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Pharmacological Characterization of the D_2 Dopamine Receptor Negatively Coupled with Adenylate Cyclase in Rat Anterior Pituitary

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SUMMARY

In male and female rat anterior pituitary homogenates dopamine inhibited basal adenylate cyclase by 30% and 50%, respectively. Dopamine also inhibited vasoactive intestinal peptide-stimulated adenylate cyclase by 50% in both sexes. Sulpiride, a specific D_2 antagonist, stereospecifically blocked with high affinity the dopamine inhibition in both males and females. RU 24926, a specific, non-catechol, non-ergot D_2 agonist, also inhibited basal adenylate cyclase of female pituitary with a higher apparent affinity than dopamine ($K_{D_{app}}$ 20 nM and 450 nM, respectively). This effect was also stereospecifically antagonized by sulpiride. Apomorphine was also more potent ($K_{D_{app}}$ 100 nM) than dopamine, whereas norepinephrine and SKF 38393, a specific D_1 agonist, were poorly active; isoproterenol and clonidine were inactive. Ergots derivatives such as CB 154, LY 14865, pergolide, and lergotrile were potent agonists. α-Dihydroergocryptine was a partial agonist of the dopamine receptor negatively coupled with an adenylate cyclase. Because of the slow association kinetics of this drug with the dopamine receptor, its $K_{D_{app}}$ (0.7 nM) for adenylate cyclase inhibition could be correctly determined only after a 30-min incubation period. All classical dopaminergic antagonists blocked dopamine inhibition of pituitary adenylate cyclase, pimozide (K_I 1 nM) and spiperone (K_I 0.8 nM) being the more potent. There were good correlations between the affinities of large series of agonists and antagonists for the anterior pituitary dopamine receptors negatively coupled with an adenylate cyclase on one hand, and for either D_2 dopamine receptors labeled with [^{3}H] dihydroergocryptine or [^{3}H]spiroperidol in both pituitary and striatum, or D_2 pituitary receptors involved in prolactin secretion on the other hand. It is concluded that the pituitary dopamine receptors negatively coupled with an adenylate cyclase are the classical D_2 receptors involved in prolactin secretion.

INTRODUCTION

One of the main concepts arising from binding studies using radiolabeled ligands is the existence of multiple receptors for a given neurotransmitter (1). In the case of dopamine, up to four pharmacologically distinct receptors (D_1, D_2, D_3, and D_4) have been described (2–6). However, only D_1 and D_2 dopamine receptors have been extensively characterized, and their existence is now well recognized. Initially D_1 dopamine receptors were defined by their ability to elicit an increase in adenylate cyclase activity, whereas D_2 receptors were supposed not to activate this enzyme (3). The main pharmacological characteristics of D_2 receptors are their high affinity for neuroleptics, in particular for those of the butyrophenone class, and their lower affinity for dopaminergic agonists (3, 4, 7–9).

Recently it has been proposed that the stimulation of D_2 receptors inhibits adenylate cyclase in both the intermediate and anterior pituitary lobes (10–16). In the intermediate pituitary lobe, the effect of various dopaminergic agonists and antagonists on adenylate cyclase inhibition appears well correlated with their effect on α-melanophore-stimulating hormone secretion (10, 12, 13).

In the anterior pituitary the debate still remains, since two reports suggest that dopamine inhibits adenylate cyclase (15, 16) while others claim that dopamine stimulates the enzyme (17) or fails to change the adenylate cyclase activity (18, 19). Despite the fact that dopamine inhibits basal prolactin secretion in both males and females, the inhibition of basal adenylate cyclase by dopamine was found only in females (15). In males dopa-

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mine inhibits only VIP[3]-stimulated adenylate cyclase (16). Moreover, the role in the prolactin secretion of the dopamine receptor coupled with the adenylate cyclase has been questioned by the observation that ergot alkaloids, which are more potent than dopamine for inhibiting hormone secretion, are weaker than the amine for inhibiting the enzyme (16). For the same reason, Onalli *et al.* (16) proposed that the dopamine receptors inhibiting VIP stimulation of pituitary adenylate cyclase are not identical with classical D_2 receptors.

Therefore, the aim of the present study was to reinvestigate the sex differences in dopamine-induced adenylate cyclase inhibition in the anterior pituitary and to characterize fully the pharmacology of the dopamine receptor involved in this response. We have also compared this pharmacology with those of the dopamine receptors involved in prolactin secretion and of D_2 binding sites labeled with radioactive ligands in both anterior pituitary and striatum.

MATERIALS AND METHODS

Homogenate preparation. Male and female Sprague-Dawley rats (200–250 g) were purchased from Charles River Breeding Laboratories. Rats were killed by decapitation. The pituitary was removed and the neuro-intermediate lobes were carefully discarded. Anterior pituitary tissue (three glands) was homogenized in a glass-Teflon Potter homogenizer in 500 μl of 1 mM Tris-maleate buffer (pH 7.2), 1 mM EGTA, and 300 mM sucrose. This homogenate was then diluted 2 times in the same medium. The homogenate was filtered through a silk screen (0.2-mm pore diameter).

