonists on cocaine self-administration under different schedules of reinforcement in the rat. J. Pharmacol. Exp. Ther. 270, 109–218.

Carlsson, M., Carlsson, A., 1990. Interaction between glutamatergic and mono-aminergic systems within the basal ganglia: implications for schizophrenia and Parkinson's disease. Trends Neurosci. 13, 272–276.

Caufield, M.P., 1993. Muscarinic receptors: characterization, coupling and function. Pharmacol. Ther. 58, 319–379.

Caufield, M.P., Birdsall, N.J.M., 1998. International Union of Pharmacology: XVII. Classification of muscarinic acetylcholine receptors. Pharmacol. Rev. 50, 279–290.

Cheng, Y.-C., Prusoff, W.H., 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Cross, A.J., Mashal, R.D., Johnson, J.A., Owen, F., 1983. Preferential inhibition of ligand binding to calf striatal dopamine D1 receptors by SCH 23390. Neuropharmacology 22, 1327–1329.

Deutch, A.Y., Cameron, D.S., 1991. Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. Neuroscience 46, 49–56.

Dreher, J.K., Jackson, D.M., 1989. Role of D_1 and D_2 dopamine receptors in mediating locomotor activity elicited from the nucleus accumbens of rats. Brain Res. 487, 267–277.

Ferre', S., Popoli, P., Gimenez-Llort, L., Finnman, U.-B., Martinez, E., Scotti de Carolis, A., Fuxe, K., 1994. Postsynaptic antagonistic interaction between adenosine A_1 and dopamine D_1 receptors. NeuroReport 6, 73–76.

Gomeza, J., Zhang, L., Kostenis, E., Felder, C., Bymaster, F., Brodkin, J., Shannon, H., Xia, B., Deng, C.-X., Wess, J., 1999. Enhancement of D1 dopamine receptor-mediated locomotor stimulation in M4 muscarinic acetylcholine receptor knockout mice. Proc. Natl. Acad. Sci. U. S. A. 96, 10483–10488.

Greengard, P., 2001. The neurobiology of slow synaptic transmission. Science 294, 1024–1030.

Groenewegen, H.J., Wright, C.I., Beijer, A.V.J., Voorn, P., 1999. Convergence and segregation of ventral inputs and outputs. Ann. N. Y. Acad. Sci. 877, 49–63.

Hajnal, A., Szekely, M., Galosi, R., Lenard, L., 2000. Accumbens cholinergic interneurons play a role in the regulation of body weight and metabolism. Physiol. Behav. 70, 95–103.

Hikida, T., Kaneko, S., Isobe, T., Kitabatake, Y., Watanabe, D., Pastan, I., Nakanishi, S., 2001. Increased sensitivity to cocaine by cholinergic cell ablation in nucleus accumbens. Proc. Natl. Acad. Sci. U. S. A. 98, 13351–13354.

Iorio, L.C., Barnett, A., Leitz, F.H., Houser, V.P., Korduba, C.A., 1983. SCH 23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. J. Pharmacol. Exp. Ther. 226, 462–468.

Kelly, E., Nahorski, S.R., 1986. Specific inhibition of dopamine D-1-mediated cyclic AMP formation by dopamine D-2, muscarinic cholinergic, and opiate receptor stimulation in rat striatal slices. J. Neurochem. 47, 1512–1516.

Lachowicz, J.E., Sibley, D.R., 1997. Molecular characteristics of mammalian dopamine receptors. Pharmacol. Toxicol. 81, 105–113.

Levey, A.I., Kitt, C.A., Simonds, W.F., Price, D.L., Brann, M.R., 1991. Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J. Neurosci. 11, 3218–3226.

McGinty, J.F., 1999. Regulation of neurotransmitter interactions in the ventral striatum. Ann. N. Y. Acad. Sci. 877, 129–139.

McGregor, A., Roberts, D.C.S., 1993. Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. Brain Res. 624, 245–252.

McKinney, M., Anderson, D., Vella-Rountree, L., 1989. Different agonist–receptor active conformations for rat brain M1 and M2 muscarinic receptors that are separately coupled to two biochemical effector systems. J. Pharmacol. Exp. Ther. 35, 39–47.

Olianas, M.C., Onali, P., Neff, N.H., Costa, E., 1983. Muscarinic receptors modulate dopamine-activated adenylate cyclase of rat striatum. J. Neurochem. 41, 1364–1369.

Olianas, M.C., Abdu, A., Karlsson, E., Onali, P., 1996. Rat striatal muscarinic receptors coupled to the inhibition of adenylyl cyclase activity: potent block by the selective m4 ligand muscarinic toxin 3 (MT3). Br. J. Pharmacol. 118, 283–288.

Onali, P., Olianas, M.C., 1998. Identification and characterization of muscarinic receptors potentiating the stimulation of adenylyl cyclase activity by corticotropin-releasing hormone in membranes of rat frontal cortex. J. Pharmacol. Exp. Ther. 286, 753–759.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd edn. Academic Press, San Diego.

Reader, T.A., Brière, R., Gottberg, E., Diop, L., Grondin, L., 1988. Specific [^{3}H]SCH 23390 binding to dopamine D_1 receptors in cerebral cortex and neostriatum: evidence for heterogeneity in affinity states and cortical distribution. J. Neurochem. 50, 451–463.

Romach, M.K., Glue, P., Kampman, K., Kaplan, H.L., Somer, G.R., Poole, S., Clarke, L., Coffin, V., Cornish, J., O'Brien, C.P., Sellers, E.M., 1999. Attenuation of the euphoric effects of cocaine by the dopamine D1/D5 antagonist ecopipam (SCH 39166). Arch. Gen. Psychiatry 56, 1101–1106.

Salomon, Y., Londos, D., Rodbell, M., 1974. A highly sensitive adenylate cyclase assay. Anal. Biochem. 58, 541–548.

Schwarz, R.D., Nelson, C.B., Augelli-Szafran, C.E., Penvoste, J.R., Jaen, J.C., Wiley, J., Frey, K.A., 1997. Pharmacological characterization of PD102807: an m4 selective muscarinic antagonist. Life Sci. 60, 1167.

Stein, R., Pinkas-Kramarski, R., Sokolovsky, M., 1988. Cloned M1 muscarinic receptors mediate both adenylate cyclase inhibition and phosphoinositide turnover. EMBO J. 7, 3031–3035.

Summers, R.J., McMartin, L.R., 1993. Adrenoceptors and their second messenger systems. J. Neurochem. 60, 10–23.

Sunahara, R.K., Guan, H.-C., O'Dowd, B.F., Seeman, P., Laurier, L.G., Ng, G., George, S.R., Torchia, J., Van Tol, H.H.M., Niznik, H.B., 1991. Cloning of the gene for a human dopamine D_5 receptor with higher affinity for dopamine than D_1. Nature 350, 614–619.

Tallarida, R.J., Murray, R.B., 1987. Manual of Pharmacologic Calculations with Computer Programs. Springer-Verlag, New York.

Wang, J.Q., Jolkkonen, M., McGinty, J.F., 1997. The muscarinic toxin 3 augments neuropeptide mRNA in rat striatum. Eur. J. Pharmacol. 334, 43–47.

Yung, K.K.L., Bolam, J.P., Smith, A.D., Hersch, S.M., Ciliax, B.J., Levey, A.I., 1995. Immunocytochemical localization of D_1 and D_2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. Neuroscience 65, 709–730.