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# Regulation of aromatic L-amino acid decarboxylase in rat striatal synaptosomes: effects of dopamine receptor agonists and antagonists

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**1** In this study we investigated the effects of dopamine receptor agonists and antagonists on rat striatal synaptosomal aromatic L-amino acid decarboxylase (AADC) activity.

**2** The results show that $10^{-5}$–$10^{-7}$ M *cis*-flupenthixol increased the striatal synaptosomal AADC activity (by 25% to 57%) in a time-dependent manner. SCH 23390 and remoxipride alone had little or no effect on striatal synaptosomal AADC activity, but in combination they increased AADC activity by 20%, suggesting that the increases in striatal synaptosomal AADC activity occurred only after blockade of both dopamine $D_1$ and $D_2$ receptors.

**3** Treatment with (+)-amphetamine and (±)-2-(N-phenylethyl-*N*-propyl)amino-5-hydroxytetralin hydrochloride ((±)-PPHT) produced a reduction of striatal synaptosomal AADC activity in a concentration- and time-dependent manner. SKF 38393 and (−)-quinpirole, however, exhibited no effect on striatal synaptosomal AADC activity, suggesting that only the mixed dopamine receptor agonists can reduce the AADC activity. Incubation with apomorphine at a concentration of $10^{-4}$ M inhibited the AADC activity by 74% and this inhibition cannot be antagonized by SCH 23390, remoxipride or *cis*-flupenthixol, suggesting that apomorphine-induced inhibition of striatal synaptosomal AADC activity was not mediated by dopamine receptors.

**4** *cis*-Flupenthixol can reverse the reduction of AADC activity induced by (+)-amphetamine and (±)-PPHT. The inhibition of AADC activity elicited by (±)-PPHT also can be reversed by SCH 23390 and remoxipride.

**5** The inhibition of striatal synaptosomal AADC activity induced by (±)-PPHT is calcium-dependent and protein kinase C may play a role in the regulation of striatal AADC activity.

**6** These studies show that striatal synaptosomal AADC activity is regulated by dopamine receptors and indicate that *in vitro* dopamine $D_1$ and $D_2$ receptors have a synergistic effect in this regulation.

**Keywords:** Aromatic L-amino acid decarboxylase; dopamine receptor; agonists; antagonists; striatum; synaptosomes

## Introduction

Aromatic L-amino acid decarboxylase (E.C. 4.1.1.28, AADC) is the enzyme which catalyzes the decarboxylation of L-phenylalanine to form 2-phenylethylamine (PE) and it is considered to have a regulatory role in the synthesis of this amine (Saavedra, 1974). AADC is also required for the formation of the catecholamines and 5-hydroxytryptamine (5-HT), but in these cases it is not rate limiting (Brodie *et al.*, 1962). Since brain AADC is thought not to be saturated with substrates and to be relatively nonselective (Bowsher & Henry, 1986), the regulation of the enzyme has not been intensively studied. There is now evidence, however, that some physiological conditions and some treatments can change AADC activity *in vivo*. In the rat retina, AADC activity increases in response to light (Hadjiconstantinou *et al.*, 1988) and these changes were mediated by dopamine $D_1$ receptors (Rossetti *et al.*, 1990). The change in AADC activity was associated with *de novo* synthesis of the protein (Hadjiconstantinou *et al.*, 1988). Both dopamine $D_1$ and $D_2$ receptor blockers such as SCH 23390, haloperidol and sulpiride increase striatal AADC activity in control mice and in mice treated with N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Hadjiconstantinou *et al.*, 1993). Using molecular biology methods, it was recently found that the AADC mRNA level of rat brain was increased after chronic treatment with the neuroleptics, haloperidol and loxapine (Buckland *et al.*, 1992a), which also produced an increase in $D_1$, $D_2$ and $D_3$ dopamine receptor mRNA level (Buckland *et al.*, 1992b). These findings suggest that AADC activity may be modulated by dopamine receptors.

Previous studies from this laboratory have shown that the administration of dopamine $D_1$ or $D_2$-like receptor blockers produced dose-dependent increases in AADC activity in striatal and mesolimbic areas in the rat (Zhu *et al.*, 1992). The dopamine receptor blockade increased the $V_{max}$ but did not affect the $K_m$ of the enzyme. The use of a protein synthesis inhibitor suggested that at least within 1 h after drug treatment, *de novo* protein synthesis was not responsible for the increases in AADC (Zhu *et al.*, 1993). AADC activity was reduced by the administration of the dopamine receptor agonist, bromocriptine (Zhu *et al.*, 1993). Moreover, there is an interaction between some dopamine receptor antagonists on the striatal AADC activity after joint administration of these compounds (Zhu *et al.*, 1993). These data suggest that *in vivo* AADC is a modulated enzyme.

AADC has been considered to be predominantly located in the soluble fraction of both kidney extracts (Lovenberg *et al.*, 1962) and rat brain (Sims *et al.*, 1973). There are reports, however, that a substantial proportion (50% or more) of AADC may be associated with membranes (Rodriguez de Lores Arnaiz & De Robertis, 1964; Sims *et al.*, 1973). Subsequently, Gardner & Richards (1981) have reported that 35% of AADC is associated with the synaptosomal pool. In the present study we have investigated the effects of some dopamine receptor agonists and antagonists on AADC activity in rat striatal synaptosomes and the possible mechanism in the AADC regulation.

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## Methods

### Animals

Male Wistar rats (Charles River Canada, Montreal, Quebec), weighing 230–250 g at the time of experimentation, were used. The animals were housed in hanging wire cages with free access to food and water on a 12 h light/dark cycle (lights on at 06 h 00 min) at a temperature of 19–21°C.

