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Postsynaptic Serotonin-Sensitive Adenylate Cyclase in the Central Nervous System

I. Development and Distribution of Serotonin and Dopamine-Sensitive Adenylate Cyclases in Rat and Guinea Pig Brain

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SUMMARY

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Postsynaptic serotonin-sensitive adenylate cyclase with an apparent affinity of 1 μM for serotonin (5-HT) was detected in various structures of the central nervous system in the rat and the guinea pig. At birth, the regional distribution of this enzyme in the rat brain was closely correlated with the topographical distribution of the serotoninergic innervation in young (9-day-old) as well as adult animals. Electrolytic raphe lesions made on the fourth day after birth, which produced 90% degeneration of serotoninergic innervation in rat colliculi, did not alter the characteristics (apparent affinity and maximal activity) of the serotonin-sensitive adenylate cyclase in this area. This suggests that the serotonin-sensitive adenylate cyclase is associated with postsynaptic serotoninergic receptors in the brain. During development, in both the rat and the guinea pig, the amount of cyclic 3',5'-AMP formed in response to an optimal concentration of serotonin in a given area remained constant. This is in contrast to the amount of cyclic AMP formed in response to an optimal concentration of dopamine, which increased 6-fold in the rat striatum between the second and 23rd days after birth. The lack of correlation between the regional distribution of the dopamine-sensitive adenylate cyclase and serotoninergic innervation and between the serotonin-sensitive adenylate cyclase and dopaminergic innervation further emphasizes that the serotonin- and dopamine-sensitive adenylate cyclases are each associated with specific receptors in the rat brain.

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INTRODUCTION

In the central nervous system, the interaction of monoaminergic neurotransmitters with their specific receptors leads to the activation of an adenylate cyclase (1). Thus Von Hungen *et al.* (2, 3) reported the existence of a serotonin-sensitive adenylate cyclase in homogenates of several brain structures of newborn rats. The maximal effect was found in colliculi. Expressed as percentage stimulation relative to basal adenylate cyclase activity, the 5-HT[3]-sensitive adenylate cyclase progressively decreased during development, being barely detectable in tissues of adult animals (2, 3). This observation was striking, since different results have been described for the dopamine-sensitive adenylate cyclase (3). Several data have indicated that the dopamine-sensitive adenylate cyclase is associated with the postsynaptic neurons. Its distribution in various structures such as striatum and cerebral cortex is identical with that of dopaminergic terminals (4, 5). Furthermore, the striatal dopamine-sensitive adenylate cyclase is still present after selective destruction of the nigro-striatal dopaminergic system (6, 7). Similar results should be found for the 5-HT-sensitive adenylate cyclase if this enzyme is linked to postsynaptic serotoninergic receptors. In the present study, using a very sensitive adenylate cyclase assay, some of the characteristics of the 5-HT-sensitive adenylate cyclase were further investigated. Particular attention was paid to analysis of the development and regional distribution of the 5-HT-sensitive adenylate cyclase in the CNS of the rat and guinea pig. These two species were selected because brain maturation occurs mainly during fetal life in the guinea pig, whereas it is largely postnatal in the rat. Parallel experiments on the dopamine-sensitive adenylate cyclase were carried out for comparison. Finally, attempts were made to measure the 5-HT-sensitive adenylate cyclase after the destruction of serotoninergic neurons in the rat. The 5-HT-sensitive adenylate cyclase could be detected in tissues of adult animals. The distribution and lesion studies revealed that the 5-HT-sensitive adenylate cyclase is associated with postsynaptic receptors, in the same manner as the dopamine-sensitive adenylate cylase.

[3] The abbreviations used are: 5-HT, serotonin (5-hydroxytryptamine) CNS, central nervous system; EGTA, ethylene glycol bis (β-aminoethyl ether)-*N,N'*-tetraacetic acid.

MATERIALS AND METHODS

Adult male and pregnant female Sprague-Dawley rats (Charles River strain) and adult male and pregnant female guinea pigs (Hartley strain) were used. The adult animals and litters were kept in a carefully controlled environment (24°, 60% humidity, alternating 12-hr cycles of light and darkness, food and water ad libitum) for at least 2 days before the experiments.

