Neuroscience Letters, 87 (1988) 247–252
Elsevier Scientific Publishers Ireland Ltd.

NSL 05266

Pharmacological characterization of D_1 and D_2 dopamine receptors in rat limbocortical areas. I. Frontal cortex

Enzo Nisoli, Mariagrazia Grilli, Maurizio Memo, Cristina Missale and PierFranco Spano

Institute of Pharmacology and Experimental Therapeutics, School of Medicine, University of Brescia, Brescia (Italy)

(Received 9 October 1987; Revised version received 8 January 1988; Accepted 12 January 1988)

Key words: Dopamine receptor; Frontal cortex; Neuroleptic; Mesolimbocortical system; Adenylate cyclase; SKF 82526

The mesolimbocortical dopaminergic system innervates several brain regions including the frontal cortex. The aim of our work was both to identify and to pharmacologically characterize the dopamine receptors located in this brain area. We found that different selective agonists for D_1 receptors were able to increase adenylate cyclase activity, and these effects were antagonized by haloperidol and SCH 23390. Moreover different agonists for D_2 receptors inhibited the cyclic AMP generating system, and these effects were prevented by (−)-sulpiride. According to the paradigm that D_1 receptors are linked with adenylate cyclase in a stimulatory way, while D_2 receptors are linked with the same enzyme in an inhibitory way, our results indicate the presence of both D_1 and D_2 receptors in rat frontal cortex.

Dopamine (DA) receptors are present in several DA-rich brain regions which are innervated by at least two main DA neuronal pathways: the mesostriatal and the mesolimbocortical systems. The latter DA system originates primarily from the ventral tegmental area (A10 cell group). Minor projections of this DA system also arise from the substantia nigra (group A9). The DA fibers ascend in the medial forebrain bundle and innervate the septum, many limbic cortical regions, such as the amygdaloid cortex and the hippocampus and the supragenual and pregenual anteromedial cortex (prefrontal cortex) [4, 7].

The aim of our work was both to identify and to pharmacologically characterize the DA receptors located in the rat frontal cortex innervated by the mesolimbocortical system. A variety of evidence suggests that DA can interact with two types of DA receptors named D_1 and D_2 [5, 12, 15, 16].

Correspondence: M. Memo, Institute of Pharmacology and Experimental Therapeutics, School of Medicine, University of Brescia, Via Valsabbina 19, I-25124 Brescia, Italy.

It is generally accepted that D_1 DA receptors are coupled with adenylate cyclase activity in a stimulatory manner, whereas D_2 DA receptors are coupled with the enzyme in an inhibitory manner. Thus, measurement of cyclic AMP production after either D_1 or D_2 stimulation has been taken as a paradigm for both identifying and pharmacologically characterizing the DA receptors located in the rat frontal cortex.

Our studies show the existence of D_1 and D_2 receptors in the rat frontal cortex. These observations may be of interest in knowledge of the mechanism of antipsychotic drugs.

Male Sprague–Dawley rats, obtained from Charles River Laboratories (Italy), each weighing 200–250 g, were used in all experiments. The animals were kept 5 to a cage in a quiet room with controlled temperature (23 ± 1°C), humidity (55 ± 5%) and light cycle (12 h light/12 h dark) with light being turned on at 07.00 h; they had free access to food and water. The rats remained in these conditions for at least one week before use. Prior to sacrifice all animals were maintained in the room for some hours to exclude the influence of environmental stress. The animals were sacrificed by decapitation. Each brain was rapidly removed and then placed into an ice-cold brain-slicer. For frontal cortex dissection, a coronal cut was made at the level of the corpus callosum using chips of razor blades, mounted in suitable holders, as knives. An additional coronal cut was made 2 mm in the rostral side. In this way a thick coronal section (2 mm thick) was made: the corpus callosum provides the main landmark in this dissection according to König and Klippel's atlas [6]. A section obtained between the 11,800 and 9800 μm planes was placed on an ice-cold slide and cortex was removed from it, weighed and then homogenized in the medium described below, using a teflon Potter-Elvenhjem.

