TOPOGRAPHICAL DISTRIBUTION OF DOPAMINERGIC INNERVATION AND OF DOPAMINERGIC RECEPTORS IN THE RAT STRIATUM. II. DISTRIBUTION AND CHARACTERISTICS OF DOPAMINE ADENYLATE CYCLASE — INTERACTION OF D-LSD WITH DOPAMINERGIC RECEPTORS

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SUMMARY

The characteristics of dopamine adenylate cyclase in the rat striatum were first studied on homogenates of fresh tissues. In the assay conditions used, dopamine (10^{-4} M) stimulated the enzyme activity by 250%. This effect was completely blocked by fluphenazine (10^{-5} M; K_t = 9 \times 10^{-9} M) and by phentolamine (10^{-5} M; K_t = 3 \times 10^{-7} M). D-LSD stimulated the adenylate cyclase activity (K_m = 1.4 \times 10^{-7} M) by interacting with dopamine receptors; indeed the dopamine effect on the enzyme activity was competitively reduced in presence of D-LSD. L-isoproterenol (K_m = 10^{-6} M) activated an adenylate cyclase through a receptor distinct from the dopaminergic receptor; this stimulation was not affected by fluphenazine or phentolamine but suppressed by DL-propranolol (10^{-4} M).

The topographical distribution of the dopamine, D-LSD and L-isoproterenol adenylate cyclase activities were examined in homogenates prepared from discs punched out on serial frozen (--7°C) slices of the striatum. Under this condition, the dopamine maximal stimulation was of 150%. A 4.8-fold progressive decrease in the amount of cyclic AMP produced in presence of dopamine (10^{-4} M) was observed in the rostrocaudal plane of the structure; the decline of the basal activity was 3.6-fold. The topographical curves of maximal activation of adenylate cyclase by dopamine and D-LSD were superimposable confirming that D-LSD acts on dopaminergic receptors. This topographical distribution of dopamine sensitive adenylate cyclase is comparable on one hand to that of endogenous dopamine and on the other hand to that of the dopamine high affinity uptake activity measured in simultaneous experiments\textsuperscript{22}. In contrast to that observed with dopamine or D-LSD, the topographical distri-
bution of the adenylate cyclase sensitive to L-isoproterenol was homogenous within the striatum.

INTRODUCTION

There is a large body of evidence which suggests that the interaction of putative neurotransmitters (such as dopamine, norepinephrine, serotonin and histamine) with their receptors leads to the stimulation of adenosine 3',5'-monophosphate (cyclic AMP) formation. So far, most investigations have been performed on slices or homogenates obtained from large brain structures. Innervation, electrical activity and finally functions differ among discrete areas of the brain; thus, it would be of particular interest to delineate with precision the topographical distribution of neurotransmitter sensitive adenylate cyclases. Recently, Palkovitz\textsuperscript{15} described a microdissection technique which allows one to punch out several discs on single brain slice. On these small samples it is possible to measure the endogenous content of neurotransmitters\textsuperscript{5,11,16,20,22}, the activity of enzymes involved in their synthesis\textsuperscript{4,7,15} and in some cases their uptake processes\textsuperscript{22}. In this report, we describe a method permitting assay of adenylate cyclase activity in homogenates of single discs (1.4 mm diameter, 500 \(\mu\)m thickness). Using this technique, the distribution of dopamine sensitive adenylate cyclase was found to be heterogenous within the striatum of the rat: the maximal activity was localized in the rostral part. By contrast, an L-isoproterenol sensitive adenylate cyclase was homogenously distributed within the structure. The main characteristics of the striatal dopamine and isoproterenol sensitive adenylate cyclases have also been analysed. Furthermore, evidence supporting the hypothesis that \(\text{D-LSI}D\) may act on dopaminergic receptors involved in adenylate cyclase activation is provided.

MATERIALS AND METHODS

**Homogenate preparation**

Male Charles River rats of the Sprague-Dawley strain weighing 350–450 g were sacrificed at 2 p.m. by decapitation. Their brains were quickly removed and the striatum dissected with the help of glass manipulators. Tissues were homogenized (5 strokes) using a Dounce homogenizer in 2 mM Tris-maleate, pH 7.2, 2 mM EGTA (usually 2 striatum in 1.5 ml) at 4 °C. Homogenates were then filtered through a silk screen (150 \(\mu\)m pore diameter).