Homogenate preparation. Animals were killed at 12:00 m. by decapitation. The brains and spinal cords were quickly removed and dissected. Tissues were homogenized using a Dounce homogenizer (four strokes) in 2 mM Tris-maleate, pH 7.2, containing 2 mM EGTA and 300 mM sucrose at 4° (40 mg in 0.8 ml). Homogenates were then filtered through a silk screen (150-μm pore diameter).

Adenylate cyclase assay. The assay mixture (40 μl) contained 25 mM Tris-maleate (pH 7.2), 0.5 mM unlabeled ATP, 1 mM $MgSO_4$, 0.5 mM EGTA (including the EGTA added with the homogenate), 0.2 mg/ml of creatine kinase, 20 mM creatine phosphate, 10 mM theophylline, 2 μCi of [α-^{32}P]ATP, and 0.001 μCi of cyclic [^{3}H]AMP. The reaction was initiated by adding 10 μl of homogenate and was allowed to proceed for 5 min at 30°. It was stopped by the 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. The cyclic [^{32}P]AMP formed and the cyclic [^{3}H]AMP added as recovery marker were isolated by two successive filtrations through a Dowex AG50W-X8 and an alumina column according to Solomon *et al.* (8). This procedure resulted in an over-all cyclic AMP recovery of 60–80%, with a reaction blank ranging from 15 to 20 cpm/10^6 cpm of labeled ATP originally added. All deter-

minations were performed in triplicate, the range of variation being less than 10%. Adenylate cyclase activities were expressed in picomoles of cyclic AMP formed per minute per milligram of protein. Protein levels were determined according to Lowry *et al.* (9).

Endogenous levels of 5-HT and dopamine. 5-HT and dopamine were determined by the microradioenzymatic assays of Saavedra *et al.* (10) and Gauchy *et al.* (11), respectively.

Raphe lesions. Electrolytic lesions of the midbrain raphe nuclei (nucleus raphe dorsalis B7 and nucleus raphe centralis superior B8) were made according to Adrien (12) on the fourth day postpartum. The extent of the lesion was determined by histological examination of the raphe area and measurement of endogenous levels of 5-HT in the whole forebrain or in various dissected forebrain areas.

Chemicals. ATP (disodium salt) was purchased from Sigma; cyclic AMP, creatine kinase, and creatine phosphate, from Boehringer/Mannheim; serotonin creatine sulfate, from Merck; and dopamine, from Calbiochem.

Radiochemicals. 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. *S*-Adenosyl[*methyl*-^{3}H]methionine (7.5 Ci/mmole) was purchased from the Radiochemical Centre.

RESULTS

Apparent affinities of 5-HT-sensitive adenylate cyclase in various brain areas of newborn rats. Homogenates of the colliculi, hypothalamus, and lumbar spinal cord (the most responsive area of this structure) of newborn rats contained an adenylate cyclase which was stimulated by low concentrations of 5-HT (Fig. 1). The apparent affinity (5-HT concentration giving half-maximal stimulation) was close to 1 μM in the three regions (Fig. 1). In this experiment the optimal concentration of 5-HT (10 μM) stimulated the basal adenylate cyclase in colliculi, hypothalamus, and lumbar spinal cord by 92%, 144%, and 78%, respectively.

Developmental pattern of collicular 5-HT-sensitive adenylate cyclase and striatal dopamine-sensitive adenylate cyclase in rats. In rat collicular and striatal homogenates, the basal adenylate cyclase activity increased during the first 2 weeks after birth (Fig. 2). In the presence of the