Adenylate cyclase assay. Adenylate cyclase activities were measured by the conversion of [α-^{32}P]ATP into [^{32}P]cyclic AMP as previously described (20). The final incubation medium (50 μl) contained 50 mM Tris-maleate (pH 7.2), 1.5 mM $MgSO_4$, 1 mM cyclic AMP, 5 mM creatine phosphate, creatine kinase (0.1 mg/ml), 0.15 mM ATP, 0.01 mM GTP, 10 mM theophylline, [^{3}H]cyclic AMP (0.001 μCi), and [α-^{32}P]ATP (1 μCi). The reaction was initiated by the addition of 10 μl of homogenate and the incubation was performed at 30° during 30 min except when stated.

Chemical. Creatine phosphate, creatine kinase, and cyclic AMP were purchased from Boehringer-Mannheim; VIP from Peninsula Laboratories; and ATP, GTP, theophylline, dopamine, apomorphine, norepinephrine, and isoproterenol from Sigma Chemical Company. The following compounds were gifts: (+)- and (−)-butaclamol (Ayerst Laboratories); clonidine (Boehringer-Engelheim) and (+)- and (−)-sulpiride (Delagrange); lergotile, pergolide, and LY 14865 (Eli Lilly); spiroperidol, (Janssen); α-flupenthixol (Labaz); haloperidol and chlorpromazine (Rhône-Poulenc); pimozide and RU 24926 (Roussel-Uclaf); α-DHEC, CB 154, and dihydroergocornine (Sandoz); SKF 38393 (Smith Kline & French); and fluphenazine (Squibb). [α^{32}P]ATP (sodium salt, 10–20 Ci/mmole) and [^{3}H]cyclic AMP (ammonium salt, 25 Ci/mmole) were purchased from New England Nuclear Corporation.

RESULTS

Time course of pituitary adenylate cyclase inhibition by dopamine. Figure 1 shows that basal adenylate cyclase activity increased linearly over 30 min and that dopamine inhibition of the enzyme was constant during the same incubation period. Therefore a 30-min incubation period was used in subsequent experiments.

[3] The abbreviations used are: VIP, vasoactive intestinal peptide; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; DHEC, dihydroergocryptine.

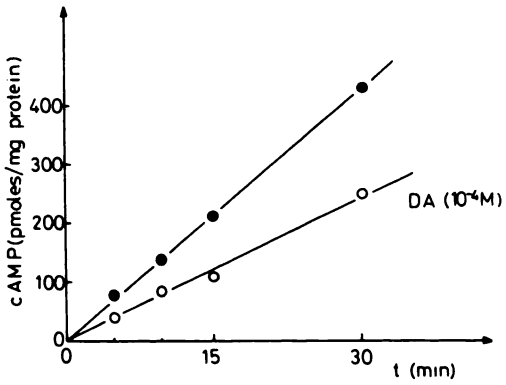

FIG. 1. *Time course of cyclic AMP production in the absence and in the presence of dopamine*

Female anterior pituitary homogenates were used for this experiment. Basal (●) and dopamine (*DA*) (○) adenylate cyclase activities.

Dopamine inhibition of basal and VIP-stimulated adenylate cyclase in males and females. Under our experimental conditions, dopamine inhibited in a dose-dependent manner basal adenylate cyclase activity in both male and female anterior pituitary. The maximal inhibition was 30% in males and 50% in females (Fig. 2). The $K_{D_{app}}$ (agonist concentration giving half-maximal inhibition) values for dopamine were 160 nM and 560 nM in males and females, respectively (Fig. 2).

In males, the adenylate cyclase activity in the presence of VIP (10^{-6} M) was 2.9-fold higher than basal activity and was inhibited 50% by dopamine (Fig. 2). Thus, in males dopamine was more potent on VIP-stimulated than on basal adenylate cyclase. This was not the case in female anterior pituitary, in which dopamine was equipotent on basal and VIP-stimulated (1.8-fold basal activity) adenylate cyclases (Fig. 2).

Since the maximal inhibition of the basal adenylate cyclase was greater in females than in males, subsequent experiments were performed on female anterior pituitary, unless indicated otherwise.

Effects of specific D_2 antagonist and agonist on basal adenylate cyclase. In both males and females sulpiride (10^{-6} M), a D_2-specific antagonist (4), stereospecifically antagonized the dopamine inhibition of pituitary adenylate cyclase (Fig. 3). At the same concentration (−)-sulpiride had no significant effect on striatal D_1-stimulated adenylate cyclase. Note that in some experiments in males, as in the one reported in Fig. 3, high concentrations of dopamine partially reversed the inhibition; this was never found in females.

Recently RU 24926, a new non-catechol, non-ergot dopaminergic agonist of the *N*-diphenethylamine class (21), was reported to be without any action on D_1 receptors (22). RU 24926 inhibited basal adenylate cyclase of female pituitary with higher apparent affinity than dopamine ($K_{D_{app}}$ = 20 nM). As in the case of dopamine, the effect of RU 24926 was stereospecifically antagonized by sulpiride (Fig. 4).