When the topographical distribution of dopamine receptors was examined, the brain was cut behind the striatum and the frontal half was fixed with sodium chloride (9%/w) on a Leitz Wetzler microtome stage refrigerated at -7 °C using a Leitz Kryomat. As soon as the brain was frozen (15 min) it was cut in slices (500 \(\mu\)m thickness). The localization of slices was determined according to the atlas of König and Klippel\textsuperscript{19} as described by Tassin \textit{et al.}\textsuperscript{22}. The first slice is delimited by the 9700 \(\mu\)m × 9200 \(\mu\)m planes and is denominated as the 9700 \(\mu\)m slice. The following serial slices are also denominated according to the rostral plane of the slices. Slices were laid on alumina
paper and discs of striatum (1.4 mm diameter) were punched out with a cool stainless steel tube sharpened at the extremity. The discs were blown into 500 μl microfuge plastic tubes containing 60 μl of the homogenization medium described above. The samples were homogenized (5 strokes) using a piston made with dental cement (Stellon from Detrey). Three discs were punched out in each slice in the dorsal, mediolateral and ventral position as indicated in Fig. 5, except for the first, second, eighth and ninth slices in which respectively only 1, 2, 2 and 1 discs were taken.

Adenylate cyclase assay

Adenylate cyclase activity was measured by conversion of [α-32P]ATP into [32P]cyclic AMP. The incubate (either 100 μl or 30 μl as indicated in figures or tables) contained 64 mM Tris-maleate, pH 7.2, 0.5 mM unlabeled ATP, 1 mM magnesium sulfate, 0.6 mM EGTA (including the EGTA added with homogenate), 0.2 mg/ml creatine kinase, 20 mM creatine phosphate, 10 mM theophylline, 1 μCi [α-32P]ATP and 0.001 μCi [3H]cyclic AMP. The reaction was initiated by addition of the homogenate and allowed to proceed for 2.5 min at 30 °C. It was stopped by addition of 100 μl of a solution containing 5 mM ATP, 5 mM cyclic AMP, 50 mM Tris–HCl, pH 7.4 and 1% sodium lauryl sulfate. [32P]Cyclic AMP formed and [3H]cyclic AMP added as recovery marker were isolated by two successive filtrations through a Dowex AG 50W-X8 and an alumina column according to Salomon et al. This procedure resulted in an overall cyclic AMP recovery of 60–80% and a reaction blank ranging from 15 to 20 counts/min per 106 counts/min of labeled ATP originally added. The range of variation between independent determinations was less than 10%.

Adenylate cyclase activities were expressed in picomole (pmole) of cyclic AMP produced/2.5 min/mg protein. Proteins were determined according to the method of Lowry et al. using bovine serum albumin as standard.

Phosphodiesterase assay

The effectiveness of theophylline (10 mM) as an inhibitor of phosphodiesterase in homogenates was examined by assaying the residual hydrolytic activities. These activities were measured under the adenylate cyclase assay conditions by conversion of a tracer amount of tritiated cyclic AMP (0.034 pmole in 100 μl, 0.68 × 10–3 μCi) to its metabolites. The reaction was stopped by addition of 100 μl of a solution containing 50 mM Tris–HCl, pH 7.6, 1 mM 5’-AMP, 1 mM adenosine, 1 mM cyclic AMP, 10 mM ATP. [3H]Cyclic AMP was isolated by thin layer chromatography on cellulose plates. The chromatograms were developed at room temperature in 1 M ammonium acetate, ethanol system (7:3 v/v). The radioactivity recovered on the cyclic AMP spot was expressed as per cent of the initial amount of [3H]cyclic AMP introduced.

