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Postsynaptic Serotonin-Sensitive Adenylate Cyclase in the Central Nervous System

II. Comparison with Dopamine- and Isoproterenol-Sensitive Adenylate Cyclases in Rat Brain

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SUMMARY

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Serotonin and dopamine stimulate distinct postsynaptic sensitive adenylate cyclases in homogenates of various brain areas in newborn rats [Enjalbert, A., Bourgoin, S., Hamon, M., Adrien, J. & Bockaert, J. (1978) *Mol. Pharmacol.*, 14, 000-000]. However, maximal effects of dopamine and serotonin on the adenylate cyclase activity in the striatum and the hypothalamus were not strictly additive. In fact, in striatum serotonin inhibited the dopamine-sensitive adenylate cyclase noncompetitively. Conversely, the maximal effects of serotonin and *l*-isoproterenol (a pure *beta* adrenergic agonist) on adenylate cyclase activity in homogenates of the cerebral cortex were strictly additive. Classical serotoninergic agonists [D-lysergic acid diethylamide (LSD), bufotenine, 5-methoxy-*N*,*N*-dimethyltryptamine] activated the adenylate cyclase in collicular homogenates. All but LSD were ineffective on the *beta* adrenergic-sensitive adenylate cyclase in C6 glioma cells. The antagonists tested were not specific for the serotoninergic or the dopaminergic receptors. Neuroleptics (clozapine, thioridazine, chlorpromazine, fluphenazine, and haloperidol) and classical serotoninergic antagonists (methiothepin, cyproheptadine, cinanserin, mianserin, and methergoline) interacted with both dopaminergic and serotoninergic receptors but not with *beta* adrenergic receptors. All these antagonists were more potent toward the dopamine- than the serotonin-sensitive adenylate cyclase. The drugs inhibited the dopamine-sensitive adenylate cyclase in a competitive manner. Methergoline was the only drug that competitively inhibited the serotonin-sensitive adenylate cyclase. The other classical serotoninergic antagonists, as well as the neuroleptics, inhibited the serotonin-sensitive enzyme by decreasing both its apparent affinity for serotonin and its maximal activity. The serotoninergic and dopaminergic receptors may exist in agonist and antagonist forms. Our results suggest that the structures of their antagonist forms may be closely related.

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INTRODUCTION

Considerable evidence suggests that serotonin is a neurotransmitter in the central nervous system of various species (1). However, the pharmacological and biochemical properties of the 5-HT[2] receptors in the CNS are not yet well defined, for at least two reasons. First, very often drugs that exhibit 5-HT agonist or antagonist properties in various peripheral preparations act differently in the CNS (2). Indeed, the classification of 5-HT receptors as M (morphine-sensitive) and D (dibenzyline-sensitive) receptors in peripheral organs (3) cannot be applied to the CNS. Second, it is only recently that two biochemical approaches have been developed to study the serotoninergic receptors in the brain. One consists of the determination of the characteristics of binding of [^{3}H]5-HT or [^{3}H]LSD to synaptosomal or microsomal membranes (4–9). However, the use of [^{3}H]LSD as a ligand for 5-HT receptors may already be criticized, since this drug also interacts with central dopaminergic receptors (10–13). The other approach is based on measurement of the activity of a 5-HT-sensitive adenylate cyclase in homogenates of brain tissues (14).

The 5-HT-sensitive adenylate cyclase and the 5-HT binding sites are both located on postsynaptic membranes (4, 15). The regional distribution of the 5-HT-sensitive adenylate cyclase in the brains of newborn rats is well correlated with that of the serotoninergic terminals in the brains both of young rats and of adult animals (15). The correlation between the regional densities of [^{3}H]5-HT binding sites and 5-HT termini is less obvious (4, 16). According to Snyder and Bennett (16), the densities of 5-HT binding sites were similar in the hypothalamus and cerebral cortex of adult rats, whereas the concentration of 5-HT was about 10 times higher in the hypothalamus than in the cerebral cortex.

As already discussed for other monoamine-sensitive adenylate cyclases (13, 17, 18), the characteristics of the 5-HT-sensitive adenylate cyclase in brain tissues (3, 15) suggest that some of the synaptic effects of 5-HT may be mediated by cyclic 3′,5′-AMP. We therefore investigated in detail the kinetic characteristics of the interactions of classical 5-HT agonists and antagonists with this 5-HT-sensitive adenylate cyclase in brain homogenates from newborn rats. The specificity of these drugs was analyzed by comparing their effects with those induced by classical dopamine antagonists such as neuroleptics. In addition, the possible interactions of 5-HT agonists and antagonists with other receptors coupled with an adenylate cyclase (dopamine-sensitive adenylate cyclase in the striatum and *beta* adrenergic-sensitive adenylate cyclase in C6 glioma cells) were investigated.

MATERIALS AND METHODS

Newborn Sprague-Dawley rats were obtained and housed as previously described (15). Homogenates of various brain structures were prepared and the adenylate cyclase assay was performed as described elsewhere (15). Briefly, fresh brain tissues were homogenized (40 mg in 800 μl containing 2 mM Tris-maleate, pH 7.2, 2 mM EGTA, and 300 mM sucrose) and filtered through a silk screen (150-μm pore diameter). The adenylate cyclase assay was performed on 10-μl aliquots of the homogenates. The assay consisted of measurement of the conversion of [α-^{32}P]ATP to cyclic [^{32}P]AMP during a 5-min incubation at 30° (15), EGTA was used to inhibit the Ca^{2+}-sensitive adenylate cyclase present in brain homogenates.

C6 glioma cells were taken from glial tumors induced by repeated injections of *N*-nitrosomethylurea into Wistar rats (19) and then cultured as previously described (20). Particulate fractions from C6 glioma cells were prepared according to a previous report (20). The adenylate cyclase assay on C6 glioma cells was performed as follows. Particulate fractions (30 μg of protein) were incubated at 30° for 5 min in 50 μl of medium containing 100 mM Tris-HCl (pH 8), 5 mM $MgSO_4$, 1 mM cyclic AMP,

[2] The abbreviations used are: 5-HT, serotonin (5-hydroxytryptamine); CNS, central nervous system; LSD, D-lysergic acid diethylamide bitartrate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

0.2 mM ATP plus [α-^{32}P]ATP, 0.2 mg/ml of creatine kinase, 20 mM phosphocreatine, 1 mM EDTA, and a tracer amount of cyclic [^{3}H]AMP. EDTA was used to stabilize the adenylate cyclase during the assay.