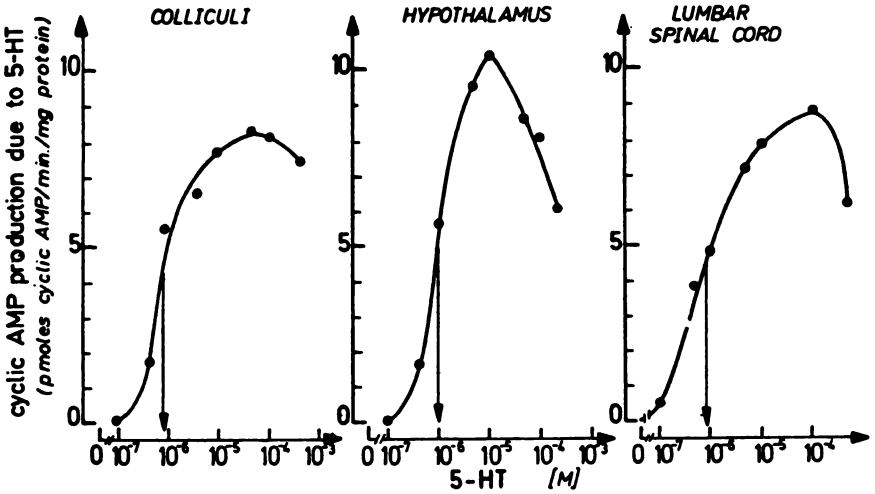

FIG. 1. *Dose-response curves for 5-HT-sensitive adenylate cyclase in colliculi, hypothalamus, and lumbar spinal cord of newborn rats*

The basal adenylate cyclase activities were 9 ± 0.35, 7.2 ± 0.25, and 11.3 ± 0.5 pmoles of cyclic AMP per minute per milligram of protein in colliculi, hypothalamus, and lumbar spinal cord, respectively. The vertical arrows indicate the 5-HT concentration giving half-maximal stimulation in each case.

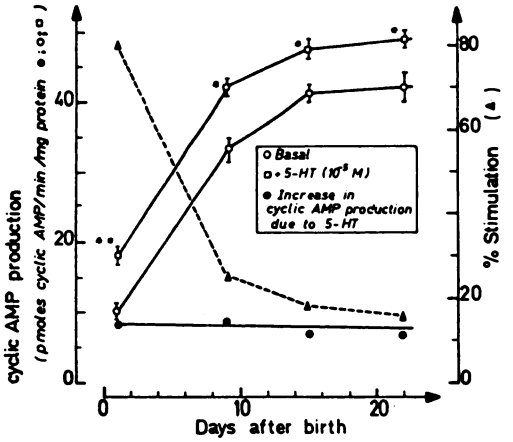

FIG. 2. *Postnatal development of basal and 5-HT-sensitive adenylate cyclases in rat collicular homogenates*

The adenylate cyclase activities were determined in the same experiment in homogenates prepared from colliculi of rats of various ages. The values are the means ± standard errors of triplicate determinations with four different homogenates. The 5-HT concentration was 10 μM.

* $p < 0.05$ compared with basal activity.

** $p < 0.01$ compared with basal activity.

optimal concentration of 5-HT (10 μM), the adenylate cyclase activity in collicular homogenates did not increase proportionally to the basal activity (Fig. 2). The increase in cyclic AMP formation induced by 5-HT remained constant during postnatal development (Fig. 2). Therefore the stimulatory effect of 5-HT, which was 80% in the newborn rats, decreased to 15% in 15-day-old animals (Fig. 2). In contrast, the absolute effect of the optimal concentration of dopamine (100 μM) on the striatal adenylate cyclase activity progressively increased as a function of age, being 6-fold higher on the 23rd day than on the first postnatal day (Fig. 3).

Some characteristics and development of 5-HT- and dopamine-sensitive adenylate cyclases in CNS of guinea pigs. A 5-HT-sensitive adenylate cyclase was detected in collicular, hypothalamic, and spinal cord homogenates of adult guinea pigs (Fig. 4). The response was maximal in the hypothalamus, where the apparent affinity of the enzyme for 5-HT was 1 μM (Fig. 5). In the three structures examined, the amounts of cyclic AMP formed in response to 5-HT were similar in adult and newborn animals (Fig. 4). Since the basal adenylate cyclase activity remained constant during postnatal development, the stimulatory effect of 5-HT, expressed as a percentage of the basal activity, was identical in newborn and adult guinea pigs (Fig. 4).