### Preparation of synaptosomes

Synaptosomal-enriched fractions were prepared from striatum by differential and Ficoll-sucrose density gradient centrifugation as described by Cotman (1974) and Wood *et al.* (1979) and modified in our laboratory. Rats were killed by decapitation and brains were removed and immediately placed in ice-cold saline followed by dissection on ice to remove striata. Striata of three rats were homogenized in 0.32 M sucrose (1%, w/v), using 10 up and down strokes at 900 r.p.m. in a glass-Teflon homogenizer (clearance 0.25 mm). The homogenate was centrifuged at 1,000 *g* for 5 min and the separated supernatant was again centrifuged at 15,000 *g* for 12 min. The pellet was collected and resuspended in 0.32 M sucrose and then applied to a Ficoll-sucrose density gradient, consisting of 4, 6 and 13% (w/v) Ficoll in 0.32 M sucrose. After centrifugation at 63,580 *g* for 45 min, the synaptosomal-enriched fraction was collected from the 6–13% interface. The particulate fraction thus obtained was resuspended in 4 volumes of 0.32 M sucrose and spun at 50,000 *g* for 20 min. The resultant pellet was resuspended in the medium to be used for incubation.

### Incubation of synaptosomes with drugs

The synaptosomal pellet was resuspended in Somjens saline (in mM: NaCl 123, KCl 3.51, $NaH_2PO_4$ 1, $MgSO_4$ 0.8, $NaHCO_3$ 26, $CaCl_2$ 1.2 and D-glucose 11, pH 7.35 and saturated with 95% $O_2$/5% $CO_2$) (Magoski & Walz, 1992). For the calcium-free experiments, $Ca^{2+}$ was omitted from the medium. For the incubation, 200 µl of the synaptosome preparation was mixed with 600 µl of oxygenated Somjens saline in the presence of different concentrations of drugs (dissolved in 200 µl water) in a total volume of 1 ml. The control group received 200 µl of water instead of drugs. All samples before incubation were undertaken at 0°C in an ice bath. After shaking, the samples were transferred to a water bath of 37°C and incubated for 1 h. The reaction was terminated by returning the samples to the ice bath following which the samples were immediately centrifuged (4°C) at 40,000 *g* for 20 min. The pellets were then resuspended in 0.01 M sodium phosphate buffer, and stored overnight at −70°C for AADC and protein assay.

### Aromatic L-amino acid decarboxylase assay

AADC activity was assayed by a modification of the method of Nagatsu *et al.* (1979) and Okuno & Fujisawa (1983). The assay was based on the enzymatic conversion of L-3,4-dihydroxyphenylalanine (L-DOPA) to dopamine with measurement of dopamine by high performance liquid chromatography with electro-chemical detection (h.p.l.c.-e.c.): 50 µl of the incubation suspension were used for the assay. The assay mixture, containing sodium phosphate buffer 50 mM, pH 7.2, L-DOPA 0.04 mM (D-DOPA for estimation of blank values), ascorbic acid 0.17 mM, pyridoxal-5-phosphate 0.01 mM, pargyline 0.1 mM, 2-mercaptoethanol 1 mM, EDTA 0.1 mM and enzyme in a total volume of 400 µl, was incubated at 37°C for 20 min and the reaction was terminated by addition of 600 µl ice cold perchloric acid (0.1 M) containing isoprenaline as an internal standard. The mixture was transferred to a small conical polypropylene test tube, centrifuged again (3,000 r.p.m. for 10 min) and 50 µl of supernatant used for h.p.l.c. assay. Protein concentration was determined (Lowry *et al.*, 1951) with bovine serum albumin used as the standard and the enzyme activity was expressed as nmol of dopamine 20 $min^{-1}$ $mg^{-1}$ of protein at 37°C.

### Drugs for incubation with synaptosomes

Drugs used in this study were obtained from following sources: (−)-quinpirole HCl, SCH 23390 (R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride), (±)-PPHT hydrochloride ((±)-2-(N-phenylethyl-*N*-propyl)amino-5-hydroxytetralin hydrochloride), SKF 38393 (1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride), H-7 HCl ((1-(5-isoquinolinesulphonyl)-2-methylpiperazine) and PDBu (phorbol 12,13-dibutyrate), Research Biochemicals Inc. (Natick, MA, U.S.A.); apomorphine hydrochloride, Sigma Chemical Co. (St. Louis, MO, U.S.A.); (+)-amphetamine sulphate, Smith, Kline and French Laboratories (Philadelphia, PA, U.S.A.). The following compounds were generous gifts: remoxipride [S-3-bromo-*N*-(1-ethyl-2-pyrrolidinyl)methyl-2,6-dimethoxybenzamide hydrochloride monohydrate], Astra Pharma Inc. (Mississauga, Ontario); *cis*- and *trans*-flupenthixol, H. Lundbeck & Co. (Kobenhavn-Valby, Denmark). Solutions were freshly prepared in nanopure water.

### Statistical methods

Data were analysed by one- and two-way analysis of variance (ANOVA) (Winer, 1971) and the Newman-Keuls tests for multiple comparisons, depending on the experimental design. Statistical analyses were performed on a Macintosh Microcomputer using the CLR ANOVA programme (Clearlake Research, Houston, TX, U.S.A.).

## Results

### The effects of dopamine receptor antagonists on AADC activity in striatal synaptosomes

cis-*Flupenthixol* The concentration-response curve for activation of striatal synaptosomal AADC by *cis*-flupenthixol is presented in Figure 1a. The results show that $10^{-6}$ and $10^{-7}$ M of *cis*-flupenthixol increased AADC activity by 56% and 57% above control values, respectively ($F_{6,25} = 5.8$, $P < 0.001$). Incubation with higher concentrations of *cis*-flupenthixol produced a lower (with $10^{-5}$ M) or no increase (with $10^{-4}$ M) in AADC activity (Figure 1a).

The time course of *cis*-flupenthixol ($10^{-6}$ M) activation of AADC activity in striatal synaptosomes shows that at 30 min of incubation AADC activity has been increased (37%) and that maximal activation (47%) occurred after 1 h of incubation. By 2 h of incubation, however, this compound reduced AADC activity by 40% below the control value (Figure 1b). This reduction in AADC activity observed with longer exposures to *cis*-flupenthixol (Figure 1b) agrees with the result of lower effects produced by higher doses (Figure 1a).