In all adenylate cyclase assays the cyclic [^{32}P]AMP formed was isolated according to Solomon *et al.* (21).

Serotonin creatine sulfate was obtained from Merck; dopamine, from Calbiochem; *l*-isoproterenol, from Sigma; LSD and thioridazine HCl, from Sandoz; bufotenine and 5-methoxy-*N*,*N*-dimethyltryptamine, from Regis; methiothepin maleate, from Hoffmann-La Roche; methergoline, from Farmitalia; cinanserin and fluphenazine, from Squibb; cyproheptadine HCl, from Merck Sharp & Dohme, Paris; mianserin, from Organon; haloperidol, thioproperazine methanesulfonate, and chlorpromazine, from Rhône-Poulenc; clozapine, from Dr. A. Wander, Bern; and α-flupenthixol HCl, from Labaz.

Cyclic [^{3}H]AMP (ammonium salt), 25 Ci/mmole, and [α-^{32}P]ATP (sodium salt), 10–20 Ci/mmole, were purchased from New England Nuclear.

RESULTS

Additivity of effects of 5-HT, dopamine, and l-isoproterenol on adenylate cyclase activity in homogenates of various brain structures. In homogenates of the cerebral cortex of newborn rats, 5-HT activated an adenylate cyclase in a concentration-dependent manner (Fig. 1). The formation of cyclic AMP was also increased by the presence of the pure *beta* adrenergic agonist *l*-isoproterenol (Fig. 1), as already observed in the cerebral cortex of the adult rat (22). The amount of cyclic AMP produced in the presence of this *beta* agonist was strictly additive with that induced by 5-HT alone (Fig. 1). In striatal homogenates of newborn rats, dopamine stimulated adenylate cyclase activity well, but 5-HT was much less effective (Fig. 2). When the dose-response curve for the dopamine-sensitive adenylate cyclase was obtained in the presence of a constant amount of 5-HT (50 μM), 5-HT partially inhibited the dopamine response in a non-

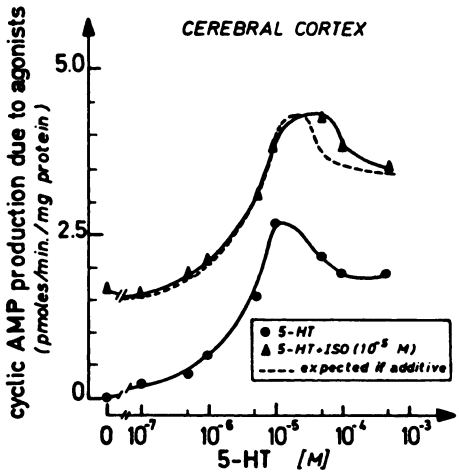


FIG. 1. *Additive effects of 5-HT and l-isoproterenol (ISO) on adenylate cyclase activity of cerebral cortex of newborn rats*

The basal adenylate cyclase activity was 5.05 ± 0.34 pmoles of cyclic AMP per minute per milligram of protein (n = 3). The dose-response curves for 5-HT activation were obtained in the absence (●) and presence (▲) of a constant concentration of isoproterenol (10 μM).

competitive manner, as shown by a Hofstee plot (23) (Fig. 2). A complementary experiment, in which the stimulatory effects of various concentrations of 5-HT on adenylate cyclase activity were determined in the absence and presence of a constant concentration of dopamine (100 μM), was performed with homogenates of hypothalamus from newborn rats. This preparation was chosen because the stimulatory effect of 5-HT was similar to that of dopamine, in contrast to the results observed in striatal homogenates (Fig. 3). In this preparation, as in the striatum, the effects of 5-HT and dopamine were not additive (Fig. 3). In the presence of 5-HT and dopamine (both at 100 μM) the production of cyclic AMP was equal to that obtained with 5-HT (100 μM) alone (Fig. 3).

Stimulation of adenylate cyclase activity in homogenates of colliculi from newborn rats by classical 5-HT agonists. The classical 5-HT agonists bufotenine (24), 5-methoxy-*N*,*N*-dimethyltryptamine (25), and LSD (26) increased the adenylate cyclase activity in collicular homogenates from newborn rats (Table 1). However, none of these compounds was as potent as 5-HT

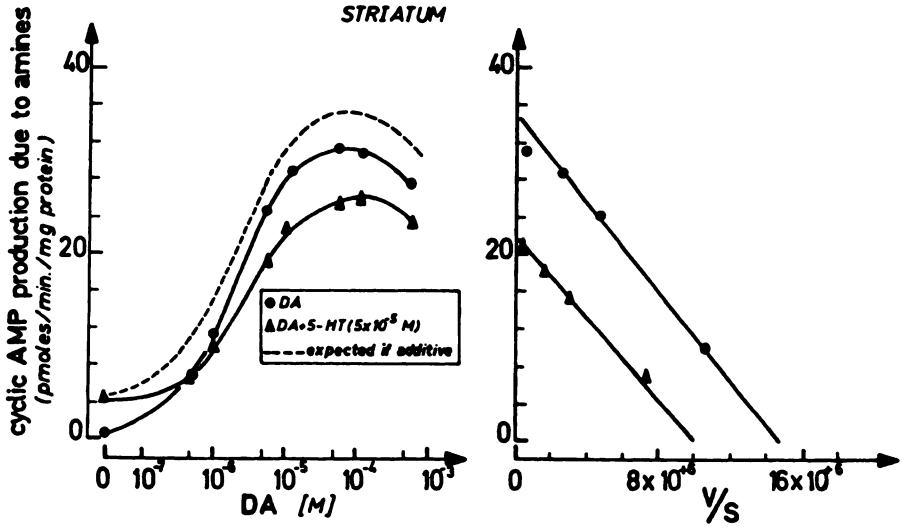


FIG. 2. *Nonadditive effects of 5-HT and dopamine (DA) on adenylate cyclase activity of striatum of newborn rats*

The basal adenylate cyclase activity was 11.3 ± 0.53 pmoles of cyclic AMP per minute per milligram of protein (n = 3). The right-hand graph presents a Hofstee plot (23) of the dose-response curves for dopamine activation, obtained in the absence (●) and presence (▲) of a constant concentration of 5-HT (50 μM) (left-hand graph).

