IS THE DOPAMINE SENSITIVE ADENYLATE CYCLASE IN THE RAT SUBSTANTIA NIGRA COUPLED WITH 'AUTORECEPTORS'?

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1. Introduction

The neurones, containing dopamine, which originate in the substantia nigra (SN) and project in the striatum constitute the nigro-striatal dopaminergic pathway. The dopamine (DA) released from the dopaminergic nerve terminals interacts with a postsynaptic dopaminergic receptor which is coupled with a specific dopamine sensitive adenylate cyclase [1–6]. This adenylate cyclase has a topographical distribution similar to that of dopaminergic terminals [6,7] and remains after degeneration of the dopaminergic nigro-striatal pathway. From pharmacological data, it has been proposed that dopaminergic terminals in the striatum possessed another categorie of dopaminergic receptors also called 'autoreceptors' [8] since they are sensitive to the neurotransmitter of the neurone on which they are localized. They are involved in the control of dopamine synthesis [8] and release [9,10]. These autoreceptors are widely distributed in all the different parts of the dopaminergic neurones and especially on the cell bodies in the substantia nigra [11–14].

The present study was done in order to know if the autoreceptors of the substantia nigra are coupled with an adenylate cyclase in the same way as the striatal postsynaptic dopaminergic receptors. For this purpose, the substantia nigra was dissected on frozen slices (−7°C) and homogenates prepared: they were found to contain a dopamine sensitive adenylate cyclase. This adenylate cyclase was also stimulated by L-norepinephrine and to a lesser extent by apomorphine. Various neuroleptics which blocked the striatal dopamine sensitive adenylate cyclase were also able to inhibit competitively the nigral dopamine sensitive adenylate cyclase. The dopamine sensitive adenylate cyclase was observed to be more concentrated in the pars reticulata (a region of the substantia nigra rich in dendrites from the dopamine neurones) than in the pars compacta which mainly contains the cell bodies of these neurones [15] and therefore the dopaminergic autoreceptors. In order to check the possibility that the dopamine sensitive adenylate cyclase could be coupled with autoreceptors localized on the dopaminergic neurones, we had specifically destroyed these neurones by a local injection of 6-hydroxydopamine (6-OH-DA). However, after this lesion, the dopamine-sensitive adenylate cyclase was still present. In contrast, after an hemisection of the brain, the dopamine sensitive adenylate cyclase of the substantia nigra completely disappeared.

These findings can support the hypothesis that the dopamine sensitive adenylate cyclase in the substantia nigra is not coupled with the dopamine autoreceptors. It is proposed that the dopamine sensitive adenylate cyclase described in this report, could be localized on the terminals of the gabaminergic neurones originating within the striatum and/or from the globus pallidus [16,17] and projecting into the substantia nigra.

2. Methods

Male Charles River rats (weighing about 350–450 g) were sacrified by decapitation. The caudal part of their brains was fixed on a Leitz Westler microtome stage refrigerated at −7°C and slices (thickness 500 μm) were made. Substantia nigra, dissected with a microscalpel, were homogeneized with a teflon Potter-Elrehjem in 2 mM Tris-Maleate pH 7.2, 2 mM EGTA pH 7.2, 300 mM sucrose. Preparation of striatal

homogenates and the conditions of the adenylate cyclase assay have been previously described [6]. The DA content in the SN homogenates was determined using a radioenzymatic assay [18]. DA neurones were destroyed by injecting 1 μl of an isotonic saline solution containing 6 OH-DA (2 μg), ascorbic acid (2 mg/ml) adjusted at pH 5 into the rostral and the caudal parts of the SN (A: 2580 μm and A: 1760 μm, respectively according to the atlas of König and Klippel [19]). The hemisections were done at the level of the plane A: 4380 μm [19].

3. Results and discussion

3.1. *Some pharmacological properties of the substantra nigra DA sensitive adenylate cyclase*

DA and NE stimulated markedly the basal adenylate cyclase of the SN. Their effects were not additive; this suggests that these catecholamines interact with the same receptor (fig.1). As observed in the striatum [1], apomorphine (10^{-4} M) was less effective. D-LSD previously shown to be a partial agonist of the striatal [6,20–22] and cerebral cortex [22] DA sensitive adenylate cyclases, was found to exhibit a poor agonist effect whereas it had a very strong antagonist effect on the SN dopamine sensitive adenylate cyclase (fig.1).

The specificity of the nigral DA sensitive adenylate cyclase towards dopaminergic agonists is therefore identical to that described for the postsynaptic striatal DA sensitive adenylate cyclase [1–6]. Isoproterenol, a pure β agonist was without effect on the basal adenylate cyclase activity (fig.1). This appears to be in contrast with that observed in rat striatum [6] and frontal cerebral cortex [23]. Serotonine (5-HT) was also ineffective on the nigral adenylate cyclase (fig.1).