Adenylate cyclase activity was measured as described by Clement-Cormier and Robinson [2] with small modifications. The frontal cortex samples were homogenized in 25 vols. of ice-cold 10 mM Tris-maleate buffer (pH 7.5), containing 1.2 mM EGTA. Aliquots of homogenate, corresponding to 2 mg of tissue, were incubated in a final volume of 500 μl for 20 min at 30°C in 80 mM Tris-maleate buffer (pH 7.4) containing 4 mM $MgSO_4$, 10 mM theophylline, 0.6 mM EGTA, 0.02% ascorbic acid, 5 mM phosphocreatine, 50 U/ml creatine phosphokinase, 1 mM ATP, plus the drugs as indicated in the results. The reaction was initiated by adding 50μl of homogenate in the incubation medium and was terminated by placing the assay tubes in a boiling water bath for 5 min. The assay tubes were then centrifuged at 1000 g for 10 min. The resulting supernatant was stored for cyclic AMP determination and the pellet was resuspended in 1 N NaOH to estimate its protein content according to the method of Lowry et al. [8]. The amount of cyclic AMP was evaluated by radioimmunoassay (New England Nuclear, DuPont).

Drugs were obtained as following: SKF 82526 from Smith Kline & French Laboratories; bromocriptine from Sandoz; (−)-sulpiride from Ravizza (Italy); haloperidol (Serenase) from Lusofarmaco (Italy); DA from Calbiochem; (−)-apomorphine from RBI (Wayland, MA, U.S.A.); lisuride and SCH 23390, (−)-*R*-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol from Schering; RU 24213 from Russel (Milano, Italy); atenolol from Ciba Geigy; ATP, creatine kinase and creatine

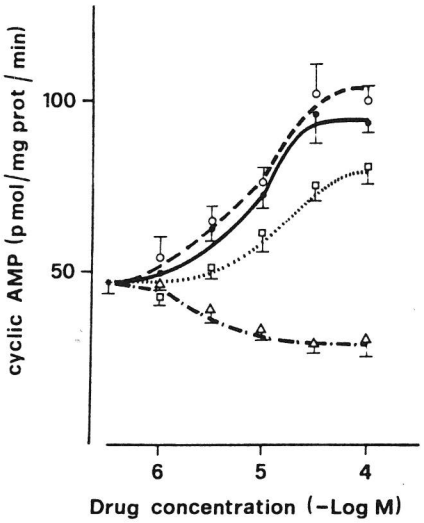

Fig. 1. Effects of atenolol (●), haloperidol (□) and SCH 23390 (△) on DA-stimulated adenylate cyclase activity in homogenates of rat frontal cortex. Each value is the mean ± S.E.M. of at least 3 separate experiments. Basal adenylate cyclase activity was 48 ± 3 pmol/mg prot./min.

phosphate from Boehringer, Mannheim; and the other drugs used in this study from Sigma.

The significance of differences between the adenylate cyclase activities in basal or stimulating/inhibiting conditions was determined by using Student's *t*-test.

Fig. 1 shows the effects of increasing concentrations of DA on the basal adenylate cyclase activity in homogenates of rat frontal cortex. As previously shown by other investigators [18], DA dose-dependently stimulated basal enzyme activity, with a maximal stimulation at 100 μM concentration (+100% over basal). The calculated EC_{50} was 10 μM. The β-blocker atenolol was not able to antagonize DA stimulation of adenylate cyclase activity. On the contrary, the increased enzyme activity was inhibited by haloperidol (50 μM), which caused a shift to the right of the DA dose–response curve. When 100 nM SCH 23390 was present in the incubation medium, DA inhibited adenylate cyclase activity, with a maximal inhibition at 50 μM (-29 ± 4 pmol/mg prot./min) and an IC_{50} value of 5 μM.

The effects of SKF 82526, DA and (−)-apomorphine, on basal adenylate cyclase activity are shown in Fig. 2. The 3 DA agonists stimulated in a dose-dependent manner basal enzyme activity with maximal effects at 50 μM. SKF 82526, DA and (−)-apomorphine stimulated adenylate cyclase activity by 85 ± 18, 64 ± 2 and 58 ± 5 pmol/mg prot./min, respectively. The EC_{50} values, calculated from the results, were 5, 11 and 9 μM for SKF 82526, DA and (−)-apomorphine, respectively.