*SCH 23390 and remoxipride* Unlike *cis*-flupenthixol, both SCH 23390 and remoxipride had no effects on striatal synaptosomal AADC activity when they were separately incubated with synaptosomal preparations (Figure 2a,b), nor was any inhibition in AADC observed with doses as high as $10^{-4}$ M. When $10^{-8}$ M of each of these compounds was incubated together with striatal synaptosomes, they increased synaptosomal AADC activity by 20% above control value ($F_{6,26} = 5.02$, $P < 0.01$, Figure 2c). This was the only dose combination found to increase the AADC activity; other lower dose ($10^{-9}$ M) or higher doses (up to $10^{-4}$ M) did not change its activity (Figure 2c).

*The effects of dopamine receptor agonists on AADC activity in striatal synaptosomes*

*(+)-Amphetamine* The incubation of different concentrations of (+)-amphetamine with striatal synaptosomes for 1 h produced a reduction in AADC activity. The effects were observed with concentrations of $10^{-7}$ M–$10^{-4}$ M of (+)-amphetamine and the reductions ranged from 29 to 35% of their respective controls ($F_{6,48} = 3.26$; $P<0.05$, Figure 3a). The time-course of the effect of (+)-amphetamine was studied with a concentration of $10^{-5}$ M and showed that by 1 h of incubation the AADC activity was 26% of controls and further reductions were observed by 2 h of incubation ($F_{3,20} = 13.2$, $P<0.001$; Figure 3b). The results indicate that (+)-amphetamine inhibits striatal synaptosomal AADC in both a concentration- and time-dependent manner.

*(±)-PPHT* Incubation of striatal synaptosomes with (±)-PPHT at concentrations of $10^{-4}$ M to $10^{-7}$ M produced a concentration-dependent reduction in AADC activity. The reductions were 37%, 36%, 31% and 30% with respect to their respective controls ($F_{6,49} = 3.3$, $P<0.01$) (Figure 3a).

Like (+)-amphetamine, (±)-PPHT ($10^{-5}$ M) also inhibited striatal synaptosomal AADC activity in a time-dependent manner (Figure 3b). This effect, however, was observed at an earlier time than that of (+)-amphetamine, reaching significance 30 min after incubation with values of 20% below control ($F_{3,19} = 8.27$, $P<0.01$).

*SKF 38393 and (−)-quinpirole* The concentration-response curves for SKF 38393 and (−)-quinpirole were measured after 1 h of incubation. The experiment indicated that neither SKF 38393 nor (−)-quinpirole (added in concentrations ranging from $10^{-9}$ to $10^{-4}$ M) changed the AADC activity in striatal synaptosomes ($F_{6,31} = 1.69$, for SKF 38393; $F_{6,57} = 0.9$, for (−)-quinpirole; data not shown), suggesting that selective dopamine $D_1$ or $D_2$-like receptor agonist does not affect AADC activity in striatal synaptosomes.

*Apomorphine* The concentration-response of apomorphine in rat striatal synaptosomes was determined after 1 h incubation. The AADC activity of controls was 5.18 ± 0.19 nmol dopamine $mg^{-1}$ of protein, 20 $min^{-1}$. Addition of $10^{-9}$ to $10^{-5}$ M of apomorphine produced no changes in synaptosomal AADC activity. AADC activity was inhibited by apomorphine at a dose of $10^{-4}$ M to values of 1.34 nmol dopamine $mg^{-1}$ of protein, 20 $min^{-1}$ (reduced by 74%, $F_{6,42} = 21.85$, $P<0.001$; data not shown). The time course indicated that incubation with $10^{-4}$ M apomorphine produced a reduction in AADC activity that was observed within 30 min (with reductions from 6.18 to 4.36 nmol dopamine

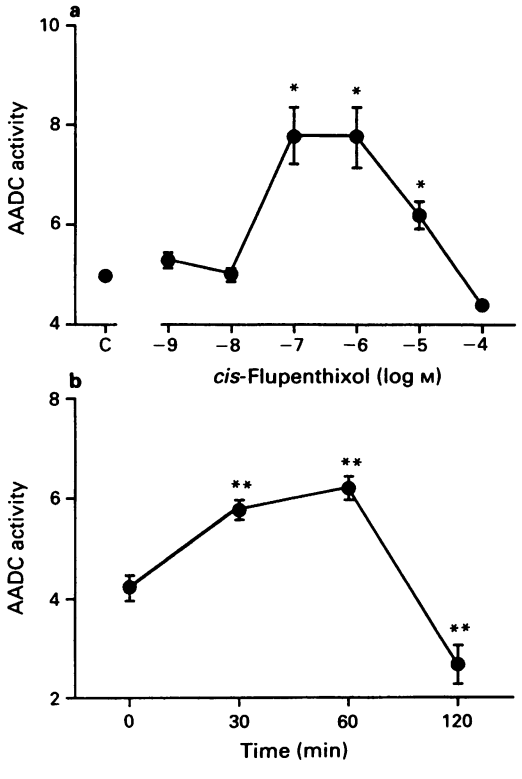


**Figure 1** Dose-response effects (a) and time course (b) of *cis*-flupenthixol on AADC activity (nmol of dopamine $mg^{-1}$ protein, 20 $min^{-1}$) in the rat striatal synaptosomes. The incubation time for dose-course was 1 h. The concentration of *cis*-flupenthixol used in the time-course was $10^{-6}$ M. The values in each experimental group were obtained from 6–8 samples. Data are mean ± s.e.mean. Significance was determined by one way ANOVA and Newman-Keuls test: $*P<0.05$; $**P<0.01$, compared with the control group (C group).