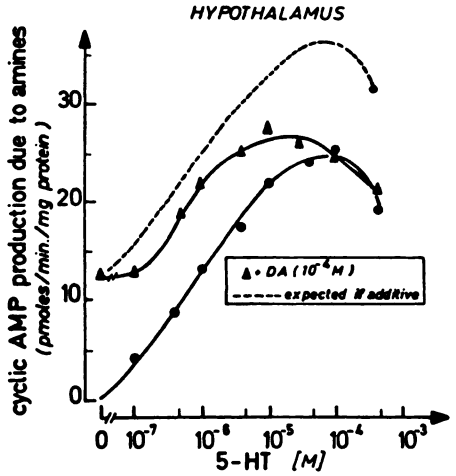


FIG. 3. *Nonadditive effects of dopamine (DA) and 5-HT on adenylate cyclase activity in hypothalamus of newborn rats*

The basal adenylate cyclase activity was 17.25 ± 0.35 pmoles of cyclic AMP per minute per milligram of protein. The dose-response curves for 5-HT activation were obtained in the absence (●) and presence (▲) of a constant concentration of dopamine (100 μM).

(Table 1). Similar results were obtained with homogenates of neonatal lumbar spinal cord, a structure rich in serotonergic termini (data not shown).

TABLE 1

Effects of 5-HT and 5-HT agonists on adenylate cyclase in collicular homogenates from newborn rats

The values are the means and standard errors of three determinations.

Compound	Concentration	Adenylate cyclase activity	Activation
	μM	*pmoles cyclic AMP/min/mg protein*	%
None (basal)		7.07 ± 0.28	
5-HT	10	13.02 ± 0.19	84
5-Hydroxy-*N,N*-dimethyltryptamine (bufotenine)	10	10.36 ± 0.42	46
	100	11.08 ± 0.26	56
5-Methoxy-*N,N*-dimethyltryptamine	10	10.21 ± 0.15	44
	100	10.64 ± 0.08	50
LSD	10	8.83 ± 0.25	25
	100	9.19 ± 0.19	30

Effect of LSD on 5-HT-, dopamine-, and beta adrenergic-sensitive adenylate cyclases. The stimulatory effects of 5-HT and dopamine on adenylate cyclase activity were greatly reduced in the presence of LSD in homogenates of colliculi and striatum, respectively (Fig. 4). In fact, LSD

acted as a noncompetitive partial agonist of the 5-HT-sensitive adenylate cyclase (Fig. 4). LSD and other 5-HT agonists, such as bufotenine and 5-methoxy-*N*,*N*-dimethyltryptamine, did not alter the basal adenylate cyclase activity in C6 glioma cells (Table 2). LSD was the only drug that reduced the *beta* adrenergic-sensitive adenylate cyclase activity (Table 2). This inhibition was competitive.[3]

Effects of classical 5-HT antagonists on 5-HT-, dopamine-, and beta adrenergic-sensitive adenylate cyclases. Methergoline, which has been proposed as a selective antagonist of central 5-HT receptors (27), inhibited the effect of 5-HT (10 μM) on cyclic AMP production in collicular homogenates (Fig. 5). This inhibition was not complete, since methergoline stimulated the basal adenylate cyclase activity at concentrations higher than 50 μM (Fig. 5). Surprisingly, methergoline was found to be a potent antagonist of the dopamine-sensitive adenylate cyclase in striatal homogenates of newborn rats. In this preparation methergoline did not alter the basal adenylate cyclase activity even at very high concentrations (Fig. 5). Methiothepin (Fig. 6) and other classical antagonists (28–40), such as cyproheptadine, mianserin, and cinanserin (data not shown), induced effects similar to those observed with methergoline. The direct stimulatory effect of methiothepin (100 μM) on the basal adenylate cyclase activity was observed not only in colliculi (2.4-fold) but also in homogenates of spinal cord (3.5-fold), hypothalamus (2.3-fold), and cerebellum (1.75-fold). However, the drug was without effect on the basal adenylate cyclase activity in striatal or cerebral cortex homogenates. Methergoline, methiothepin, cyproheptadine, cinanserin, and mianserin did not interfere with the stimulation of the *beta* adrenergic-sensitive adenylate cyclase induced by *l*-isoproterenol in C6 glioma cells (Table 2).

Effects of neuroleptics on 5-HT-, dopamine-, and beta adrenergic-sensitive adenylate cyclases. Dopamine- and 5-HT-sensitive adenylate cyclases, measured in striatal and collicular homogenates, respectively, were both inhibited by classical neuroleptics. The orders of potency of these drugs in these two systems were completely different (Table 3). The neuroleptics tested had no effect on the *beta* adrenergic-sensitive adenylate cyclase of C6 glioma cells (Table 3).

Kinetic analysis of inhibition of 5-HT- and dopamine-sensitive adenylate cyclases by 5-HT antagonists and neuroleptics. Methergoline (10 μM) competitively inhibited the 5-HT-sensitive adenylate cyclase in collicular homogenates (apparent K_i = 1 μM) (Fig. 7). In contrast, the other 5-HT antagonists tested induced mixed competitive and noncompetitive inhibition of the 5-HT-sensitive adenylate cyclase. The results obtained with methiothepin are illustrated in Fig. 8. In the presence of this drug (as with cyproheptadine, mianserin, or cinanserin) both the maximal stimulation produced by 5-HT and its apparent affinity were reduced.