In the hypothalamic homogenates of fetal guinea pigs (50th day of gestation), the basal and 5-HT (10 μM)-sensitive adenylate cyclase activities were 6.01 ± 0.52 and 9.74 ± 0.84 pmoles of cyclic AMP per minute per milligram of protein, respectively. The stimulation of the adenylate cyclase by 5-HT was thus 62% in fetal homogenates. This is 3 times higher than observed in newborn and adult guinea pigs (20%, Fig. 4). The dopamine-sensitive adenylate cyclase was also estimated in collicular, hypothalamic, and spinal cord homogenates of adult and newborn animals. In contrast to the observations with 5-HT, pronounced response was found in the colliculi; it was similar to that detected in the hypothalamus. The stimulatory effect of the optimal concentration of dopamine (100 μM) was identical in brain homogenates of newborn and adult animals, the basal adenylate cyclase activity remaining constant (Fig. 4).

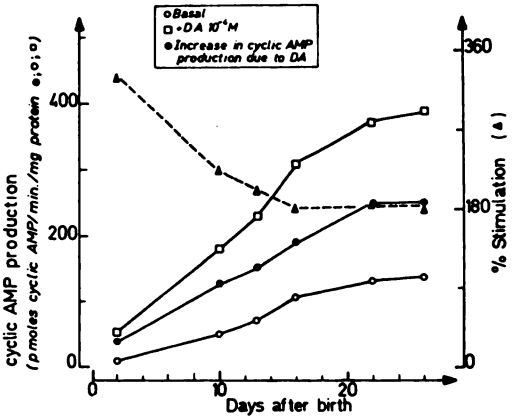

FIG. 3. *Postnatal development of basal and dopamine (DA)-sensitive adenylate cyclases in rat striatal homogenates*

The adenylate cyclase activities were all determined in the same experiment and are the means of triplicate determinations, with a range of variation of 3–5%.

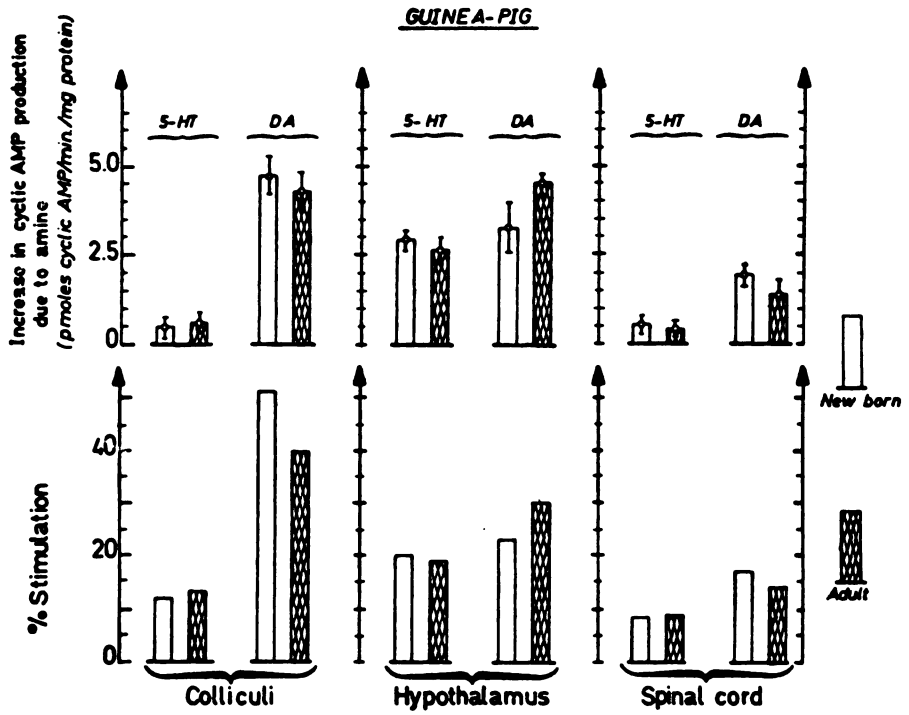

FIG. 4. *5-HT and dopamine (DA)-sensitive adenylate cyclases in newborn and adult guinea pigs*
The following basal adenylate cyclase activities (picomoles of cylic AMP per minute per milligram of protein) of each structure are those for newborn and adult guinea pigs, respectively: 8.21 ± 0.18 and 8.07 ± 0.53 in colliculi; 14.22 ± 0.22 and 13.22 ± 0.34 in hypothalamus; 11.32 ± 1.13 and 9.4 ± 0.92 in lumbar spinal cord.