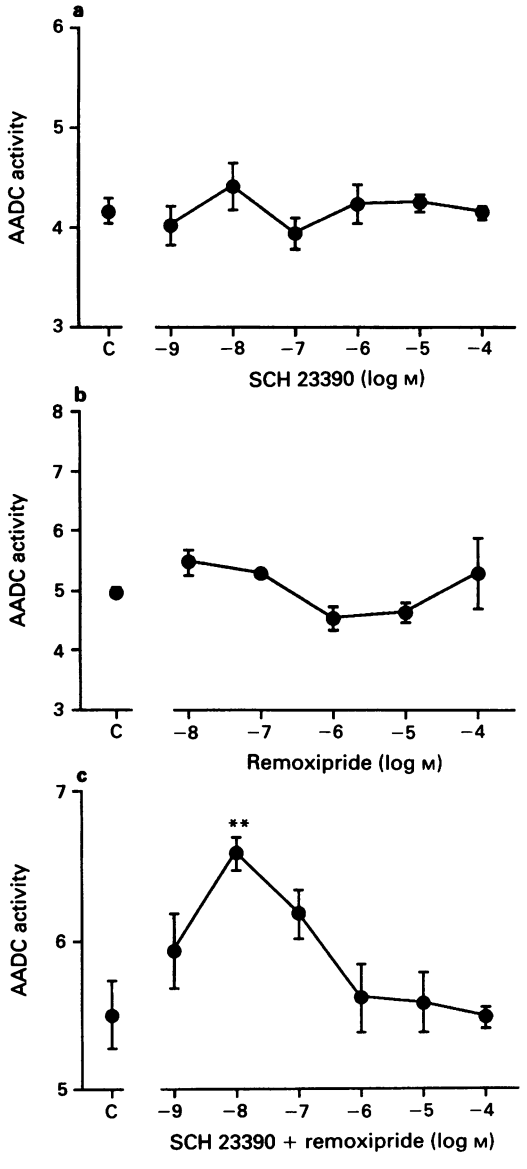


**Figure 2** Dose-response effects of SCH 23390 (a), remoxipride (b) and their combination (c) on AADC activity (nmol dopamine $mg^{-1}$ protein, 20 $min^{-1}$) in the rat striatal synaptosomes. The incubation time was 1 h. The values in each experimental group were obtained from 6 samples. Data are means ± s.e.mean. Significance was determined by one-way ANOVA and Newman-Keuls test: $**P<0.01$, compared with the control group (C).

$mg^{-1}$ of protein, 20 $min^{-1}$, 30% below controls). The AADC inhibition increased with time and by 2 h of incubation, almost all AADC activity (reaching values of 0.39 nmol dopamine $mg^{-1}$ of protein, 20 $min^{-1}$) was inhibited ($F_{6,28} = 94.5$, $P < 0.001$; data not shown). Treatment with SCH 23390, remoxipride or *cis*-flupenthixol did not reverse the inhibition of AADC activity induced by $10^{-4}$ M of apomorphine (data not shown). The findings suggested that the inhibition of AADC activity produced by apomorphine ($10^{-4}$ M) in striatal synaptosomes is unspecific or acts by a different mechanism.

### *The reversal of dopamine receptor agonist-mediated reduction in AADC activity by dopamine receptor blockers*

Exposure of synaptosomes to *cis*-flupenthixol abolished the (+)-amphetamine- or (±)-PPHT-induced reduction in AADC activity. The results showed that $10^{-5}$ and $10^{-6}$ M of *cis*-flupenthixol can effectively antagonize the effects of (+)-amphetamine ($10^{-5}$ M) on striatal synaptosomal AADC activity (Figure 4a). In another experiment, the action of (±)-PPHT ($10^{-5}$ M) was antagonized by $10^{-5}$–$10^{-8}$ M of *cis*-flupenthixol (Figure 4b).

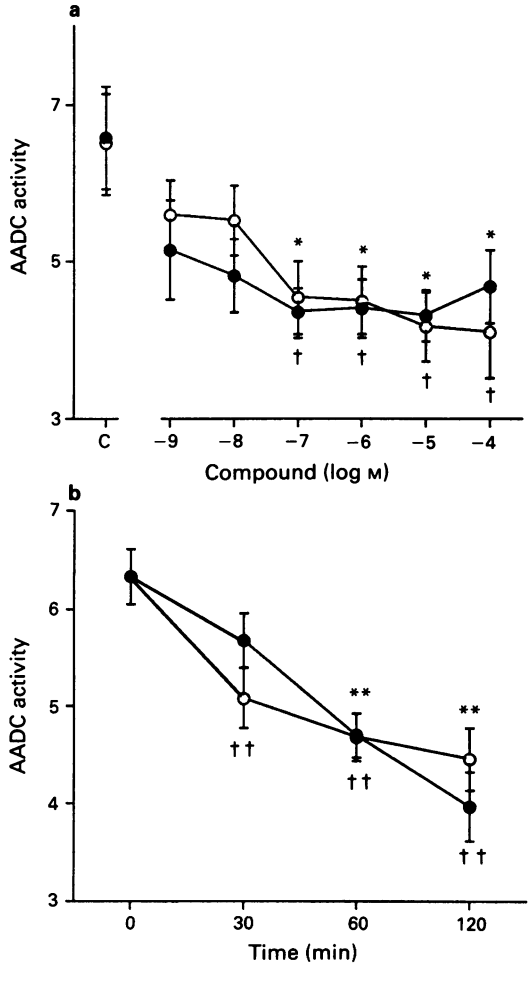


**Figure 3** Dose-response effects (a) and time course (b) of (+)-amphetamine (●) and (±)-PPHT (○) on AADC activity (nmol of dopamine $mg^{-1}$ protein, 20 $min^{-1}$) in the rat striatal synaptosomes. The incubation time for dose-course was 1 h. The concentrations of (+)-amphetamine and (±)-PPHT used in the time-course were $10^{-5}$ M. The values in each experimental group were obtained from 10 samples. Data are mean ± s.e.mean. Significance was determined by one way ANOVA and Newman-Keuls test: *,†$P < 0.05$; **††$P < 0.01$, compared with the control group (C and 0 groups).