All the 5-HT antagonists inhibited the dopamine-sensitive adenylate cyclase in a competitive manner. Their inhibitory effects were even more pronounced than those observed with the 5-HT-sensitive adenylate cyclase of collicular homogenates. For example, the apparent K_i values for methergoline were 1 and 0.05 μM for 5-HT- and dopamine-sensitive adenylate cyclases, respectively.

The characteristics of the inhibition by cyproheptadine of the 5-HT- and dopamine-sensitive adenylate cyclases were also determined in hypothalamic homogenates, since 5-HT and dopamine exert similar stimulatory effects on cyclic AMP production in this structure (Fig. 9). As before, the inhibition of the 5-HT-sensitive adenylate cyclase was mixed competitive and noncompetitive and that of the dopamine-sensitive adenylate cyclase was competitive (Fig. 9).

Fluphenazine, a highly potent neuroleptic that competitively inhibited the dopamine-sensitive adenylate cyclase in striatal homogenates of newborn animals (apparent K_i = 0.03 μM) (Fig. 10), also induced mixed inhibition of the 5-HT-sensitive adenylate cyclase in collicular homog-

[3] A. Dolphin, A. Enjalbert, J. P. Tassin, M. Lucas, and J. Bockaert, in press.

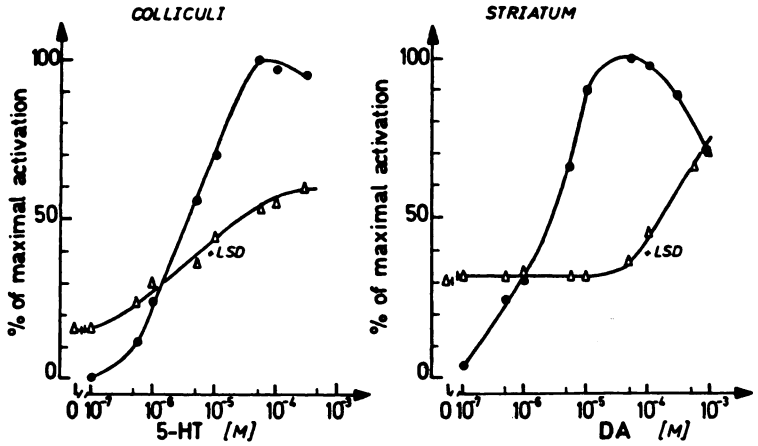


FIG. 4. *Effect of LSD on 5-HT- and dopamine (DA)-sensitive adenylate cyclase activities*

The basal adenylate cyclase activities were 8.9 ± 0.4 and 12.4 ± 0.26 pmoles of cyclic AMP per minute per milligram of protein in collicular and striatal homogenates, respectively (n = 3). Those measured in the presence of optimal concentrations of 5-HT and dopamine were 13.6 ± 0.54 and 30.3 ± 2.4 in collicular and striatal homogenates, respectively. The LSD concentration was 10 μM. LSD alone stimulated basal adenylate cyclase in both structures.

TABLE 2

Effects of 5-HT and 5-HT agonists and antagonists on beta adrenergic-sensitive adenylate cyclase in C6 glioma cells

The basal adenylate cyclase activity was 5.4 ± 0.42 pmoles of cyclic AMP per minute per milligram of protein (n = 3).

Drug (10 μM)	Cyclic AMP production due to *l*-isoproterenol (1 μM)
	pmoles/min/mg protein (% control)
None	82.5 (100)
5-HT	82.4 (100)
5-Hydroxy-*N*,*N*-dimethyltryptamine (bufotenine)	85.8 (104)
5-Methoxy-*N*,*N*-dimethyltryptamine	79.2 (96)
LSD	48.5 (58)
Methiothepin	80.5 (98)
Methergoline	76.6 (93)
Cyproheptadine	76.8 (95)
Cinanserine	78.8 (95)
Mianserine	75.9 (92)

enates. Similar results were obtained with haloperidol (data not shown).

DISCUSSION

The present data suggest that some structural similarities exist between the 5-HT and dopamine receptors coupled with an adenylate cyclase: first, in various structures the stimulatory effects of 5-HT and dopamine on adenylate cyclase activity were not strictly additive; second, 5-HT antagonists tested blocked the dopamine-sensitive adenylate cyclase and, conversely, neuroleptics inhibited the 5-HT-sensitive adenylate cyclase. The stimulatory effects of dopamine and 5-HT on adenylate cyclase activity were not additive in hypothalamic and striatal homogenates. Indeed, in the presence of 5-HT (50 μM), the maximal stimulation induced by dopamine was reduced by 30% in striatal homogenates (Fig. 2). Similarly, 5-HT may partially antagonize the stimulatory effect of dopamine in hypothalamic homogenates (Fig. 3). It could also be proposed that dopamine interferes with the stimulation of 5-HT-sensitive adenylate cyclase.

Classical 5-HT antagonists (methergoline, methiothepin, cyproheptadine, cinanserin, and mianserin) (28–40) inhibited the 5-HT-sensitive adenylate cyclase (Figs. 5 and 6). This adenylate cyclase was also strongly inhibited by neuroleptics, which are considered to be classical dopamine antagonists in the CNS; however, their potencies in the two systems were different (Table 3 and Fig. 10). These results confirm previous data indicating