Fig. 3 shows the blockade of SKF 82526 effect by haloperidol and SCH 23390. At a concentration of 50 μM, haloperidol blocked SKF 82526 stimulated adenylate cyclase activity. The selective D_1 DA receptor antagonist, SCH 23390 (100 nM), was also able to completely antagonize the SKF 82526 effect on cyclic AMP production.

The effect of bromocriptine, lisuride and RU 24213 on basal adenylate cyclase activity are reported in Fig. 4. All the drugs used were able to inhibit dose-dependently the basal enzyme activity. The maximal inhibition of cyclic AMP production was obtained at 100 μM (-24 ± 5, -18 ± 3 and -18 ± 4 pmol/mg prot./min for bromocriptine, lisuride and RU 24213, respectively). The IC_{50} values were 10, 20 and 20 μM for bromocriptine, lisuride and RU 24213, respectively.

Fig. 5 shows that the maximal bromocriptine effect on cyclic AMP basal production was antagonized by 50 μM (−)-sulpiride.

It is generally accepted that antipsychotic drugs produce their therapeutic effects by blocking DA receptors [11]. It should seem reasonable that the DA system involved in these antipsychotic effects is the mesolimbocortical one. This DA system, indeed, connects many superior cerebral areas, such as limbic cortical regions and discrete areas of the prefrontal cortex.

The present study shows the existence of both D_1 and D_2 DA receptors in homogenates from the rat frontal cortex. This has been demonstrated by measuring the ability of selective D_1 and D_2 agonists to respectively stimulate and inhibit cyclic AMP production. Until now the evidence supporting the presence of DA receptors in frontal cortex was based on the observation of a DA-sensitive adenylate cyclase activity, in addition to classical α- and β-adrenergic ones, which was stimulated in a dose-dependent manner by DA and blocked by DA antagonists, such as haloperidol and chlorpromazine [1, 18].

Indications for the presence of D_1 DA receptors in the rat frontal cortex came from

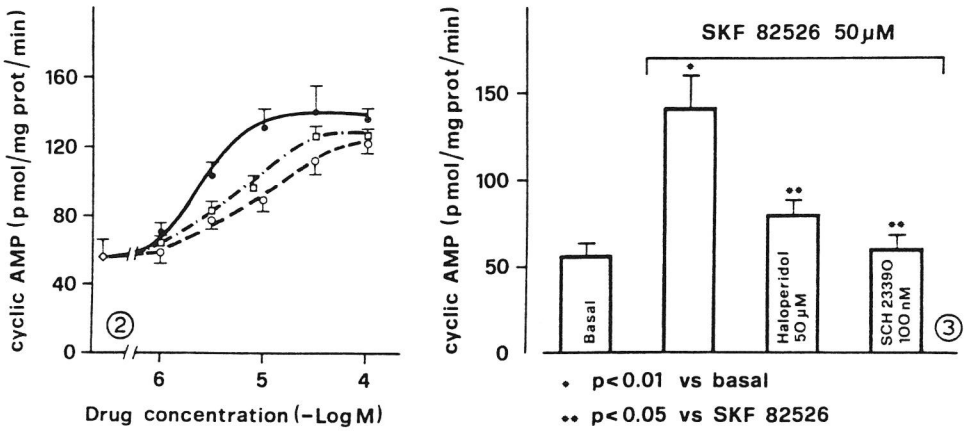

Fig. 2. Dose–response for SKF 82526 (●), DA (□) and (−)-apomorphine (○) activation of adenylate cyclase activity in frontal cortex. Each value is mean ± S.E.M. of 3 experiments run in triplicate. Basal enzyme activity was 56 ± 5 pmol/mg prot./min.