Effect of antagonists on dopamine inhibition of basal adenylate cyclase. Dopamine antagonists of various classes competitively inhibited dopamine-induced inhibition with high potencies (Fig. 5). As in the case of sulpiride, the inhibition by butaclamol appeared stereo-

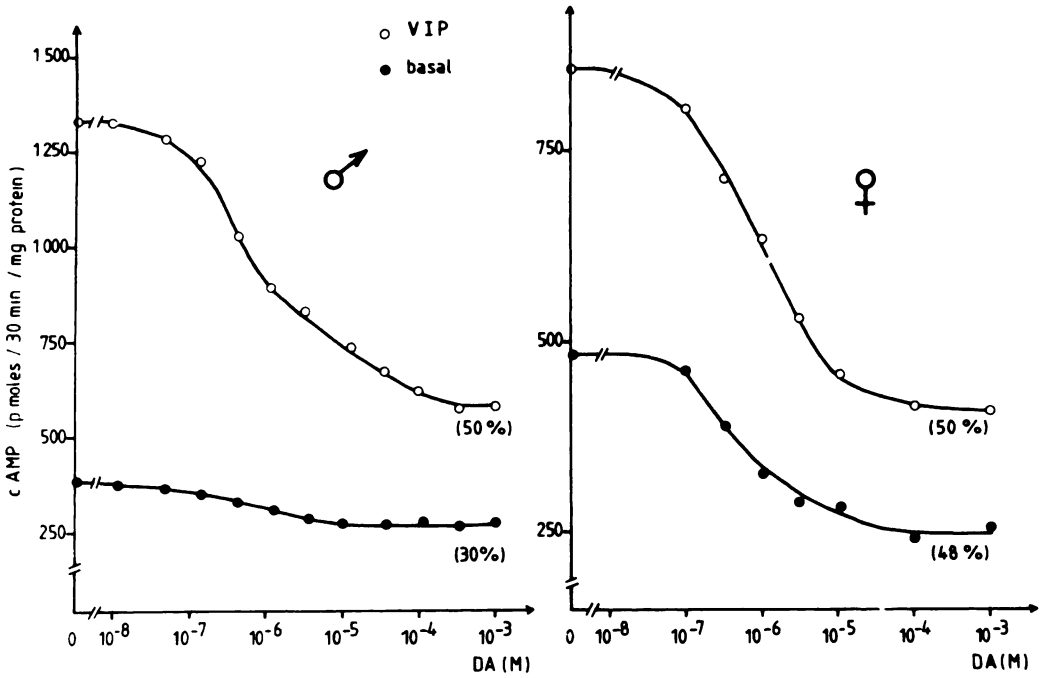

FIG. 2. *Inhibition by dopamine of basal and VIP-stimulated adenylate cyclases of male and female anterior pituitary homogenates* The VIP concentration was 10^{-6} M.

specific, (−)-butaclamol being more than 600 times less potent than (+)-butaclamol (Fig. 5). Spiroperidol and pimozide were the most active antagonists. We compared the apparent affinities of a series of 11 antagonists on pituitary dopamine receptors involved in the adenylate cyclase inhibition and those of the literature on pituitary D_2 receptors labeled with [^{3}H]DHEC and on dopamine inhibition of prolactin secretion (Fig. 6). There was a very good correlation between adenylate cyclase and [^{3}H]DHEC data on one hand and adenylate cyclase and prolactin response on the other hand. There was also a good correlation between adenylate cyclase and [^{3}H]spiroperidol data when sulpiride was excluded (Table 1). In fact, the affinities given by Cronin and Weiner (25) for sulpiride in the pituitary were more than 10 times lower than that reported by Leysen *et al.* (9) in the striatum.

The specificity of the receptor mediating inhibition of pituitary adenylate cyclase was similar to that of D_2 (Table 1) striatal receptors identified by [^{3}H]spiroperidol binding in striatum. In contrast, the orders of potency of the dopamine antagonists for pituitary adenylate cyclase and for the D_1-stimulated adenylate cyclase in the striatum were not correlated (Table 1).

Effects of agonists on basal adenylate cyclase. Apomorphine was more potent than dopamine ($K_{D_{app}}$ = 100 and 300 mM, respectively) (Fig. 7). Norepinephrine was poorly active ($K_{D_{app}}$ = 18,000 nM). A specific D_1 agonist, SKF 38 393, which is more potent than dopamine on D_1

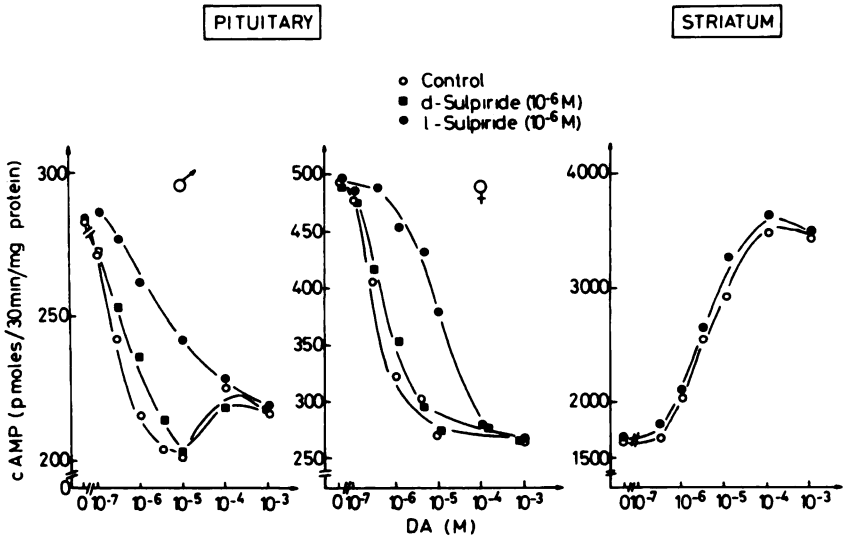

FIG. 3. *Effect of sulpiride on dopamine (DA)-sensitive adenylate cyclases of male and female anterior pituitary and striatal homogenates* Two striata were homogenized in 4 ml of 1 mM Tris-maleate (pH 7.2), 1 mM EGTA, and 300 mM sucrose.