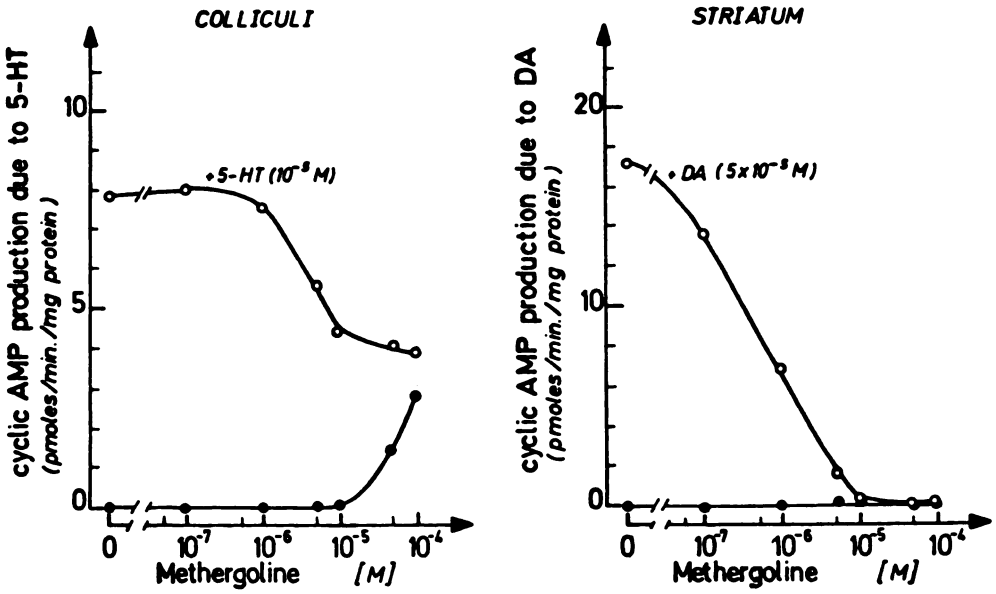


FIG. 5. *Effect of methergoline on basal and dopamine (DA)- and 5-HT-sensitive adenylate cyclase activities*

The effect of various concentrations of methergoline was tested in the absence (●) and presence (○) of a constant concentration of 5-HT (10 μM) or dopamine (50 μM). The basal adenylate cyclase activities measured in collicular and striatal homogenates were 7.08 ± 0.41 and 8.74 ± 0.33 pmoles of cyclic AMP per minute per milligram of protein, respectively (n = 3).

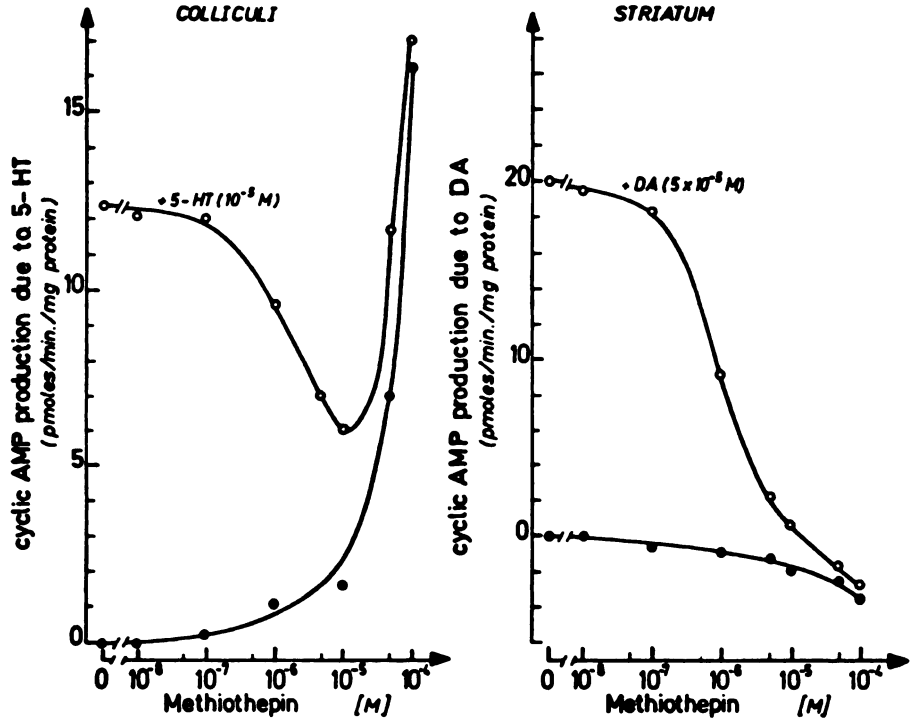


FIG. 6. *Effect of methiothepin on basal and 5-HT- and dopamine (DA)-sensitive adenylate cyclase activities*

The effect of various concentrations of methiothepin was tested in the absence (●) and presence (○) of a constant concentration of 5-HT (10 μM) or dopamine (50 μM). The basal adenylate cyclase activities measured in collicular and striatal homogenates were 7.08 ± 0.41 and 8.74 ± 0.33 pmoles of cylic AMP per minute per milligram of protein, respectively (n = 3).

TABLE 3

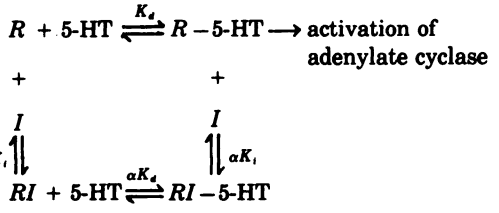
Effects of neuroleptics on 5-HT-, dopamine-, and beta adrenergic-sensitive adenylate cyclases

The basal adenylate cyclase activities were 6.6 ± 0.6, 7.8 ± 0.14, and 19.2 ± 0.5 pmoles of cyclic AMP per minute per milligram of protein in collicular homogenates, striatal homogenates, and C6 glioma cell particulate preparations, respectively. In colliculi the agonist was 5-HT (10 μM) and the concentration of neuroleptics was 10 μM; in striatum the agonist was dopamine (50 μM) and the neuroleptics were used at 5 μM; and in C6 glioma cells the agonist was *l*-isoproterenol (0.1 μM) and the neuroleptics were added at 10 μM.