Moreover, when SCH 23390 ($10^{-6}$ M) or remoxipride ($10^{-4}$–$10^{-6}$ M) was incubated alone with $10^{-5}$ M of (±)-PPHT and striatal synaptosomes, both counteracted the inhibition of AADC activity elicited by (±)-PPHT (Figure 5a,b).

### *The effects of calcium on AADC activity in striatal synaptosomes*

Somjens saline contains 1.2 mM calcium. Striatal synaptosomes were incubated with modified Somjens solution containing no calcium, low calcium (0.6 mM) and high calcium (2.4 mM). The effects of calcium on (±)-PPHT-induced inhibition of synaptosomal AADC activity were investigated and the results were analysed by two-way ANOVA. Incubation of striatal synaptosomes in Somjens solution in the absence of calcium or with lower (0.6 mM) calcium concentrations had no effect on synaptosomal AADC activity, compared to synaptosomes incubated in normal Somjens saline (1.2 mM calcium). The increase in calcium content of Somjens saline (2.4 mM) increased AADC activity by 41% ($F_{3,40} = 37.77$, $P < 0.001$) (Figure 6).

(±)-PPHT significantly reduced AADC activity in normal (1.2 mM calcium) medium as described above ($F_{1,40} = 16.52$, $P < 0.001$), but had no effects on synaptosomal AADC activity in calcium-free and lower calcium medium (0.6 mM) (Figure 6). In the higher calcium (2.4 mM) medium, incubation with (±)-PPHT counteracted the increase in AADC activity (Figure 6). These results indicate that (±)-PPHT-induced inhibition of AADC activity was calcium-dependent. There was a significant interaction between calcium and (±)-PPHT treatment ($F_{3,40} = 5.72$, $P < 0.001$).

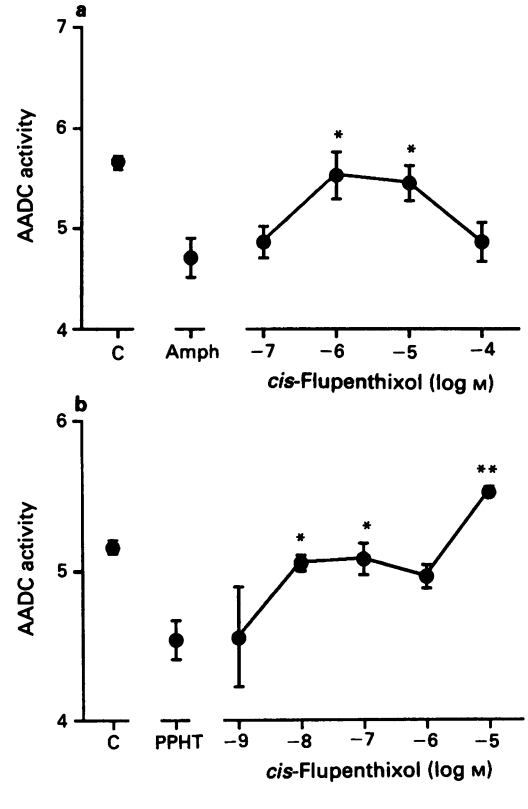


**Figure 4** The antagonism by *cis*-flupenthixol of (+)-amphetamine (Amph) (a) and (±)-PPHT (b)-induced inhibition of AADC activity (nmol of dopamine $mg^{-1}$ protein, 20 $min^{-1}$) in rat striatal synaptosomes. The incubation time was 1 h. The concentration of (+)-amphetamine and (±)-PPHT used in the experiments was $10^{-5}$ M. The values in each experimental group were obtained from 6 samples. Data are means ± s.e.mean. Significance was determined by one-way ANOVA and Newman-Keuls test: *$P < 0.05$; **$P < 0.01$, compared with the Amph and (±)-PPHT group, respectively.

### *Synaptosomal AADC activity after stimulation or inhibition of protein kinase C*

Striatal synaptosomal preparations were incubated with H-7 or PDBu, which respectively inhibit or activate protein kinase C activity. Activation of protein kinase C with PDBu alone significantly inhibited synaptosomal AADC activity at concentrations of $10^{-6}$–$10^{-7}$ M ($F_{5,24} = 3.68$, $P<0.05$, Figure 7b). H-7 alone had no effect on AADC activity ($F_{6,28} = 1.72$, $P<0.015$, Figure 7a), but $10^{-6}$ M H-7 attenuated the inhibition of synaptosomal AADC activity produced by $10^{-6}$ M PDBu ($F_{1,21} = 7.47$, $P<0.05$, as determined by two-way ANOVA).

Following a two-way experimental design, the addition of $10^{-5}$ M of (±)-PPHT alone reduced the synaptosomal AADC activity ($F_{1,10} = 43.93$, $P<0.001$) (Figure 7d). Treatment with $10^{-6}$ M H-7 neither affected AADC activity ($F_{1,10} = 1.05$, $P<0.33$), nor interfered with the inhibitory action of (±)-PPHT on synaptosomal AADC activity