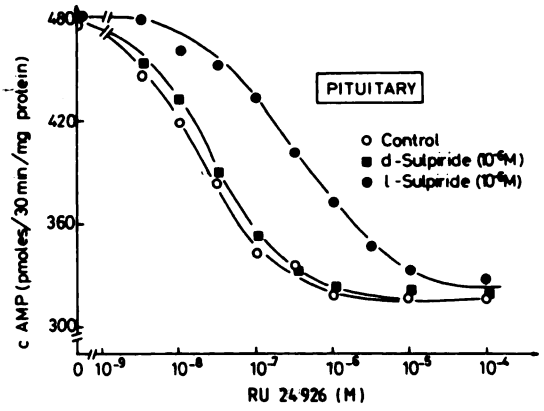

FIG. 4. *Effect of RU 24926 on basal adenylate cyclases of anterior pituitary*

The effect of RU 24926 on female anterior pituitary adenylate cyclase was competitively and stereospecifically inhibited by sulpiride.

striatal receptors (27) was found to be 2,000 times less potent than dopamine on pituitary dopamine receptor coupled with adenylate cyclase. The *beta-* and *alpha-*adrenergic agonists isoproterenol and clonidine were inactive (Fig. 7).

Effects of ergot derivatives on basal adenylate cyclase. DHEC is known to be one of the most potent dopaminergic agonists on prolactin secretion (23) and has been used in a tritiated form to label dopamine receptors in anterior pituitary (23, 24). Thus, the effect of this compound on pituitary adenylate cyclase was of particular interest. When adenylate cyclase activities were measured during a 5-min period, α-DHEC produced a biphasic inhibition with a plateau between 10^{-6} and 10^{-5} M. The first component represented 30% of the maximal dopamine inhibition, and the $K_{D_{app}}$ of α-DHEC was about 100 nM (Fig. 8). When the incubation was performed during 30 min, a biphasic curve was also obtained with a plateau between 10^{-7} and 10^{-5} M. The apparent affinity of α-DHEC for the first component was higher ($K_{D_{app}}$ = 1 nM) and represented 55% of the total dopamine inhibition. (−)-Sulpiride antagonized competitively only the first component, indicating the nonspecific nature of the inhibition obtained with the highest concentrations of α-DHEC. Thus α-DHEC appears to be a partial agonist (Fig. 8).

That α-DHEC interacted with the same receptor as dopamine to inhibit the adenylate cyclase was further demonstrated by the experiment presented in Fig. 9. In the presence of α-DHEC (10^{-7} M) the basal adenylate cyclase was inhibited and the dose-response curve for dopamine was shifted to the right. A theoretical dose-response curve (*broken line* in Fig. 9) was calculated assuming a direct competition between α-DHEC and dopamine and taking the following parameters: V_{max} = 210 and 85 pmoles of cyclic AMP per 30 min/mg of protein, $K_{D_{app}}$ = 400 and 0.7 nM for dopamine and α-DHEC, respectively. This curve fitted well with the experimental results (Fig. 9).