Relationship between regional localization of 5-HT-sensitive adenylate cyclase and serotoninergic innervation in rat brain: comparison with distribution of dopamine-sensitive adenylate cyclase and dopaminergic innervation. The adenylate cyclase responses to the optimal concentration of 5-HT (10 μM) measured in several structures of the CNS of newborn rats (2 days old) were compared with the respective amounts of 5-HT in these structures in 2-, 9-, and 90-day-old rats. The absolute stimulatory effect of 5-HT on the 5-HT-sensitive adenylate cyclase correlated poorly (r = 0.58) with the amounts of 5-HT in the structures of the 2-day-old rats (Fig. 6). However, a good correlation (r = 0.96) was seen with the amounts of 5-HT in the structures of the 9-day-old rats (Fig. 6). The correlation with the 5-HT levels found in adult rats was also good (r = 0.92) (Table 1). A significant correlation could also be established when the regional distribution of the 5-HT-sensitive adenylate cyclase (Table 2) was compared with that of [^{3}H]5-HT high-affinity uptake, determined by Bennett and Snyder (13) (Table 1). A similar experiment was performed to compare the adenylate cyclase response to the optimal concentration of dopamine (100 μM) in the newborn rat (2 days old) with the amounts of dopamine in several structures of the CNS of newborn (Table 3) and adult animals (14). In this case an excellent correlation (r = 0.9) was also found between these two parameters in the various structures, in both newborn and adult animals (Table 1).

No correlations were found between the regional distribution of the 5-HT-sensitive adenylate cyclase and dopamine levels or between the distribution of the dopamine-sensitive adenylate cyclase and the levels of 5-HT (Table 1).

Postsynaptic localization of 5-HT recep-

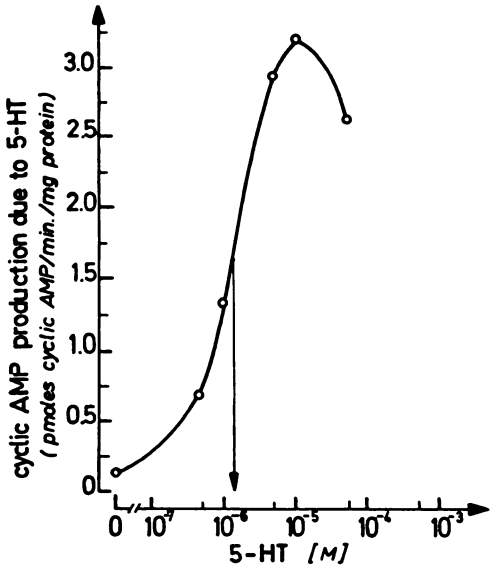

FIG. 5. *Dose-response curve for 5-HT-sensitive adenylate cyclase in hypothalamus of adult guinea pigs*

The basal adenylate cyclase activity was 9.6 ± 0.45 (n = 3) pmoles of cyclic AMP per minute per milligram of protein.

tors coupled with adenylate cyclase. Electrolytic lesions of B7 and B8 raphe nuclei were made on the fourth day of postnatal life. The 5-HT content of the lumbar spinal cord remained unchanged, while that of colliculi was decreased by 90%, on the fifth day after the lesion (Table 4). In both colliculi and lumbar spinal cord, however, the adenylate cyclase stimulation by 5-HT (10 μM) followed the same pattern during postnatal ontogenesis in control and raphe-lesioned rats (Table 4). Furthermore, the apparent affinity of the 5-HT-sensitive adenylate cyclase in the collicular homogenates measured on the fifth day after surgery was unaffected by the treatment (data not shown).