Chemicals

ATP (disodium salt), DL-propranolol, and L-isoproterenol were purchased from Sigma. Cyclic AMP, creatine kinase and creatine phosphate were obtained from Boehringer Mannheim. Theophylline originated from Rhone Poulenc and dopamine
RESULTS

General properties of adenylate cyclase from rat striatum

Under our assay conditions the amount of cyclic AMP produced increased linearly both with incubation time (0–10 min) and enzyme concentration (10–70 µg of protein). The adenylate cyclase activities measured in the absence or presence of dopamine (10^{-4} M) increased as a function of Mg^{2+} concentration. However, the basal adenylate cyclase activity increased more rapidly than the dopamine sensitive activity. This resulted, as previously described, for other hormonally sensitive adenylate cyclases, in a decrease in the relative stimulation of the enzyme by dopamine: the dopamine stimulated over basal adenylate cyclase activities ratios were 3.2, 2.9, 1.8 and 1.3, respectively, in the presence of 1, 1.5, 2, 5 and 10 mM Mg^{2+}. Similarly
Interaction of D-LSD with dopaminergic receptors

As recently demonstrated by Von Hungen and Roberts, D-LSD stimulated the adenylate cyclase in striatal homogenates. The basal adenylate cyclase activity was increased by 40% in the presence of $10^{-5} M$ D-LSD (Fig. 2). A significant stimulation was observed for a concentration as low as $10^{-8} M$ and the apparent affinity of the adenylate cyclase for D-LSD was $1.4 \times 10^{-7} M$ which is about thirty times...
that of dopamine since the activation was completely suppressed by DL-propranolol.
TABLE I
EFFECT OF FLUPHENAZINE ON THE INCREASE OF CYCLIC AMP PRODUCED IN PRESENCE OF DOPAMINE AND D-LSD IN HOMOGENATE OF RAT STRIATUM

Inhibition constant of fluphenazine: (1) for the dopamine sensitive adenylate cyclase < 9 × 10⁻⁹ M; (2) for the D-LSD sensitive adenylate cyclase < 6 × 10⁻⁹ M. The adenylate cyclase activity was measured in homogenates from whole fresh striatum. The incubation medium (100 µl) contained 56 µg of protein. The basal adenylate cyclase activity was 220 pmoles/2.5 min/mg of protein. The increase in cyclic AMP produced in presence of dopamine and D-LSD alone were respectively 472 and 60 pmoles/2.5 min/mg of protein. The results are expressed as percentage of these increases. Each value represents the mean of two independent determinations.

<table>
<thead>
<tr>
<th>Fluphenazine (M)</th>
<th>Dopamine (5 × 10⁻⁴ M)</th>
<th>D-LSD (10⁻⁵ M)</th>
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<tr>
<td>0</td>
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<td>100</td>
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<tr>
<td>10⁻⁹</td>
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<td>10⁻⁵</td>
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Fig. 4. Specificity of dopaminergic and noradrenergic receptors in rat striatum. Thirty discs were punched out from frozen slices cut between 7500 µm and 5700 µm. They were pooled and homogenized in 2 ml of homogenization medium. The incubation medium (100 µl) contained 30 µg protein. The basal adenylate cyclase activity was 204 pmoles/2.5 min/mg protein. Each value represents individual determination. A: activation of adenylate cyclase by 1-isoproterenol; the 1-isoproterenol giving the half maximal stimulation was 10⁻⁴ M. B: effects of blocking agents on adenylate cyclase activation by 1-isoproterenol and dopamine. The concentration of different drugs were: 10⁻⁴ M 1-isoproterenol (ISO); 10⁻⁴ M 1-propranolol (PRO); 10⁻⁴ M fluphenazine (FLU); 10⁻⁴ M ephedrine (PHENT). The basal adenylate cyclase activity was decreased by 7%, 11% and 15% respectively in presence of 1-propranolol, fluphenazine or phenolamine alone. Similar results were obtained in two other experiments.
TABLE 1

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TABLE II

TOPOGRAPHICAL DISTRIBUTION OF L-ISOPROTERENOL SENSITIVE ADENYLYL CYCLASE ACTIVITY IN THE STRIATUM

The experimental protocol used was the same as described in Fig. 7. Values for cyclic AMP formed are the means ± S.E.M. for triplicate samples in typical experiments. P values were calculated for the difference between the cyclic AMP formed in presence and absence of L-isoproterenol using the Student's t-test.