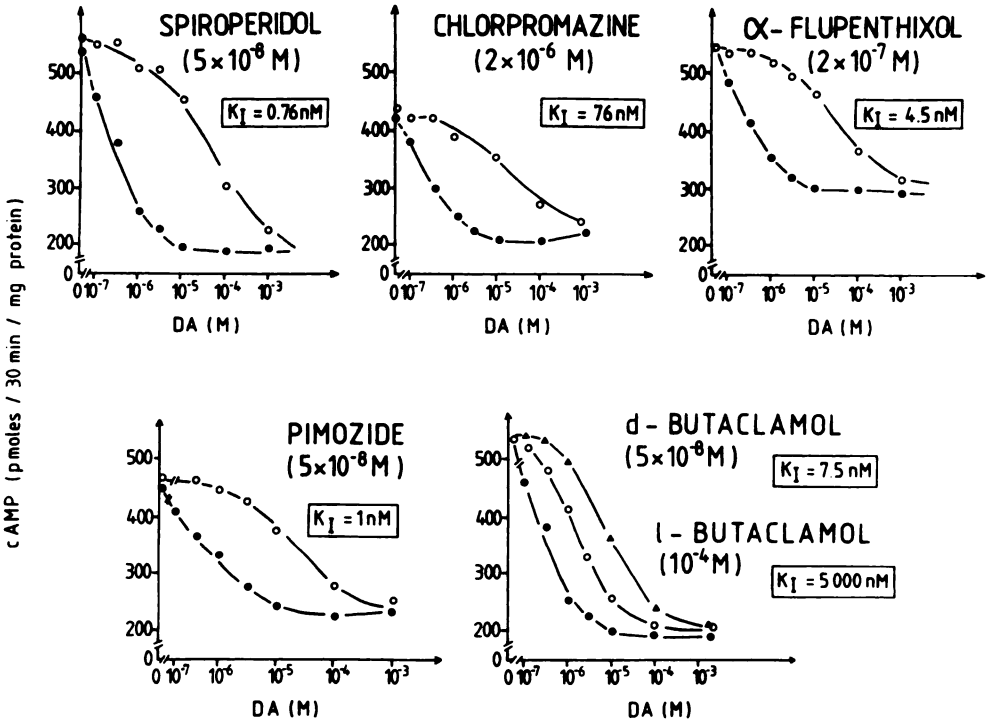

FIG. 5. *Competitive inhibitions by classical neuroleptics of dopamine-sensitive adenylate cyclase of female anterior pituitary*

K_I values (affinity of neuroleptics for the dopamine receptor) were calculated with the formula: $K_{D_{app_2}} = K_{D_{app_1}}\left(1 + \frac{[I]}{K_I}\right)$, where $K_{D_{app_1}}$ and $K_{D_{app_2}}$ are the concentrations of dopamine (*DA*) giving half-maximal inhibition of basal adenylate cyclase activities in the absence and in the presence of neuroleptic, respectively. [*I*] = concentration of neuroleptic.

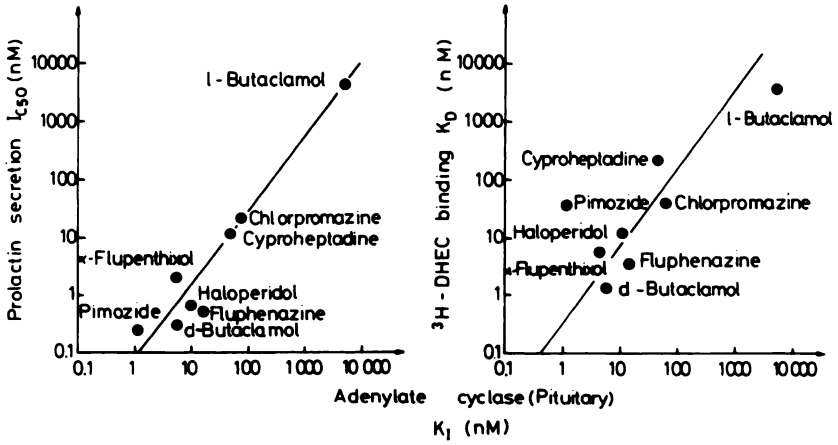

FIG. 6. *Correlation between the affinities of a series of neuroleptics for the anterior pituitary dopamine receptors involved in adenylate cyclase inhibition and for either those involved in prolactin secretion or labeled with [^{3}H]DHEC*

Affinities of neuroleptics for [^{3}H]DHEC binding and prolactin secretion are taken from Caron *et al.* (23) except for (−)-butaclamol (24).

Other ergot derivatives, except dihydroergocornine (data not shown) were full agonists (Fig. 10).

The agonist specificity of the dopamine receptors, coupled with an adenylate cyclase in the pituitary, was identical with (a) the specificity of D_2 receptors labeled with [^{3}H]spiroperidol or [^{3}H]DHEC in pituitary, (b) the specificity of the dopamine receptor involved in prolactin secretion, and (c) the specificity of the D_2 receptors labeled with [^{3}H]spiroperidol in the striatum. In contrast, numerous compounds active on pituitary adenylate cyclase did not activate striatal D_1 dopamine receptors (Fig. 11; Table 2).

DISCUSSION

Our results confirm and extend previous reports showing that dopamine inhibits the anterior pituitary adenylate cyclase through a specific receptor (15, 16). We and others (15, 16) were unable to reproduce the results reported by Ahn *et al.* (17) of a stimulation by dopamine of the anterior pituitary adenylate cyclase. In previous reports (15, 16), the relationship between dopamine inhibition of the adenylate cyclase and the well-defined D_2 receptors involved in prolactin secretion was questionable for two main reasons: (a) Dopamine inhibition was

TABLE 1

Comparison between affinities of dopamine antagonists on dopamine-sensitive adenylate cyclase and [^{3}H]spiroperidol binding in pituitary and striatum

Pituitary: A, inhibition of basal adenylate cyclase by dopamine [values are those of this study]; B, [^{3}H]spiroperidol binding [values are taken from Cronin and Weiner (25)]. Striatum: C, [^{3}H]spiroperidol binding [values are taken from Leysen *et al.* (9) except for (−)-butaclamol (8)]; D, dopamine-sensitive adenylate cyclase stimulation [values are taken from Iversen (2) except for spiroperidol (26)].