DISCUSSION

Like other monoamines, 5-HT stimulates the production of cyclic AMP in rat brain homogenates. In the newborn rat, the 5-HT-sensitive adenylate cyclase was maximal in homogenates from hypothalamus and colliculi and progressively decreased in other structures (spinal cord ≅ brain stem > hippocampus ≅ striatum > cerebral cortex > cerebellum). These findings confirm and extend the original observations by Von Hungen *et al.* (2, 3). The apparent affinity of the adenylate cyclase for 5-HT was about 1 μM whatever the structure considered (Fig. 1). The high sensitivity of the assay used to measure adenylate cyclase activitity made it possible to estimate 5-HT-sensitive adenylate cyclase in collicular homogenates of the adult rat (Fig. 2). In this structure the amount of cyclic AMP produced per milligram of protein in response to an optimal concentration of 5-HT remained constant during postnatal development (Fig. 2), in contrast to the basal adenylate cyclase

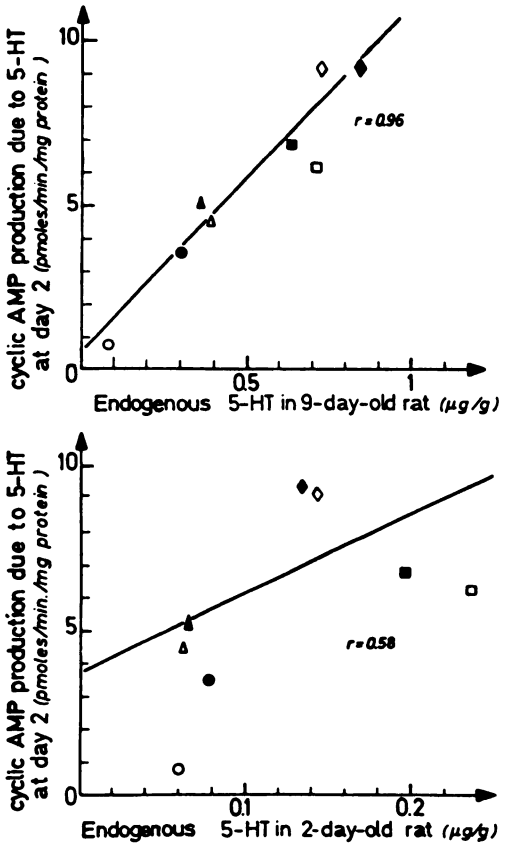

FIG. 6. *Correlations between regional distribution of 5-HT-sensitive adenylate cyclase and endogenous content of 5-HT*

Cyclic AMP production due to 5-HT (10 μM) in various CNS structures of 2-day-old rats were correlated with endogenous 5-HT content in 9-day-old (upper) and 2-day-old (lower) rats: ○, cerebellum; ●, cerebral cortex; △, striatum; ▲, hippocampus; □, brain stem; ■, spinal cord; ◇, colliculi; ◆, hypothalamus.

activity, which increased during ontogenesis (Figs. 2 and 3). This explains why the stimulatory effect of 5-HT (expressed as a percentage of the basal level) on adenylate cyclase activity decreased as a function of age (Fig. 2). In fact, if the occupation of one 5-HT receptor coupled with an adenylate cyclase produces a constant amount of cyclic AMP at various ages, it may be concluded that the concentration of serotoninergic receptors coupled with an adenylate cyclase is similar in newborn and adult rats. However, further experiments on coupling between the sensitive adenylate cyclase and 5-HT receptors are required to substantiate this conclusion. Possible changes in coupling fac-

TABLE 1

Correlations between cyclic AMP production due to optimal concentrations of 5-HT (10 μM) or dopamine (100 μM) in homogenates of various CNS structures of newborn rats, and content of 5-HT, dopamine, and [³H]5-HT uptake sites in CNS of rats of various ages

The values for cyclic AMP production and the 5-HT and dopamine concentrations were those reported in Tables 2 and 3, except for the [³H]5-HT uptake values, which are from Bennett and Snyder (13), and the dopamine concentrations in the CNS of adult rats, which are from Versteeg *et al.* (14).