Neuroleptic	Cyclic AMP production due to agonist	Inhibition
	pmoles/min/mg protein	%
Colliculi		
None	6.17	0
Clozapine	0.93	85
Thioproperazine	1.67	73
Chlorpromazine	1.80	71
Thioridazine	2.57	58
α-Flupenthixol	3.20	48
Fluphenazine	3.63	41
Haloperidol	3.93	36
Striatum		
None	20.13	0
α-Flupenthixol	0.57	97
Fluphenazine	1.00	95
Haloperidol	2.60	87
Chlorpromazine	4.97	75
Thioridazine	6.73	67
Clozapine	7.30	64
Thioproperazine	13.60	33
Glioma cells		
None	131.2	0
Thioridazine	114.8	12
Chlorpromazine	116.2	11
Fluphenazine	118.7	9
Haloperidol	125.0	5
Clozapine	131.8	0
α-Flupenthixol	133.0	0
Thioproperazine	133.4	0

that neuroleptics interact with the high-affinity binding of [^{3}H]5-HT on synaptosomal membranes (4). There is no available information about the type of inhibition by the 5-HT antagonists or neuroleptics toward either 5-HT binding sites or the 5-HT-sensitive adenylate cyclase in the CNS. The present data indicate that the type of inhibition of the 5-HT-sensitive adenylate cyclase induced by all the 5-HT antagonists tested, except methergoline, was mixed competitive and noncompetitive (Figs. 8 and 9). It did not differ from that observed with neuroleptics such as fluphenazine (Fig. 10). Recently Dray *et al.* (41) reported that the highly potent neuroleptic α-flupenthixol was able to block the effects of dopamine as well as those of 5-HT on the firing of neurons in the substantia nigra. On the other hand, our studies show that the dopamine-sensitive adenylate cyclase could be inhibited competitively not only by neuroleptics but also by putative 5-HT antagonists (Figs. 5–10). Similarly, high-affinity binding of [^{3}H]dopamine on striatal membranes was inhibited by methiothepin, cyproheptadine, and mianserin (42).

A Lineweaver-Burk (43) plot of the dose-response curves of 5-HT-sensitive adenylate cyclase for 5-HT in the presence of various concentrations of inhibitors revealed that the straight lines crossed at the same point ($1/v > 0$; $1/5\text{-HT} < 0$). This is indicative of a linear mixed type of inhibition. The simplest representation of this type of inhibition is shown in Scheme 1. This representation implies that the 5-HT receptor (R) has two different sites, one for the agonist (5-HT) and the other for the antagonist (I). The presence of I on the receptor changes the dissociation constant for the agonist from K_d to αK_d. Similarly, the presence of 5-HT on the receptor changes the affinity of I for its binding site by a factor α, $R-5\text{-HT}$ is the only form that induces activation of the adenylate cyclase. On the basis of binding studies, it has been proposed that 5-HT receptors, like many other receptors for neurotransmitters in the CNS, may exist in two forms, one with a high affinity for the agonist and the other exhibiting a high affinity for the antagonist (16), their



SCHEME 1

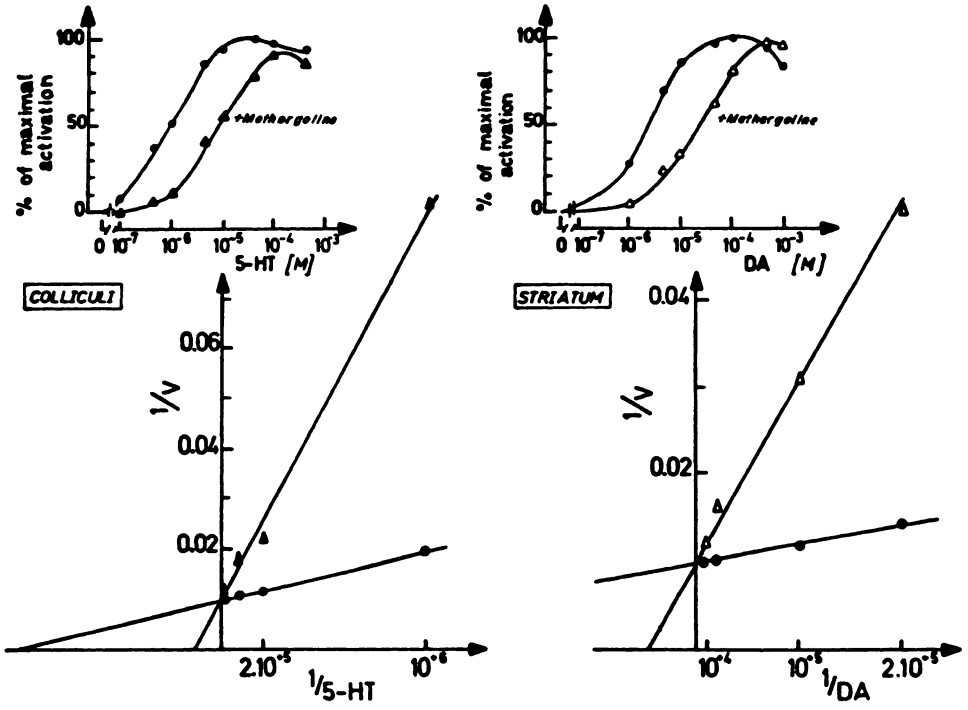

% of maximal activation
100
50
0
+Methergoline
0 10⁻⁷ 10⁻⁶ 10⁻⁵ 10⁻⁴ 10⁻³
5-HT [M]
DA [M]
COLLICULI
STRIATUM
1/v
0.06
0.04
0.02
2.10⁻⁵
10⁻⁶
1/5-HT
10⁻⁴
10⁻⁵
1/DA