Dopamine antagonist	Pituitary[a]		Striatum[a]	
	Adenylate cyclase (A)	[^{3}H]Spiroperidol binding (B)	[^{3}H]Spiroperidol binding (C)	Adenylate cyclase (D)
	nM	*nM*	*nM*	*nM*
Butyrophenone				
Haloperidol	10.0	11.5	1.4	220.0
Spiroperidol	0.8	0.8	0.1	100.0
Phenotiazine				
Fluphenazine	16.0		2.8	4.3
Chlorpromazine	64	16.0	10.0	48.0
Benzamide				
(−)-sulpiride	47.0	2,200.0	120.0	
(+)-sulpiride	2,000.0	>40,000.0		
Thioxantine				
α-flupenthixol	4.5		4.2	1.0
Aralkylpiperidine				
Pimozide	1.0	4.0	1.1	140.0
Miscellaneous				
(+)-butaclamol	7.5	1.6	1.4	8.8
(−)-butaclamol	5,000.0	17,000.0	3,000.0	
Cyproheptadine	50.0			

[a] Correlation coefficients: A-B with sulpiride, $r = 0.66$; A-B without sulpiride, $r = 0.99$; A-C, $r = 0.99$; A-D, $r = -0.18$.

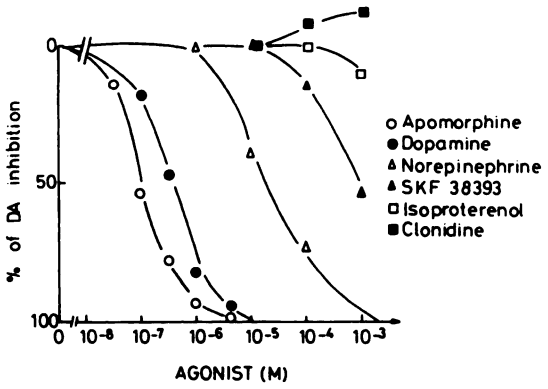

FIG. 7. *Effects of various agonists on the dopamine receptor involved in adenylate cyclase inhibition*

The basal and dopamine (*DA*) (10^{-4} M)-sensitive adenylate cyclase activities were 430 and 202 pmoles/30 min/mg of protein, respectively. Inhibitions are expressed as percentages of the inhibition obtained with dopamine. All drugs were tested in a single experiment.

found only in females (15) or after VIP stimulation of the adenylate cyclase in males (16), whereas D_2 binding sites and dopamine inhibition of basal prolactin release have been described in both sexes. We have shown in this report that dopamine was able to inhibit the basal adenylate cyclase of male anterior pituitary. The reason for the discrepancy between our results and those of Giannattasio *et al.* (15) and Onalli *et al.* (16) are unclear. It should be noted that the addition of sucrose in the homogenization medium stabilizes the enzyme and also increases the intensity of dopamine inhibition (data not shown). However, even under these experimental conditions the dopamine inhibition of basal activity was lower than the inhibition of VIP-stimulated adenylate cyclase (Fig. 2). This phenomenon was not apparent in females. (b) Ergot derivatives were found to be poorly active on pituitary adenylate cyclase (16), whereas these compounds are known to be among the most potent agonists on D_2 receptors and prolactin release (23). Present data show that these drugs are indeed the most potent ago-

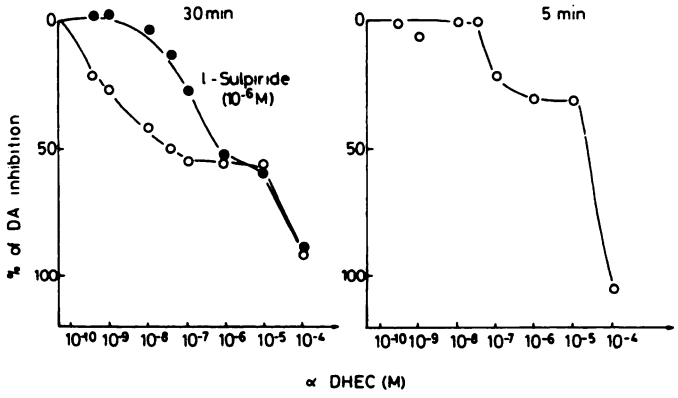

FIG. 8. *Effect of incubation time and of sulpiride on α-DHEC-induced adenylate cyclase inhibition*

After 5 min of incubation, the basal and dopamine (*DA*) (10^{-4} M)-sensitive adenylate cyclase activities were 120 and 52 pmoles/mg of protein, respectively; after 30 min they were 632 and 308 pmoles/mg of protein, respectively.

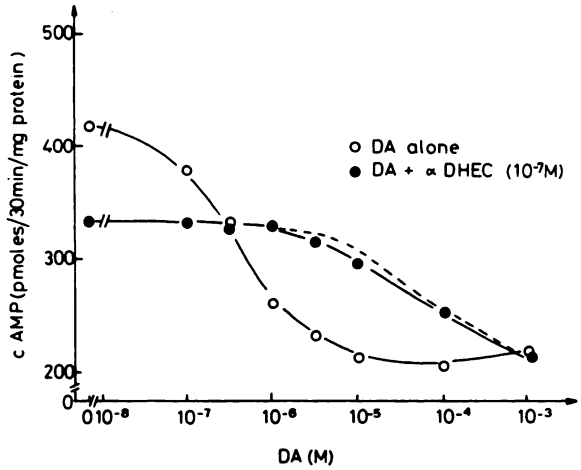

FIG. 9. *Evidence that α-DHEC is a partial agonist of the dopamine receptor involved in adenylate cyclase inhibition of anterior pituitary*