3.2. *Relative affinities of the nigral and striatal DA sensitive adenylate cyclases for dopamine, norepinephrine and neuroleptics*

The apparent affinity of the nigral dopaminergic receptor for DA was about 2-fold lower than that of the striatal dopaminergic receptor. (K_d = 9.5 ± 0.23 μM, N = 5 and 3.9 ± 0.39 μM, N = 5 respectively). The difference between the relative apparent affinities of these two receptors for NE was even higher (K_d = 175 ± 2.5 μM, N = 3 in SN and 42.5 ± 2.5 μM, N = 3 in striatum). Thus, in these two structures NE was 10 to 20 times less potent than DA, suggesting that DA is the physiological agonist. Haloperidol a high potent neuroleptic inhibited in a competitive manner the nigral and striatal DA sensitive adenylate cyclases (fig.2).

The apparent affinity for haloperidol of the SN dopaminergic receptor was two times lower than that of the striatal DA receptor (K_i = 108 ± 2.7 nM,

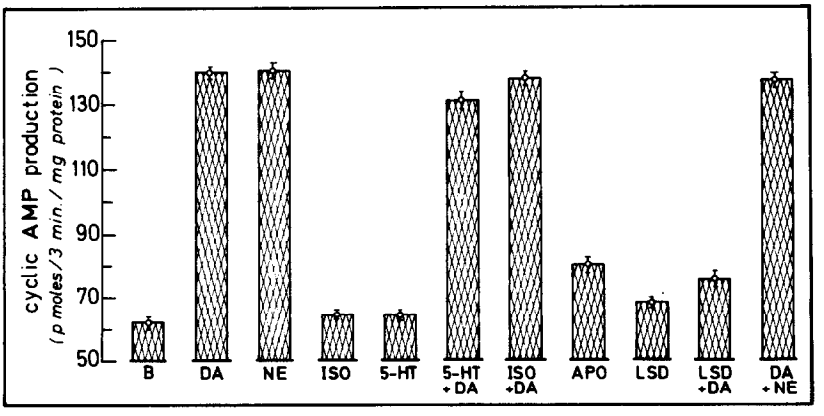

Fig.1. Some pharmacological properties of the SN dopamine sensitive adenylate cyclase. Drugs were tested at the following concentrations: dopamine (DA; 5 × 10^{-4} M), L-norepinephrine (NE; 5 × 10^{-4} M), L-isoproterenol (ISO; 5 × 10^{-4} M), serotonine (5 HT; 5 × 10^{-4} M), apomorphine (APO; 10^{-4} M) and D-LSD (LSD; 10^{-5} M). B = basal activity in the absence of drug. Each value is the mean ± SEM of three determinations.

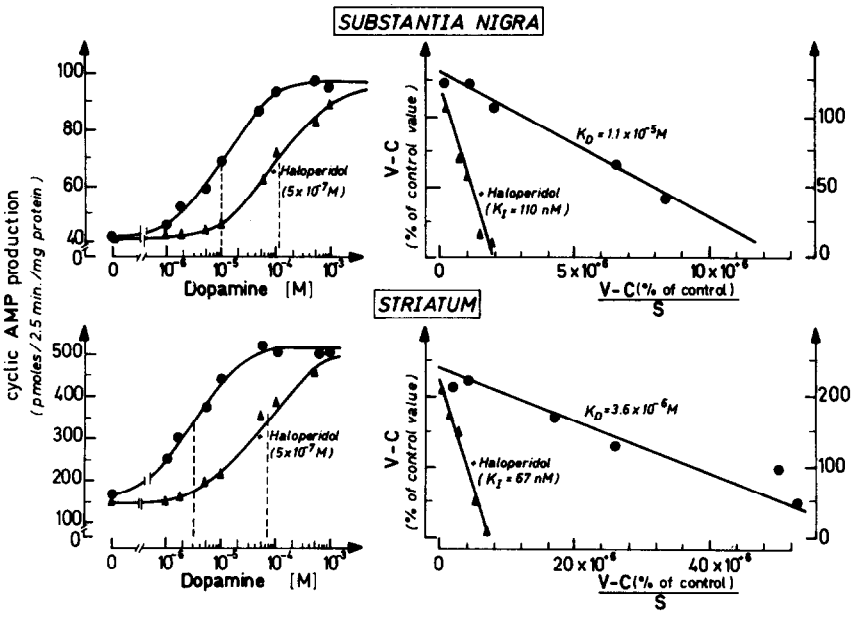

Fig.2. Competitive inhibition of SN and striatal dopamine sensitive adenylate cyclases by haloperidol. The left part represents the dose response curves for the DA sensitive adenylate cyclases of the SN and of the striatum made in the absence (•) or presence (▲) of haloperidol (5×10^{-7} M). The right part represents the Hofstee plot of the dose response curves. The value of respective apparent K_D and K_I calculated from this plot are indicated on the figure. V = adenylate cyclase activity in presence of DA alone or DA + haloperidol; C = basal adenylate cyclase activity; S = DA concentration.

N = 3 in the SN and 62.7 ± 2.6 nM, N = 3 in the striatum).

The relative potency of various neuroleptics to antagonize the nigral DA sensitive adenylate cyclase was comparable to that observed for the striatal DA sensitive adenylate cyclase (table 1) [23].