<table>
<thead>
<tr>
<th>Distance (μm)</th>
<th>Basal (pmoles of cyclic AMP/2.5 min/mg protein)</th>
<th>+ L-isoproterenol (10⁻⁶ M) (pmoles of cyclic AMP)</th>
<th>Increase in cyclic AMP production in presence of L-isoproterenol (pmoles of cyclic AMP/2.5 min/mg protein)</th>
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<td>9180</td>
<td>143 ± 2.5</td>
<td>167.8 ± 1.05*</td>
<td>24.8</td>
</tr>
<tr>
<td>8680</td>
<td>152.1 ± 2.7</td>
<td>181.3 ± 0.73*</td>
<td>29.2</td>
</tr>
<tr>
<td>8180</td>
<td>163.2 ± 2.6</td>
<td>189.4 ± 2.18**</td>
<td>26.2</td>
</tr>
<tr>
<td>7680</td>
<td>104.0 ± 3.4</td>
<td>133.0 ± 1.61*</td>
<td>29</td>
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<tr>
<td>7180</td>
<td>91.3 ± 3.4</td>
<td>115.1 ± 1.6**</td>
<td>21.8</td>
</tr>
<tr>
<td>6680</td>
<td>63.9 ± 1.05</td>
<td>88.3 ± 2*</td>
<td>24.4</td>
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</table>

* P < 0.001.
** P < 0.01.

and only slightly affected by either fluphenazine or an α-adrenergic blocking agent (phenolamine) (Fig. 4). By contrast the dopamine activation of adenylate cyclase was not affected by DL-propranolol and was markedly reduced by either fluphenazine or phenolamine (Fig. 4).

Topographic distribution of basal adenylate cyclase within the striatum

Distribution of basal adenylate cyclase is shown in Fig. 5. The basal adenylate cyclase activity decreased by 3.6-fold from the rostral to the caudal part of the striatum. Furthermore, a non-homogeneous distribution of this adenylate cyclase activity appeared between the dorsal, mediolateral and ventral areas of serial slices. In most slices, the activity on the mediolateral disc was higher than those of the other parts. The activity of the ventral disc was always the lowest one. Since the protein content of disc was very similar in each disc (90–102 μg protein/disc), the fall of basal adenylate cyclase activity reflected a decrease of the number of catalytic sites. This distribution was not related to the presence of different amounts of endogenous dopamine in homogenates since the addition of fluphenazine (10⁻⁶ M) did not modify the results.

Topographic distribution of dopamine sensitive adenylate cyclase

Assuming that the stimulation by dopamine of the basal adenylate cyclase catalytic activity coupled with dopaminergic receptors is constant along the striatum, the increase in cyclic AMP produced in the presence of a saturating dose of dopamine can be taken as a measure of the density of dopaminergic receptor sites. As shown in Fig. 6, the amount of dopaminergic receptor sites decreased by 4.8-fold from the rostral to the caudal part of the structure. As observed for the distribution of basal
Fig. 5. Topographic distribution of basal cyclase activity in the striatum. Discs were punched out on serial slices in the dorsal, mediolateral and ventral parts of the striatum. The abscissae indicate the rostrocaudal localization of discs according to the atlas of König and Klippel. The incubation medium (30 μl) contained 15-17.5 μg protein. Each value represents the mean of two independent determinations obtained with homogenates of single disc: sl, nucleus septi lateralis; cp, caudate putamen (striatum); gcc, genu corporis callosi.

Fig. 6. Topographic distribution of dopamine sensitive adenylate cyclase activity in the striatum. Homogenates obtained in the experiment described in Fig. 5 were used to measure the dopamine sensitive adenylate cyclase activity. The ordinate represents the difference between the amount of cyclic AMP produced in presence or absence of 10 -4 M dopamine. The results presented in Figs. 5 and 6 were representative of a group of 3 experiments done with 3 different rats.

Adenylate cyclase, the activity in the ventral disc of serial slices was lower than those found in mediolateral and dorsal discs. Layering the rostral, instead of the caudal part of the brain directly on the refrigerated plate, did not modify this distribution.