The dopamine (*DA*) dose-response curves were obtained in the absence (○) and in the presence (●) of a constant concentration (10^{-7} M) of α-DHEC. The theoretical dose-response curve (– – –), which is expected if one supposes an interaction of dopamine and α-DHEC at the same sites, was calculated as follows: the maximal decreases in cyclic AMP production due to dopamine ($V_{max\,1}$) and α-DEHC ($V_{max\,2}$) are 210 and 85 pmoles/30 min/mg of protein, respectively. The decrease in adenylate cyclase activity due to dopamine (V_{DA}) is equal to:

$$V_{DA} = \frac{[DA]}{[DA] + K_{D_{app\,DA}}(1 + [\alpha\text{-DHEC}]/K_{D_{app\,\alpha\text{-DHEC}}})}$$

[DA] and [α-DHEC] are the concentrations of dopamine and α-DHEC, respectively. $K_{D_{app\,DA}}$ = concentration of dopamine giving half-maximal inhibition and, as taken from this experiment, equals 4×10^{-7} M; $K_{D_{app\,\alpha\text{-DHEC}}}$ = concentration of α-DHEC giving half-maximal inhibition and equals 7×10^{-10} M (see Table 2). The decrease in adenylate cyclase activity due to α-DHEC ($V_{\alpha\text{-DHEC}}$) is equal to:

$$V_{\alpha\text{-DHEC}} = V_{max\,2}\frac{[\alpha\text{-DHEC}]}{[\alpha\text{-DHEC}] + K_{D_{app\,\alpha\text{-DHEC}}}(1 + [DA]/K_{D_{app\,DA}})}$$

The theoretical dose-response curve (y) is described by y = basal activity − (V_{DA} + $V_{\alpha\text{-DHEC}}$).

nists on dopamine receptors coupled with an adenylate cyclase in anterior pituitary. We demonstrate in the case of α-DHEC that the use of short incubation times leads to an underestimation of the apparent affinity (from 1 to

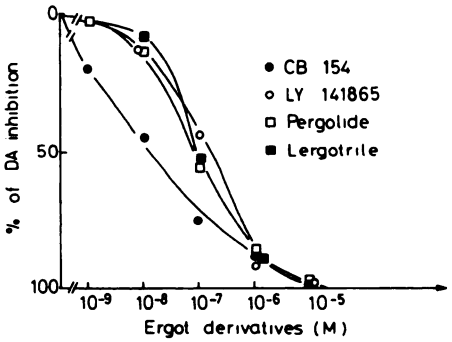

FIG. 10. *Effect of some ergot derivatives on dopamine-sensitive adenylate cyclase of anterior pituitary*

The basal and dopamine (*DA*)-sensitive adenylate cyclase activities were 530 and 238 fmoles/30 min/mg of protein, respectively.

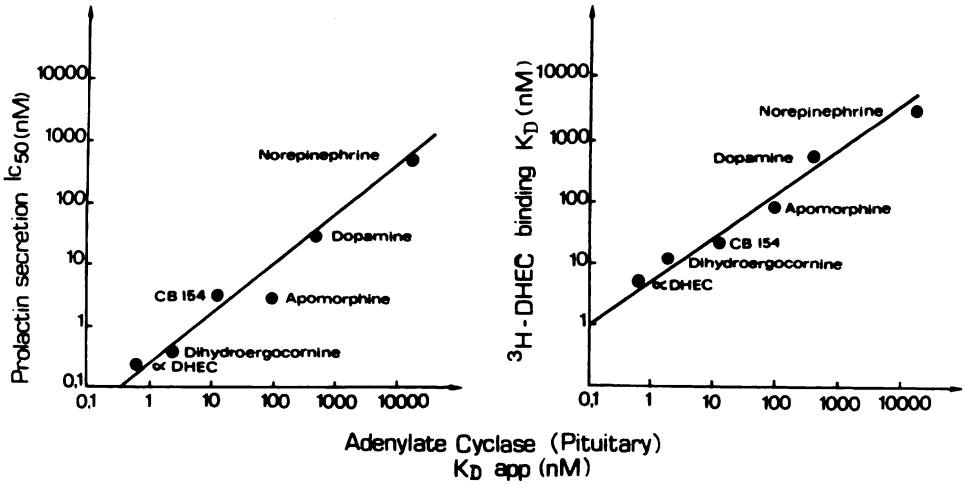

FIG. 11. *Correlation between the affinities of a series of agonists for the anterior pituitary dopamine receptors involved in adenylate cyclase inhibition and for either those involved in prolactin secretion or labeled with [^{3}H]DHEC*

Affinities of dopamine agonists for [^{3}H]DHEC binding and prolactin secretion are taken from Caron *et al.* (23), except for lergotrile (28).

100 nM). This observation is easily explained by the kinetics of [^{3}H]DHEC binding described by Caron *et al.* (23); 30 min at 25° are needed to reach equilibrium.