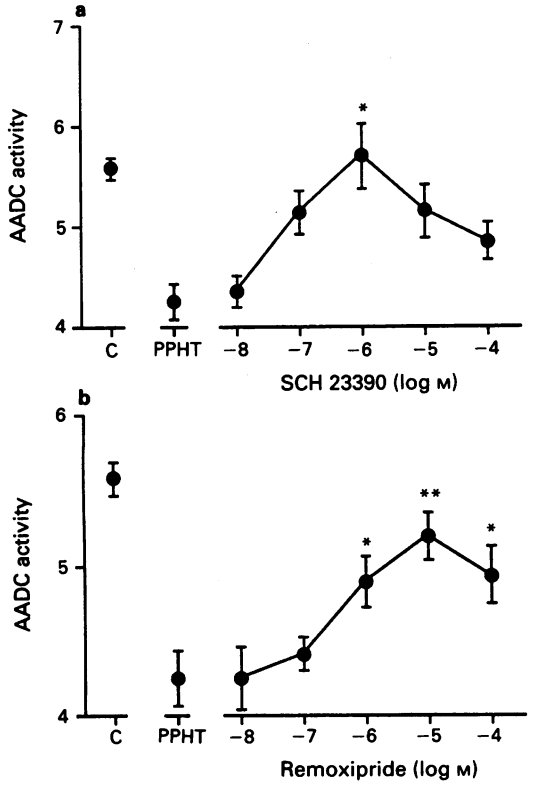


**Figure 5** The antagonism by SCH 23390 (a) and remoxipride (b) of (±)-PPHT-induced inhibition of AADC activity (nmol of dopamine $mg^{-1}$ protein, 20 $min^{-1}$) in rat striatal synaptosomes. The incubation time was 1 h. The concentration of (±)-PPHT used in the experiments was $10^{-5}$ M. The values in each experimental group were obtained from 6 samples. Data are means ± s.e.mean. Significance was determined by one-way ANOVA and Newman-Keuls test: $*P<0.05$; $**P<0.01$, compared with the (±)-PPHT group.

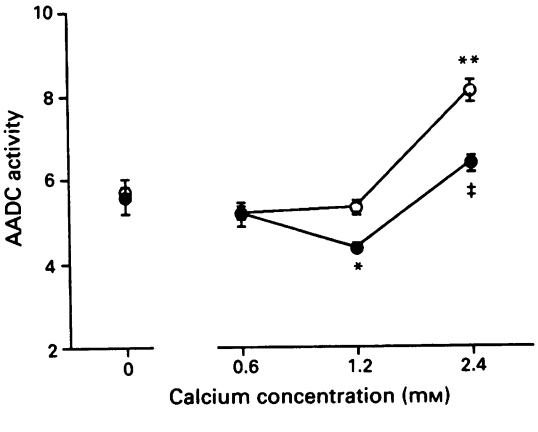


**Figure 6** The effects of different calcium concentrations on AADC activity (nmol of dopamine $mg^{-1}$ protein, 20 $min^{-1}$) in the presence (●) and absence (○) of (±)-PPHT ($10^{-5}$ M) in striatal synaptosomes. The incubation time was 1 h. The values in each group were obtained from 6 samples. Data are means ± s.e.mean. Significance was determined by two-way ANOVA and Newman-Keuls test: $*P<0.05$; $**P<0.01$, compared with the no-(±)-PPHT-1.2 mM group. $\dagger\dagger P< 0.01$, compared with the no-(±)-PPHT-2.4 mM group.

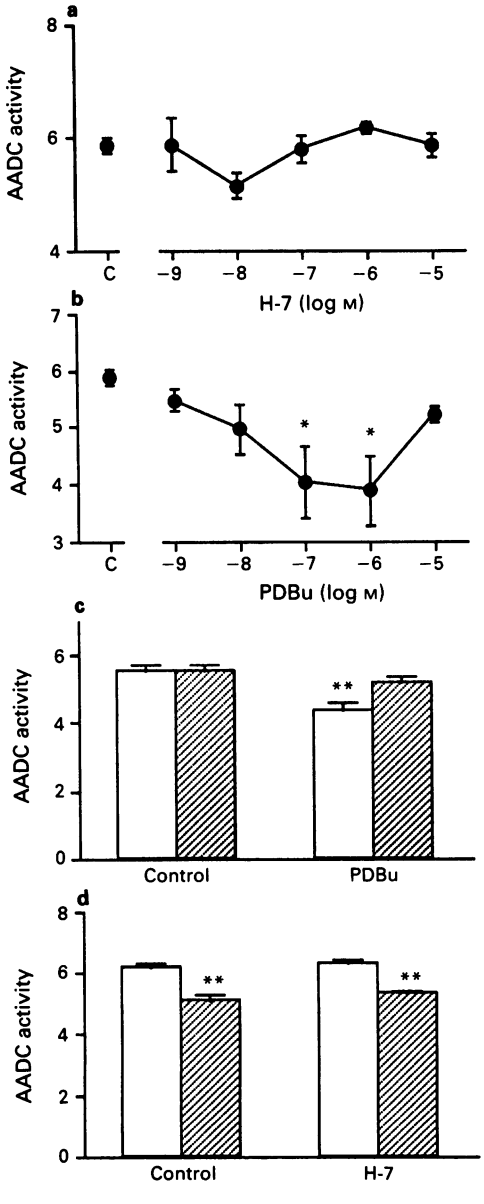


**Figure 7** Dose-response effects of H-7 (a), PDBu (b) and combinations of H-7 and PDBu (c, open columns represent control group; hatched columns represent H-7), H-7 and (±)-PPHT (d, open columns represent control group; hatched columns represent (±)-PPHT) on AADC activity (nmol dopamine $mg^{-1}$ of protein, 20 $min^{-1}$) in the rat striatal synaptosomes. The concentrations of H-7, PDBu and (±)-PPHT in (c) and (d) experiments were $10^{-6}$, $10^{-6}$ and $10^{-5}$ M respectively. Data are means ± s.e.mean (bars) from 9 samples. Statistical significances are determined by one-way ANOVA and Newman-Keuls test (a,b) or two-way ANOVA (c,d). $*P<0.05$, $**P<0.01$, compared with control group (c and d) groups.

($F_{1,10} = 0.1$, $P < 0.75$). This finding suggests that inhibition of synaptosomal AADC by (±)-PPHT is not related to protein kinase C.