Table 1
Effect of different neuroleptics on basal, and DA sensitive adenylate cyclase activities

Neuroleptic	Adenylate cyclase activities		Increase of cyclic AMP production of presence of DA (% of control)
	Basal	+ DA (10^{-4} M)	
Control	62.9	122.6	100
α Flupenthixol	58.2	61.2	4.0
Fluphenazine	58.3	64.5	9.5
Haloperidol	59.4	70.8	19.0
Chlorpromazine	58.3	77.8	33.0
Thioridazine	59.1	84.0	43.0
Clozapine	58.2	84.4	44.0
Thioproperazine	56.6	95.4	65.0

The increase of cyclic AMP produced in the presence of DA are expressed in % of the control value in absence or neuroleptic. Each value is the mean of two independent determinations which were within less than 10% of a mean determination.

3.3. *The nigral DA sensitive adenylate cyclase is not localized on dopaminergic cell bodies*

The DA sensitive adenylate cyclase was determined in homogenates of the pars reticulata and of the pars compacta. The increase in cyclic AMP induced by DA (10^{-4} M) was about 2.5-fold higher in the pars reticulata which is rich in dopaminergic cell deutrites, than in the pars compacta where the dopaminergic cell bodies are mainly concentrated (table 2). Three weeks after the unilateral injection of 6-OH-DA into the SN, most of neurones had degenerated as indicated by the marked decrease in the nigral DA content (85%) (table 3). However, the cyclic AMP production of the SN devoid of the DA cell bodies remained identical to that observed in the contralateral side or in nigral homogenates of control rats (table 3).

These results suggested that the DA sensitive adenylate cyclase was not coupled with the DA autoreceptors. This conclusion is further supported by the absence of a DA sensitive adenylate cyclase in the A_{10} area (Bockaert et al., umpublished data) which contains most of the cell bodies of the mesocortical [24–26] and mesolimbic [27] dopaminergic neurones.

The unilateral hemisection of the brain made between the SN and the striatum led to the complete disappearance of the ipsilateral nigral DA sensitive adenylate cyclase but did not affect the DA sensitive adenylate cyclase of the contralateral SN (table 4). This may indicate that this DA sensitive adenylate cyclase is not localized on interneurones in the SN. It could be associated to terminals of neurones originating rostrally to the SN. It is tempting to

Table 2
Repartition of the DA sensitive adenylate cyclase between the pars compacta and the pars reticulata of the SN

	Adenylate cyclase activities (pmol/3 min/mg protein)					
	Pars compacta			Pars reticulata		
Exp. No.	Basal	+ DA (10^{-4} M)	△	Basal	+ DA (10^{-4} M)	△
1	78.8	130.6	51.8	60.8	152.6	91.6
2	99.2	132.6	33.4	197.0	299.8	102.8

△ = Cyclic AMP production due to the presence of DA. Each value is the mean of two independent determinations which were within less than 10% of a mean determination.

Table 3
Effect of the destruction of dopaminergic cell bodies of SN on the DA sensitive adenylate cyclase activity

	Increase of cyclic AMP in presence of DA (10^{-4} M) (pmol/6 min/mg protein)		Dopamine content (pg/mg protein)	
Ipsilateral (6-OH-DA lesioned)	170.5 ± 15.3	(N = 4)	942 ± 292	(N = 4)
Contralateral	171.3 ± 24.5	(N = 4)	6334 ± 466	(N = 4)
Control	159.7 ± 15	(N = 6)	8184 ± 718	(N = 4)

Three weeks after the local unilateral injection of 6-OH-DA, the ipsilateral and contralateral SN of the lesioned rats and SN of control rats were dissected and homogeneized in 100 μl of the homogeneization medium. DA was estimated in 50 μl aliquot and 50 μl were used for measuring the DA sensitive adenylate cyclase. The basal adenylate cyclase activity did not change after lesions. The values are the mean ± SEM of the results obtained with 4 or 6 animals.

Table 4
Effect of unilateral brain hemisection on the DA sensitive adenylate cyclase of SN

	Adenylate cyclase activities (pmol/3 min/mg protein)			
	Basal		+ DA (10^{-4} M)	
Ipsilateral (hemisectioned)	90.9 ± 0.3	(N = 3)	87.8 ± 13	(N = 3)
Contralateral	92.3 ± 11.3	(N = 3)	163.7 ± 12	(N = 3)
Control	106 ± 6.6	(N = 4)	183.7 ± 11.3	(N = 4)

Four weeks after the unilateral hemisection, the ipsilateral and contralateral SN of lesioned rat and SN of control animals were dissected and homogenized individually in 60 μl. The values are the mean ± SEM of the results obtained with 3 or 4 animals.

propose that the DA sensitive adenylate cyclase in the SN is located on terminals of the gabaminergic neurones which control the activity of the dopaminergic neurones (for review see [17]).

There is increasing evidence that dopamine may be released from dentrites of the dopaminergic neurones in the SN [28,29]. The DA sensitive adenylate cyclase described in this report associated to DA receptors not located on dopaminergic neurones might correspond to one of the sites of action for dopamine released from the dopaminergic dentrites.

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