**Distribution of D-LSD sensitive adenylate cyclase**

The increased production of cyclic AMP measured in presence of LSD followed
DISCUSSION

The dopamine stimulation of adenylate cyclase in rat striatum homogenates has been reported to be variable: 110% (ref. 9), 73% (ref. 5), 79% (ref. 25), 107% (ref. 13), 50-90% (ref. 23), 80-110% (ref. 8), and 185% (ref. 14). In our assay conditions, a higher and reproducible stimulation was observed (150-250%), similar to the increase in cyclic AMP content induced by dopamine in intact striatal slices (140%)10. In homogenates, the fluctuations in dopamine adenylate cyclase stimulation reported by different laboratories could be related to the assay conditions, and especially to the Mg²⁺ concentration used. Tell et al.23, who measured the adenylate cyclase activity in the presence of 6 mM Mg²⁺ instead of 1 mM in our assay, found a lower stimulation (50-90%). Our results confirm that the activation of the dopamine sensitive adenylate cyclase can be antagonized by low concentration of neuroleptics (apparent \( K_f \approx 9 \times 10^{-9} M \) for fluphenazine); although less effective, a similar effect can be seen with an \( \alpha \)-adrenergic blocking agent such as phentolamine (apparent
As expected propranolol, a β-adrenergic blocking agent, was ineffective. D-LSD which stimulated the adenylate cyclase of rat striatum and antagonized competitively the dopamine stimulation was thus acting as a partial agonist of the dopaminergic receptor. This was substantiated by the observation that fluphenazine abolished the D-LSD activation of adenylate cyclase. The inhibition constants of fluphenazine for dopamine and LSD sensitive adenylate cyclase were very similar to those determined by Clement-Cormier et al.\(^5\) in homogenates of rat striatum and olfactory tubercles (8 × 10\(^{-9}\) M). The high affinity (1.4 × 10\(^{-7}\) M) of dopaminergic sensitive adenylate cyclase for D-LSD could indicate that some of the psychomimetic effects of this agent, thought to be mediated by interactions with serotoninergic receptors, could also be evoked by interaction with postsynaptic dopaminergic receptors. A marked heterogenous distribution of the dopamine sensitive adenylate cyclase was found within the striatum. Indeed, the amount of dopaminergic receptors appeared to decrease by 4.8-fold from the rostral to the caudal part of the striatum; although less pronounced, differences were also seen between the dorsal, medio-lateral and ventral parts of the structure. Whereas cyclic AMP produced in presence of dopamine (10\(^{-4}\) M) decreased by 4.8-fold in the rostrocaudal plane, the basal activity of the adenylate cyclase declined only by 3.6-fold. This difference could be explained by a progressive drop of dopamine stimulatory capacity and/or by an increasing amount of a basal adenylate cyclase(s) uncoupled with dopamine receptors in the caudal part of the structure. In simultaneous experiments Tassin et al.\(^2,2\) using a similar procedure to punch out discs of striatal frozen slices observed very close rostrocaudal distributions of endogenous dopamine and of the dopamine high affinity uptake activity. These parallelisms further support the idea that the dopamine sensitive adenylate cyclase is a component of dopaminergic synapses. The close superimposition of the distribution curves of D-LSD and dopamine sensitive adenylate cyclase strongly support the hypothesis of the D-LSD interaction with dopaminergic receptors.

The high sensitivity of our assay allowed detection of adenylate cyclase stimulated by L-isoproterenol in homogenates of fresh as well as frozen tissues. The L-isoproterenol induced activation was completely suppressed by DL-propranolol and weakly affected either by an α-blocking agent (phenolamine) or by a highly potent neuroleptic (fluphenazine). These results indicate that the adenylate cyclase stimulation by L-isoproterenol was mediated through a β-adrenergic receptor distinct from the dopamine receptor. The homogenous distribution of the L-isoproterenol sensitive adenylate cyclase within the striatum, non-superimposable on the dopamine one, reinforced this statement (Table II). The adenylate cyclase activation by L-isoproterenol in homogenates was 10 times lower than that produced by dopamine. This is not in accordance with the observations made on striatal intact slices by Forn et al.\(^6\) who recently reported that L-isoproterenol was more potent than dopamine in increasing cyclic AMP formation. The simplest explanation would be that the striatal tissue loses much of its isoproterenol adenylate cyclase sensitivity during homogenization.

In conclusion, the present study reveals that it is now possible to study the
fine topographic distribution of catecholamine sensitive adenylyl cyclases in structures of the brain.

ACKNOWLEDGEMENT

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REFERENCES