Correlate	Day after birth	Correlation coefficient (r)	
		Cyclic AMP production due to 5-HT, 2nd day after birth	Cyclic AMP production due to dopamine, 2nd day after birth
Endogenous 5-HT	2	0.58	−0.49
	9	0.96	−0.14
	Adult	0.92	0.03
Endogenous dopamine	2	−0.16	0.90
	Adult	−0.11	0.93
[³H]5-HT uptake	Adult	0.86	0.30

TABLE 3

Regional distribution of endogenous dopamine and of cyclic AMP production due to dopamine in CNS of newborn rats

The values for endogenous dopamine are the means of at least three duplicate determinations, with a range of variation of 5–10%. For the adenylate cyclase assay the dopamine concentration was 100 μM.

Region	Endogenous dopamine	Cyclic AMP production due to dopamine (100 μM)
	μg/g, wet wt	*pmoles/min/mg protein*
Cerebellum	0.015	0.2 ± 0.25
Cerebral cortex	0.056	11.0 ± 0.75
Striatum	0.445	24.0 ± 1.70
Hippocampus		6.0 ± 0.25
Brain stem	0.089	4.0 ± 0.75
Spinal cord	0.012	3.0 ± 0.40
Colliculi	0.014	5.0 ± 0.25
Hypothalamus	0.047	6.5 ± 0.40

TABLE 2

Regional distribution of endogenous 5-HT in CNS of rats of various ages and of cyclic AMP production due to 5-HT in newborn rat CNS

The values for endogenous 5-HT are the means of at least three duplicate determinations, with a range of variation of 3–5%. For the adenylate cyclase assay the 5-HT concentration was 10 μM, and the values are means and standard errors of triplicate determinations.

Region	Endogenous 5-HT			Basal adenylate cyclase (newborn)	Cyclic AMP production due to 5-HT (10 μM) (newborn)
	Newborn	9 days old	Adult		
	μg/g, wet wt			*pmoles/min/mg protein*	
Cerebellum	0.060	0.08	0.085	6.0 ± 0.4	0.8 ± 0.20
Cerebral cortex	0.079	0.30	0.246	5.2 ± 0.35	3.6 ± 0.15
Striatum	0.066	0.38	0.588	9.1 ± 0.15	4.6 ± 0.20
Hippocampus	0.062	0.36	0.435	6.0 ± 0.25	5.0 ± 0.25
Brain stem	0.238	0.70	0.759	9.5 ± 0.20	6.2 ± 0.25
Spinal cord	0.197	0.63	0.775	10.8 ± 0.25	6.8 ± 0.35
Colliculi	0.142	0.73	0.724	7.0 ± 0.40	9.0 ± 0.30
Hypothalamus	0.139	0.84	1.022	12.2 ± 0.25	9.2 ± 0.35

TABLE 4

Effect of midbrain raphe lesions on stimulatory effect of 5-HT on adenylate cyclase activity in collicular and lumbar spinal cord homogenates

Electrolytic lesions were made on the fourth day after birth. For the adenylate cyclase assay the 5-HT concentration was 10 μM. Each value is the mean of results obtained with two different animals in each age group. 5-HT levels are the means ± standard errors of five duplicate determinations.

Region	Day after birth	Basal adenylate cyclase activity		Adenylate cyclase stimulation by 5-HT		Endogenous 5-HT	
		Control	Lesioned	Control	Lesioned	Control	Lesioned
		pmoles/min/mg protein		*% basal*		*μg/g*	
Colliculi	6	24.6	25.2	45	52		
	9	33.2	32.4	55	52	0.346 ± 0.031	0.032 ± 0.004
	15	40.8	35.6	32	24		
	25	41.6	47.4	15	13		
Lumbar spinal cord	6	15.2	13.8	19	21		
	9	18.7	16.4	20	20	0.393 ± 0.029	0.404 ± 0.019
	15	22.3	21.6	9	8		

tors during development, such as ions (Ca^{2+}) or GTP, might explain the apparently constant concentration of 5-HT receptors associated with an adenylate cyclase in brain during ontogenesis in the rat.