## Discussion

This study has shown that treatment of rat striatal synaptosomes with the direct dopamine receptor agonist, (±)-PPHT and the indirect dopamine receptor agonist, (+)-amphetamine, inhibits AADC activity in rat striatal synaptosomes in a concentration- and time-dependent manner (Figure 3). A mixed dopamine receptor antagonist such as *cis*-flupenthixol not only increased AADC activity (Figure 1), but also reversed the inhibition of AADC produced by either (+)-amphetamine or (±)-PPHT (Figure 4). The dopamine $D_1$ receptor antagonist, SCH 23390 and the dopamine $D_2$ receptor antagonist, remoxipride alone had no effect on the synaptosomal AADC activity (Figure 2a,b), but when simultaneously administered, they increased the AADC activity (Figure 2c). Furthermore, both antagonized the (±)-PPHT-induced inhibition of AADC activity (Figure 5). These results extend our previous findings *in vivo* (Zhu *et al.*, 1992; 1993), and reveal that in rat striatal synaptosomes AADC is regulated by dopamine $D_1$-like and $D_2$-like receptors.

Both AADC and tyrosine hydroxylase are involved in the synthesis of dopamine. It is well known that apomorphine can act directly and indirectly on tyrosine hydroxylase. High concentrations of apomorphine ($10^{-4}$ M) produced a complete inhibition of tyrosine hydroxylase activity in a striatal enzyme preparation (Hurata & Shibata, 1991), striatal slices (Delanoy & Dunn, 1982) or in synaptosomal preparations (Christiansen & Squires, 1974), and DOPA producing cell lines (Bräutigram *et al.*, 1982). In most cases the inhibition of tyrosine hydroxylase caused by apomorphine can be completely antagonized by haloperidol and other neuroleptics *in vivo* (Kehr *et al.*, 1972; Walters & Roth, 1976; Westerink & Horn, 1979). The mechanism of the direct action of apomorphine on tyrosine hydroxylase is believed to be due to the catechol structure present in the apomorphine molecule, which competes for the pterin cofactor (Goldstein *et al.*, 1970; Birtan & Bustos, 1982). In this study we observed that a high concentration of apomorphine inhibited the striatal synaptosomal AADC activity and this inhibition was not mediated by dopamine receptors, since it was not reversed by dopamine receptor antagonists. In the control experiment we found that apomorphine ($10^{-4}$ M) had no direct effect on freely presented AADC activity (Zhu, 1993) so it is most likely that apomorphine is having an effect by an unspecific action.

Since it was first reported that both dopamine $D_1$ and $D_2$ receptor agonists were required to reverse the hypokinesia produced by reserpine in mice (Gershanik *et al.*, 1983), evidence has been accumulated suggesting that there is an interaction between dopamine $D_1$ and $D_2$ receptors. It has been proposed that this interaction possesses both synergistic or opposing effects (Clark & White, 1987). Most of the evidence about synergistic effects, however, was obtained from behavioural (Gershanik *et al.*, 1983; Rosengarten *et al.*, 1986), electrophysiological (White & Wang, 1986) and clinical studies (Lieberman *et al.*, 1981). Furthermore, the investigations were limited to the nucleus accumbens (White & Wang, 1986), globus pallidus (Carlson *et al.*, 1986; Walters *et al.*, 1987) and substantia nigra (Weick & Walters, 1987), and few investigations were done in the striatum (Stoof & Kebabian, 1981; 1984).

Although (±)-PPHT is considered as a selective dopamine $D_2$-like receptor agonist (Seeman *et al.*, 1985; Seiler *et al.*, 1986), there is evidence that when its concentration is over $10^{-6}$ M, it can act on dopamine $D_1$ high and low affinity sites (Berry, 1993). In the present study, it was found that among the dopamine receptor agonists, only nonselective agonists such as (+)-amphetamine and (±)-PPHT produced inhibition of striatal synaptosomal AADC activity (Figure 3), while no effects were observed with either the dopamine $D_1$-like receptor agonist, SKF 38393 or the dopamine $D_2$-like receptor agonist, (−)-quinpirole. Furthermore, only a mixed dopamine $D_1$ and $D_2$-like receptor antagonist such as *cis*-flupenthixol increased the striatal synaptosomal AADC activity (Figure 1). Blockade of dopamine $D_1$ receptors by SCH 23390 or dopamine $D_2$ receptors by remoxipride produced no changes in striatal synaptosomal AADC activity (Figure 2a,b). It was interesting to find that the combination of SCH 23390 and remoxipride dramatically increased the AADC activity (Figure 2c). The results clearly demonstrate that the activation and inhibition of AADC activity in striatal synaptosomes requires the participation of both dopamine $D_1$ and $D_2$ receptors. This is the first time that a biochemically synergistic interaction between dopamine $D_1$ and $D_2$ receptors in striatal synaptosomes has been reported.

The synergistic action of dopamine $D_1/D_2$ receptors has been interpreted as dopamine $D_1$ receptor activation enabling the functional expression of dopamine $D_2$ receptor stimulation (Clark & White, 1987). There has been a report that dopamine $D_1$ and $D_2$ receptor synergism at the level of a single cell may also occur via activation of the arachidonic acid cascade (Piomelli *et al.*, 1991). At present, however, the mechanism of the regulation of striatal AADC by dopamine receptors remains unknown. Since the administration of (+)-amphetamine inhibited striatal synaptosomal AADC activity (Figure 3), and *in vivo* acute and chronic administration of reserpine, which depletes endogenous dopamine storage (Bertler, 1961), increased the striatal AADC activity (Zhu, 1993), endogenous dopamine may act as a negative feedback for striatal AADC. Similar results were recently observed by Hadjiconstantinou *et al.* (1993). This finding that in striatal synaptosomes dopamine $D_1$ and $D_2$ receptors appear to be synergistic with respect to AADC activity may possess important clinical significance. Administration of a selective and potent dopamine $D_1$-like receptor agonist with a dopamine $D_2$-like receptor agonist may lead to therapeutic improvement in the treatment of Parkinsons's disease. Recently, apomorphine has been investigated as a rescue therapy for Parkinson's disease; it was administered subcutaneously and sublingually to reverse 'off' periods occurring during oral L-DOPA therapy (Deffond *et al.*, 1993), and as a continual therapy, delivered subcutaneously by minipump (Frankel *et al.*, 1990).