FIG. 7

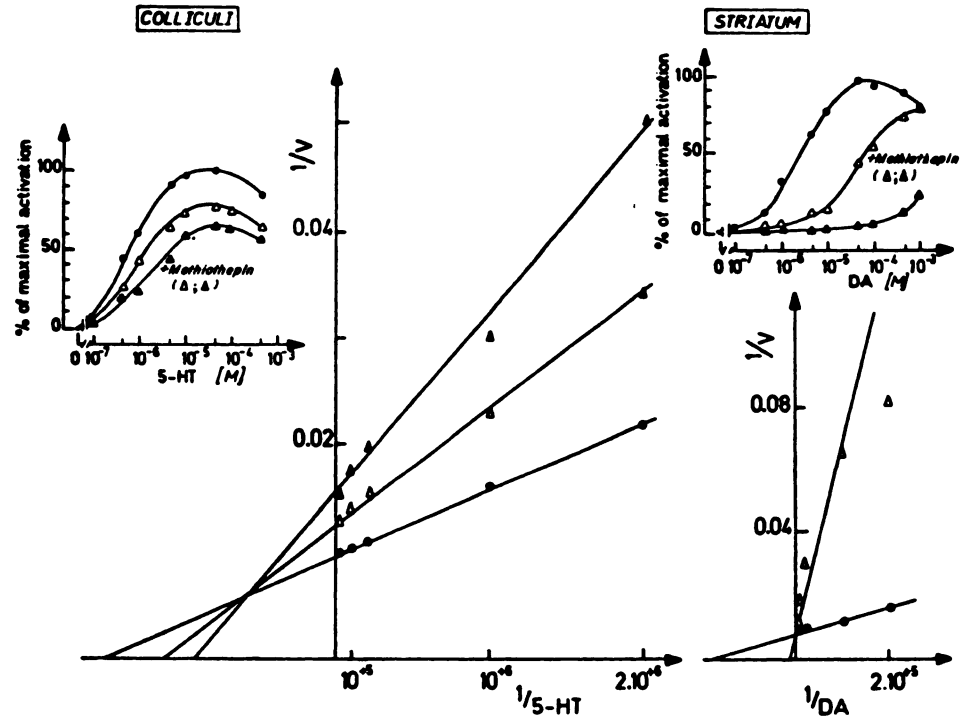

COLLICULI
STRIATUM
% of maximal activation
100
50
0
+Methiothepin (Δ;Δ)
0 10⁻⁷ 10⁻⁶ 10⁻⁵ 10⁻⁴ 10⁻³
5-HT [M]
DA [M]
1/v
0.04
0.02
0.08
10⁻⁶
2.10⁻⁶
1/5-HT
2.10⁻⁵
1/DA

FIG. 8

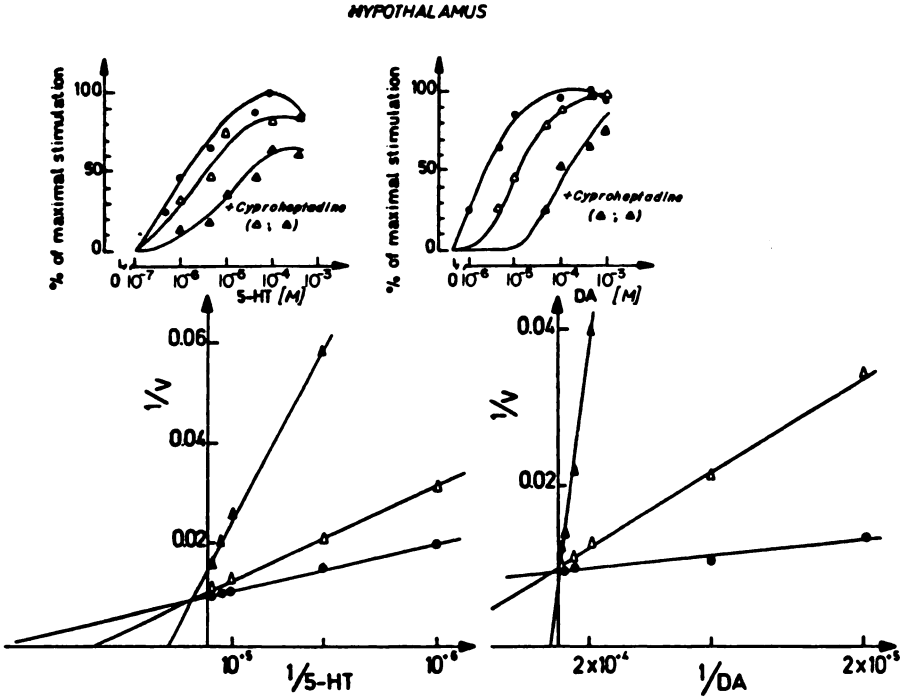


FIG. 9. *Kinetic analysis of cyproheptadine inhibition of 5-HT- and dopamine (DA)-sensitive adenylate cyclase activities in hypothalamic homogenates from newborn rats.*

The basal adenylate cyclase activity was 10.05 ± 0.54 pmoles of cyclic AMP per minute per milligram of protein, whereas the activity in presence of an optimal concentration of 5-HT or dopamine was 20.15 ± 0.62 and 15.8 ± 0.7 pmoles/min/mg of protein, respectively (n = 3). The dose-response curves for 5-HT and dopamine were obtained in the absence (●) and presence of either 0.5 μM (△) or 10 μM (▲) cyproheptadine. Cyproheptadine when used at these concentrations was without effect on basal adenylate cyclase activities.

interconversion not being energetically favorable (16). According to the model proposed in Scheme 1, these two forms of the 5-HT receptor may be analogous to the two sites (for 5-HT and *I*) of the 5-HT receptor coupled with an adenylate cyclase. On the basis of biochemical and behavioral studies performed on rats, methergoline has been proposed as a selective blocker of 5-HT receptors in the CNS

FIG. 7. *Kinetic analysis of methergoline inhibition of 5-HT- and dopamine (DA)-sensitive adenylate cyclase activities*

The basal adenylate cyclase activities measured in collicular and striatal homogenates were 5.9 ± 0.25 and 9.2 ± 0.44 pmoles of cyclic AMP per minute per milligram of protein, respectively (n = 3). Those measured in the presence of an optimal concentration of 5-HT or dopamine were 11.25 ± 0.55 and 33.6 ± 1.34 pmoles/min/mg of protein, respectively (n = 3). The dose-response curves for 5-HT and dopamine were obtained in the absence (●) and presence of either 0.5 μM (△) or 10 μM (▲) methergoline. Methergoline when used at these concentrations was without effect on basal adenylate cyclase activities.

FIG. 8. *Kinetic analysis of methiothepin inhibition of 5-HT- and dopamine (DA)-sensitive adenylate cyclase activities*

The basal adenylate cyclase activities measured in collicular and striatal homogenates were 7.8 ± 0.12 and 13.4 ± 0.32 pmoles of cyclic AMP per minute per milligram of protein, respectively (n = 3). Those measured in the presence of an optimal concentration of 5-HT or dopamine were 16.4 ± 0.92 and 42.8 ± 1.41 pmoles/min/mg of protein, respectively. The dose-response curves for 5-HT and dopamine were obtained in the absence (●) and presence of either 0.5 μM (△) or 10 μM (▲) methiothepin. Methiothepin when used at these concentrations was without effect on basal adenylate cyclase activities.