As in the rat, a 5-HT-sensitive adenylate cyclase was detected in homogenates from various brain areas in the guinea pig. In contrast to what was observed in the rat, not only the absolute amount of cyclic AMP formed in response to an optimal concentration of 5-HT (10 μM) but also the percentage stimulation was comparable in newborn and adult guinea pigs in all structures examined (Fig. 4). This might be related to the earlier maturation of the CNS in the guinea pig compared with the rat (15, 16). Indeed, during fetal life (50th day of gestation), when the degree of maturity of the guinea pig CNS is comparable to that of the rat at birth, the percentage stimulation over basal adenylate cyclase by 5-HT in hypothalamic homogenates was higher than that observed in newborn and adult guinea pigs. However, as discussed previously for the rat, these percentage changes were related only to the developmental increase of basal adenylate cyclase activity. The 5-HT receptors coupled with an adenylate cyclase seem to differ from those detected by binding studies. According to Bennett and Snyder (17), the affinity of the binding sites for [^{3}H]5-HT is 8 NM. This affinity is thus about 100 times higher than that of the 5-HT receptors coupled with an adenylate cyclase (1 μM). Furthermore, the concentration of the high-affinity binding sites for [^{3}H]5-HT in the brain increased 10-fold from the first to the 21st day after birth (17), in contrast to the apparent stability of the concentration of 5-HT receptors coupled with an adenylate cyclase observed in our study (Fig. 2). One cannot exclude the hypothesis that within a single population of 5-HT receptors only a limited, constant number of these receptors are functionally coupled with an adenylate cyclase. In fact, receptors may exist in different states. For instance, the acetylcholine receptor in the electric organ of *Torpedo* appears to be present in two states, one coupled with a sodium ionophore having a low affinity for the transmitter, and the other, not involved in sodium permeability, exhibiting a higher affinity (18).

Electrolytic lesions of the midbrain raphe nuclei in 4-day-old rats induced rapid degeneration of the serotoninergic neurons projecting to the forebrain but had no effect on the serotoninergic neurons innervating the spinal cord (19, 20). In such lesioned rats, the characteristics of the 5-HT-sensitive adenylate cyclase were

identical with those found in nonlesioned animals, not only in the spinal cord but also in the colliculi, a forebrain structure. These results indicated not only that the 5-HT-sensitive adenylate cyclase was localized on the postsynaptic site but also that the receptors associated with this enzyme did not become supersensitive following the selective degeneration of serotoninergic neurons in brain. It is of interest to recall that the [^{3}H]5-HT binding sites measured by Bennett and Snyder also remained unaffected following degeneration of serotoninergic neurons (17). Taking either the concentration of 5-HT or the quantity of the high-affinity 5-HT uptake sites [as determined by Bennett and Snyder (13)] in various CNS structures as indices of serotoninergic innervation, an excellent correlation was found between the regional distribution of the 5-HT nerve terminals in adult animals and the distribution of the 5-HT-sensitive adenylate cyclase measured in newborn rats (Fig. 5 and Table 1). This suggests that the 5-HT receptors coupled with an adenylate cyclase are closely associated with serotoninergic terminals.

The comparison between the presently described 5-HT-sensitive adenylate cyclase and the well-known dopamine-sensitive adenylate cyclase in rat brain revealed striking differences. Thus, whereas the 5-HT-sensitive adenylate cyclase activity did not change during development, the dopamine-sensitive enzyme increased about 6-fold in dopamine-containing areas such as the striatum. Furthermore, although the regional distribution of the dopamine-sensitive adenylate cyclase correlated well with that of the dopaminergic terminals, it was quite distinct from the topographical localization of the 5-HT-sensitive adenylate cyclase in the CNS structures of the rat. These findings show that each adenylate cyclase is in fact associated with a specific receptor in brain.

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