Several arguments presented in this report strongly suggest the D_2 nature of the receptor involved in adenylate cyclase inhibition in anterior pituitary: (a) A specific D_2 agonist (RU 24926) and a specific D_2 antagonist (sulpiride) which do not interact with D_1 dopamine receptors were found to be highly potent on dopamine receptors negatively coupled with an adenylate cyclase in anterior pituitary (Figs. 3 and 4). (b) The orders of potency of large series of agonists and antagonists on pituitary adenylate cyclase were well correlated with those found for D_2 binding sites labeled with [^{3}H]spiroperidol in both pituitary (25) and striatum (8, 9) or labeled with [^{3}H]DHEC in pituitary (23, 24) (Tables 1 and 2; Figs. 6 and 11). Moreover, the specificity toward agonists and antagonists of the dopamine receptors negatively coupled with an adenylate cyclase appears completely different from the specificity of the D_1 receptors positively coupled with the enzyme (2, 26) (Tables 1 and 2). The high stereospecificity of neuroleptic blockade excludes an effect at the calmodulin level (29). (c) The binding of various agonists on pituitary D_2 receptors has been shown to be GTP-sensitive (30–32). This result is compatible with a coupling of these dopamine receptors with an adenylate cyclase (33). However, bromocryptine (CB 154), which inhibits with high potency the pituitary adenylate cyclase, does not bind to the [^{3}H]spiroperidol receptor site in a GTP-dependent manner in both pituitary and striatum (26, 30, 32). It thus seems that the two states of the D_2 dopamine receptor (uncoupled or coupled with the GTP binding protein), although having different affinity for most dopaminergic agonists, have the same affinity for bromocriptine.

Since the pharmacological properties of the recogni-

TABLE 2

Comparison between affinities of dopamine agonists on adenylate cyclase and [^{3}H]spiroperidol binding in pituitary and striatum

Pituitary: A, inhibition of basal adenylate cyclase [values are those of this study]; B, [^{3}H]spiroperidol binding [values are taken from Cronin and Weiner (25)]. Striatum: C, [^{3}H]spiroperidol binding [values are taken from Seeman (4) except for dopamine and CB 154 (26)]; D, dopamine-sensitive adenylate cyclase stimulation [values are taken from Seeman (4) except for LY 14865 (22) and SKF 38 393 (27).

Dopamine agonist	Pituitary[a]		Striatum[a]	
	Adenylate cyclase (A)	[^{3}H]Spiroperidol binding (B)	[^{3}H]Spiroperidol binding (C)	Adenylate cyclase (D)
	nM	*nM*	*nM*	*nM*
Dopamine	450	1,330	550	4,000
Apomorphine	100	220	500	1,500
Norepinephrine	18,000	18,000	46,000	40,000
SKF 38393	1,000,000.0			100
CB 154	12	18.5	56	NA[b]
α-DHEC	0.7		5	NA
Dihydroergocornine	2.0			NA
Lergotrile	90.0		20	NA
LY 14865	150.0			NA
RU 24926	21			NA

[a] Correlation coefficients: A-B, $r = 0.98$; A-C, $r = 0.97$.

[b] NA, Not an agonist.

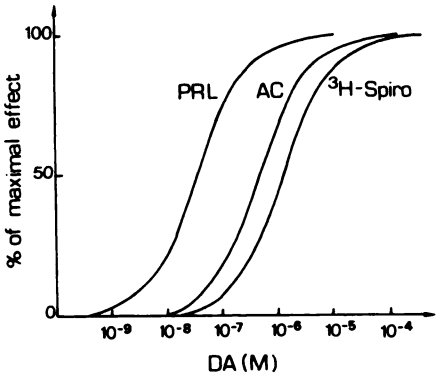

FIG. 12. *Relationship between dopamine dose-response curves for prolactin secretion, adenylate cyclase inhibition, and [^{3}H]spiroperidol binding*

The theoretical curves were calculated assuming a simple Michaelis-Menten kinetics and taking the apparent affinities given in Table 2 and Fig. 11. *DA*, dopamine; *PRL*, prolactin secretion; *AC*, adenylate cyclase; *^{3}H-Spiro*, [^{3}H]spiroperidol binding.

tion sites of the D_2 receptors in the pituitary are identical with those of the D_2 receptors present in the striatum, the question arises whether or not the latter are also negatively coupled with an adenylate cyclase. So far, the only argument in favor of such a coupling is that the release of cyclic AMP from striatal slices is lower under D_2 dopamine stimulation (34). However, one cannot exclude the possibility that two receptors with the same recognition sites have different transducing mechanisms, although this has not as yet been reported for any systems.

The D_2 receptors negatively coupled with an adenylate cyclase are likely to be the dopamine receptors involved in prolactin secretion (23, 28) (Tables 1 and 2; Figs. 6 and 11). Increasing intracellular cyclic AMP by pharmacological agents or hormones results in an increase in prolactin release and synthesis (35–40). Conversely, dopamine agonists have been shown to decrease cyclic AMP production and to inhibit prolactin synthesis or release in anterior pituitary cells (37, 40).

Figure 12 presents the relationships between the dopamine dose-reponse curves for prolactin release, adenylate cyclase, and [^{3}H]spiroperidol binding. As classically observed, the affinity for the receptor sites is 3 times lower than the $K_{D_{app}}$ for adenylate cyclase, which is more than 10 times lower than the IC_{50} for the physiological response. The occupation of 10% of receptors resulted in 23% of maximal inhibition of adenylate cyclase and in 80% of maximal inhibition of prolactin secretion. Therefore, it is not surprising that a partial agonist on adenylate cyclase, such as α-DEHC, can be a full agonist on prolactin response.

In conclusion, we suggest that D_2 dopamine receptors on lactotroph cells are negatively coupled with adenylate cyclase. Purification of lactotroph cells, however, is necessary to demonstrate this point definitively.

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