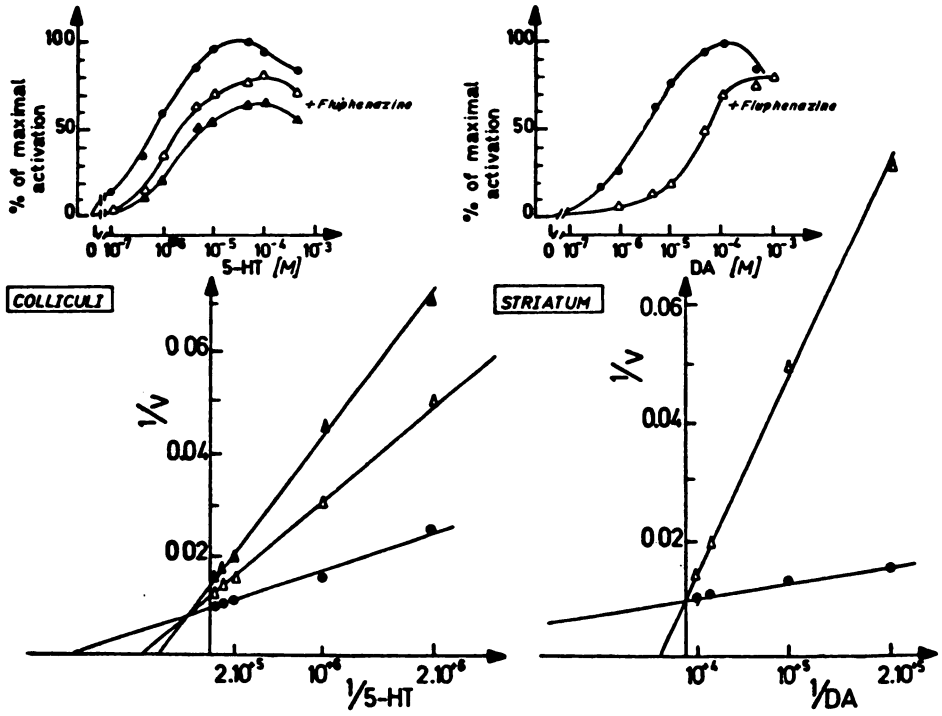


FIG. 10. *Kinetic analysis of fluphenazine inhibition of 5-HT- and dopamine (DA)-sensitive adenylate cyclase activities*

The basal adenylate cyclase activities measured in collicular and striatal homogenates were 6.8 ± 0.18 and 10.3 ± 0.29 pmoles of cyclic AMP per minute per milligram of protein, respectively (n = 3). Those measured in the presence of an optimal concentration of 5-HT or dopamine were 15.6 ± 0.34 and 38.5 ± 1.9 pmoles/min/mg of protein, respectively (n = 3). The dose-response curves for 5-HT and dopamine were obtained in the absence (●) and presence of either 0.5 μM (△) or 10 μM (▲) fluphenazine. Fluphenazine when used at these concentrations was without effect on basal adenylate cyclase activities.

(27). In fact, like other putative 5-HT antagonists, it also blocked the dopamine receptors coupled with adenylate cyclase (Fig. 7). Its apparent K_i was even lower (0.05 μM) in this case than for the 5-HT-sensitive adenylate cyclase (K_i = 1 μM). Among the 5-HT antagonists, methergoline was the only one that induced competitive inhibition of the 5-HT receptor. An alternative explanation might be that methergoline interacts with the antagonist form but also induces a very important reduction of the affinity of 5-HT for the agonist form ($\alpha \gg 1$). The inhibition of the dopamine-sensitive adenylate cyclase by neuroleptics or by putative 5-HT antagonists, as well as that induced by LSD, was competitive in all cases (Figs. 6–10). Binding studies suggest that the dopaminergic receptors may also exist in an agonist and an antagonist form (42). Therefore our results could be explained as already proposed for the mechanism of interaction of methergoline on the 5-HT receptor; the binding of neuroleptics, 5-HT antagonists, and LSD to the antagonist form of the dopamine receptor should considerably decrease the affinity of dopamine for the agonist form ($\alpha \gg 1$). Although the dopaminergic and serotoninergic receptors are distinct (15), we failed to find any specific inhibitor for either receptor. These results may indicate that some structural similarities exist between the two receptors. Such a hypothesis has been proposed for 5-HT and dopamine receptors in isolated canine arteries (38). In our hypothesis, the structural similarities between the two receptors may involve the antagonist forms. Like 5-HT, the 5-HT agonists

tested, such as bufotenine, 5-methoxy-*N,N*-dimethyltryptamine, and LSD, activated the basal adenylate cyclase activity in collicular homogenates, but their effects were less pronounced (Table 1). However, since dopamine, like 5-HT, also stimulates the adenylate cyclase activity in collicular homogenates with lower efficancy (15), the possibility cannot yet be excluded that 5-HT agonists may partially activate the adenylate cyclase by interacting with a dopamine receptor.

If the characteristics of the 5-HT and dopamine receptors are in some way similar, the properties of the 5-HT receptors differ compeltely from those of the *beta* adrenergic receptors coupled with an adenylate cyclase. First, the stimulatory effects of 5-HT and *l*-isoproterenol were strictly additive in cerebral cortex homogenates (Fig. 1). 5-HT agonists and 5-HT antagonists were without effect on the *beta* adrenergic-sensitive adenylate cyclase in C6 glioma cells (Tables 2 and 3). There was only one exception—LSD, which produced marked inhibition of the *beta* adrenergic-sensitive adenylate cyclase. This may raise some further questions about the central mechanism of action of this drug. Finally, neuroleptics, like 5-HT antagonists, did not inhibit the *beta* adrenergic-sensitive adenylate cyclase (Table 3).

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