Fig. 3. Maximal activation of adenylate cyclase by SKF 82526 (50 μM) and its blockade by haloperidol (50 μM) and SCH 23390 (100 nM) in frontal cortex. Values are the means ± S.E.M. of at least 3 separate experiments. Basal enzyme activity was 56 ± 5 pmol/mg prot./min. The statistical significance of data was performed by Student's *t*-test.

binding studies using either [^{3}H]SCH 23390 or [^{3}H]SKF 38393 as ligand [3, 14]. Our results demonstrate the presence of the D_1 receptors coupled with stimulation of cyclic AMP generating system. Indeed, SKF 82526 and (−)-apomorphine, two D_1 DA receptor agonists, were able to dose-dependently stimulate adenylate cyclase activity. Haloperidol, an antagonist for both D_1 and D_2 DA receptors, and more specifically the selective D_1 DA receptor antagonist, SCH 23390, were able to block SKF 82526 stimulation of cyclic AMP formation.

The existence of D_2 DA receptors was supported by binding studies with [^{3}H]sulpiride [13, 17, 19] or more recently by ^{125}I-iodosulpiride [9, 10]. Our results demonstrate the presence of the D_2 receptors coupled with inhibition of cyclic AMP production. Indeed, bromocriptine, lisuride and RU 24213, which are all selective D_2 DA receptor agonists, were able to dose-dependently inhibit adenylate cyclase activity in homogenates from rat frontal cortex. Furthermore, (−)-sulpiride, a specific D_2 receptor antagonist, blocked bromocriptine inhibition of cyclic AMP production.

Interestingly, selective stimulation of D_1 receptors by maximal effective concentrations of SKF 82526 produced an increase of 85 ± 5 pmol/mg prot./min, whereas selective stimulation of D_2 receptors by maximal effective concentrations of bromocriptine produced a decrease of −24 ± 5 pmol/mg prot./min. These results might suggest that rat frontal cortex has more D_1 than D_2 receptors. Indeed, the net response of adenylate cyclase activity after stimulation of D_1 and D_2 receptors by DA per se is an enhancement of cyclic AMP formation.

In summary this study shows the presence of both D_1 and D_2 receptors in rat fron-

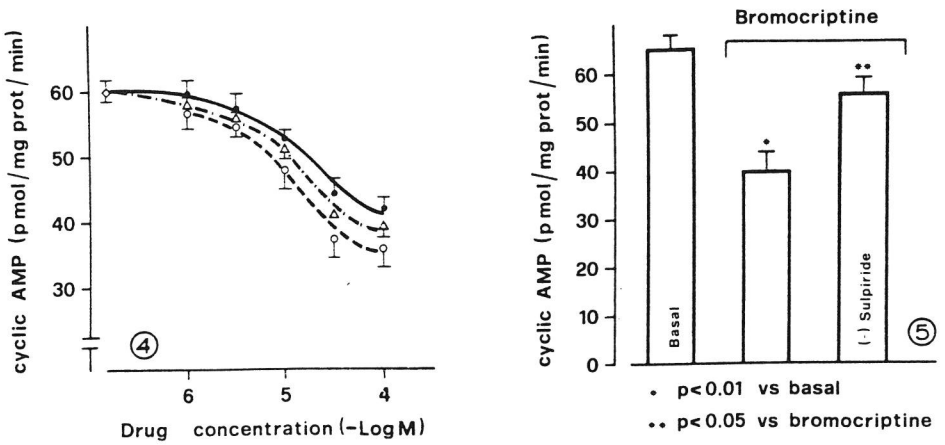

Fig. 4. Dose–response curves for bromocriptine (○), lisuride (△) and RU 24213 (●) inhibition of frontal cortex adenylate cyclase activity. Each value is the mean ± S.E.M. of 3 experiments run in triplicate. Basal enzyme activity was 60 ± 3 pmol/mg prot./min.

Fig. 5. Maximal inhibition of frontal cortex adenylate cyclase by bromocriptine (50 μM) and its blockade by (−)-sulpiride (50 μM). Values are the means ± S.E.M. of triplicate determinations of at least 3 separate experiments. Basal enzyme activity was 65 ± 3 pmol/mg prot./min.