*In vivo* the dopamine $D_1$-like receptor antagonist, SCH 23390 and the dopamine $D_2$-like receptor antagonist, pimozide, as well as remoxipride alone increased the AADC activity in rat striatum (Zhu *et al.*, 1992; 1993). Synaptosomal preparations showed no increase in AADC activity after selective blockade of dopamine $D_1$ or $D_2$ receptors (Figure 2a,b). These differences may be due to the different experimental conditions, and the regulation of striatal synaptosomal AADC activity may be more specific than in *in vivo* brain tissues. In the intact animal, the dopaminergic nigrostriatal neurones have perikarya in the substantia nigra with their terminals in the striatum. The substantia nigra is an important action site for dopamine receptor antagonists. Also, dopamine receptors may be localized on dendrites, terminals, or glial cells adjacent to dopaminergic terminals (Creese, 1987). All these factors may affect the appearance of action of dopamine receptor antagonists *in vivo*. In contrast, in synaptosomes nerve terminals in sufficiently dilute suspension may be considered as an isolated structure where interactions with other brain regions do not exist.

The inhibition of striatal synaptosomal AADC activity induced by (+)-amphetamine and (±)-PPHT can be reversed by the mixed dopamine $D_1$ and $D_2$-like receptor antagonist *cis*-flupenthixol (Figure 4). Although SCH 23390 and remoxipride alone had no effect on AADC activity (Figure 2a,b), they can reverse the inhibition of AADC activity induced by (±)-PPHT (Figure 5). These findings suggest that the changes in striatal synaptosomal AADC

activity by (+)-amphetamine and (±)-PPHT may be receptor-mediated. Although the precise mechanism to explain this regulation has not been identified, one interesting finding is that the absence of calcium or the presence of a lower than normal concentration of calcium has no effect on the synaptosomal AADC activity, but it does prevent the inhibition of AADC activity by (±)-PPHT (Figure 6). On the other hand, higher concentrations of calcium increased the AADC activity, but did not block the inhibition of AADC activity induced by (±)-PPHT. This suggests that the regulation of striatal synaptosomal AADC by dopamine receptors is calcium-dependent. At present, it may be speculated that stimulation or blockade of dopamine receptors in striatal synaptosomes by dopamine receptor agonists or antagonists produced a change in the second messenger system, which in turn caused other changes like phosphorylation and led to the changes in AADC activity.

Other experiments were conducted to examine whether phosphorylation was involved in the regulation of AADC via dopamine receptors. The results show that PDBu, a phorbol ester which selectively activates protein kinase C (Castagna *et al.*, 1982), significantly reduced synaptosomal AADC activity (Figure 7b). This reduction in AADC activity was prevented by the simultaneous administration of H-7, a protein kinase inhibitor (Hidaka *et al.*, 1984) (Figure 7c), although H-7 alone did not influence the AADC activity (Figure 7a). This suggests that protein kinase may participate in the regulation of striatal AADC *in vitro*, which is supported by the finding that AADC regulation requires the presence of calcium (Figure 6), since activation of protein kinase C is dependent on calcium (Nishizuka, 1986). However, when H-7 was co-administered with the dopamine receptor agonist (±)-PPHT, H-7 could not prevent the reduction of synaptosomal AADC activity produced by (±)-PPHT (Figure 7d). This latter experiment indicates that although protein kinase C may participate in the regulation of AADC, it may not be related to the regulation of AADC by dopamine receptors. Other neurotransmitters or compounds, which act on protein kinase C via their second messenger system, may therefore regulate AADC activity.

Our previous papers (Zhu *et al.*, 1992; 1993) indicated that at least within 1 h after administration of dopamine receptor antagonist the change of striatal AADC is not due to *de novo* protein synthesis. Other investigators demonstrated that 3 or 6 h after injection of dopamine receptor antagonists, protein synthesis was responsible for the increase of striatal AADC activity (Hadjiconstantinou *et al.*, 1988; 1993). These observations suggest that there may be different mechanisms for regulation of AADC: a short-term regulation of AADC that occurs within 1 h after dopamine receptor blockade and that is not dependent on protein synthesis (Zhu *et al.*, 1992; 1993), and a long-term regulation that is dependent on protein synthesis (Hadjiconstantinou *et al.*, 1988; 1993). Since phosphorylation of tyrosine hydroxylase is the main mechanism for its short term regulation (Masserano *et al.*, 1989), it is reasonable to assume that phosphorylation may be responsible for the short term regulation of striatal AADC activity. The data demonstrate that a phorbol ester can reduce the AADC activity in striatal synaptosomes and this reduction may be mediated by activation of protein kinase C. In conclusion, protein kinase C may play a role in the regulation of striatal AADC. This preliminary result, however, cannot rule out the participation of other protein kinases such as adenosine 3':5'-cyclic monophosphate (cyclic AMP)-dependent protein kinase or $Ca^{2+}$/calmodulin-dependent protein kinase, both of which as well as protein kinase C, participate in the regulation of tyrosine hydroxylase (Joh *et al.*, 1978; Vulliet *et al.*, 1984; Raese *et al.*, 1979). Consistent with the present findings, the AADC activity in the mouse striatum and midbrain has been reported to be increased by the intracerebroventricular injection of either forskolin, which activates adenylate cyclase and increases cyclic AMP, or 8-Br-cyclic AMP (Young *et al.*, 1993), suggesting that a cyclic AMP-dependent protein kinase may phosphorylate AADC and increase its activity.

In conclusion, the present *in vitro* study provides evidence that the regulation of striatal AADC occurs in synaptosomes and that it may play a role in the mechanism of the effects of drugs that interact with dopamine receptors. The data also suggest that synaptosomes may provide a useful system to study the effects of drugs on AADC activity.

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