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SEROTONIN-SENSITIVE ADENYLATE CYCLASE AND [^{3}H]SEROTONIN BINDING SITES IN THE CNS OF THE RAT—II

RESPECTIVE REGIONAL AND SUBCELLULAR DISTRIBUTIONS AND ONTOGENETIC DEVELOPMENTS

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Abstract—The 5-HT receptor linked to adenylate cyclase and the high affinity binding site of [^{3}H]5-HT were compared on the basis of their localization and ontogenetic changes in the CNS of the rat. Subcellular fractionation of cerebral tissues from newborn rats showed a good correlation between the distributions of 5-HT-sensitive cyclase and [^{3}H]5-HT binding sites, with the mitochondrial fraction exhibiting the highest specific adenylate cyclase activity and density of binding sites. There was also a good correlation between the regional distributions of the cyclase and the binding in the CNS of newborn rats. However, when the regional distribution of [^{3}H]5-HT binding in newborns was compared to that of adults, no correlation was found, showing that large changes were occurring during ontogenesis. In the cortex and hippocampus, there was little change in the amount of 5-HT-sensitive adenylate cyclase during development whereas [^{3}H]5-HT binding increased approximately 7-fold from birth to adulthood. Only in the striatum was there a positive correlation between the changes in the cyclase and the binding. The injection of kainic acid into the striatum of 10-day-old rats produced large decreases in both DA- and 5-HT-sensitive adenylate cyclase activities. The specific binding of [^{3}H]5-HT was also reduced in the injected striatum but to a smaller extent. Therefore, the 5-HT-sensitive adenylate cyclase but not the [^{3}H]5-HT-high affinity binding site appeared to be preferentially associated with neurons destroyed by kainic acid, i.e. neurons intrinsic to the striatum. The findings that the 5-HT-sensitive adenylate cyclase and the [^{3}H]5-HT binding sites can develop independently and are localized, at least partly, on different types of cells provide additional evidence for the existence of multiple types of 5-HT receptors in the CNS of the rat.

In the accompanying paper [1], we have provided evidence that the serotonin (5-HT) receptor linked to adenylate cyclase in the central nervous system (CNS) of the rat [2, 3] exhibits different properties from those of the binding sites for [^{3}H]5-HT [4–6]. In particular, their respective affinities for 5-HT and sensitivities to various pharmacological agents were markedly different [1].

These findings strongly suggest that the 5-HT-sensitive adenylate cyclase and the [^{3}H]5-HT binding sites correspond to two distinct classes of 5-HT receptors. However, other criteria must be fulfilled to confirm this hypothesis. The best would consist of the physical separation of the 5-HT receptor–cyclase complex from the [^{3}H]5-HT high affinity binding sites. Attempts in this direction were made in the present study by examining carefully the respective regional and subcellular distributions of these two markers of central 5-HT receptors. In addition, their patterns of evolution were compared during ontogenesis in defined areas such as the hippocampus, the striatum and the cerebral cortex. Finally, since kainic acid-induced lesions appeared to be very useful for demonstrating the heterogeneity of other receptor types in the CNS [7–9], such lesions were realized in young rats, at a period when both the activity of 5-HT-sensitive adenylate cyclase and the specific [^{3}H]5-HT binding could be accurately measured [1–3, 10].

The large differences observed in the respective changes in [^{3}H]5-HT binding and 5-HT-sensitive adenylate cyclase activity during ontogenesis on the one hand and following kainic acid-induced lesions on the other hand further support the concept of multiple classes of central 5-HT receptors already proposed in the accompanying paper [1].

MATERIALS AND METHODS

Chemicals. The following compounds were used: 5-hydroxytryptamine creatinine sulfate (5-HT, Merck); ATP (disodium salt), cyclic AMP, creatine kinase and creatine phosphate were from Boehringer–Mannheim; kainic acid, ethylene glycol bis-(β-aminoethylether)-*N*,*N*′-tetraacetic acid (EGTA)

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and theophylline were purchased from Sigma. Dopamine (DA) and L-glutamic acid were from Calbiochem. Pargyline was generously donated by Abbott. [G-^{3}H]5-HT (generally labelled, 10.7–15.2 Ci/mmole), [α-^{32}P]ATP (sodium salt, 10–20 Ci/mmole) and cyclic [^{3}H]AMP (ammonium salt, 25 Ci/mmole) were obtained from the Radiochemical Centre, Amersham, U.K. [^{3}H]5-HT was purified [11] just before each binding experiment. All other compounds (salts, etc.) were the purest commercially available.

Animals

Sprague–Dawley rats were housed in a controlled environment (24°, 60% humidity, alternate cycles of 12 hr light and 12 hr darkness, food and water *ad lib.*) before being used. Newborn animals were used regardless of sex, whereas only male adults (250–350 g) were taken. Animals were decapitated, and the brains were dissected in the cold (4°) according to the procedure of Glowinski and Iversen [12]. All subsequent steps in the tissue preparations (see below) were also carried out at 0–4°.

Injection of kainic acid into the striatum of newborn rats. Six- or ten-day-old rats were anaesthetized with ether and placed in a stereotaxic apparatus. Then, 2 μg of kainic acid in 0.25 μl of an artificial cerebral spinal fluid (with or without a saturating concentration of L-glutamic acid) was injected during 2 min at each of two sites in the right striatum according to the following coordinates: (1) $A = 3.8$ mm, $L = 3.0$ mm, $H = +5.0$ mm; (2) $A = 3.2$ mm, $L = 3.7$ mm, $H = +4.0$ mm. These coordinates are based on a zero taken at the intersection of a line through the auditory canals with the midline (see Ref. 13). After the injections, the rats were returned to their litter mates and, if necessary, nourished by intra-oesophagal feeding with powdered milk (Neocarnis).

The addition of L-glutamic acid to the injection solution was based on the report by Bizière and Coyle [14] that this amino acid potentiates the neurotoxicity of kainic acid in animals lacking the glutamatergic corticostriatal innervation, like newborn rats [15].

Preparations of membranes

Lysed P_2 fractions. The complete procedure has been previously described [6]. These fractions corresponded to membranes from lysed synaptosomes [P_2] which were preincubated to remove endogenous 5-HT.

Crude membrane preparation. Tissues were homogenized in 70 vol. of 50 mM Tris–HCl, pH 7.4, using a Polytron disrupter (type PT 10 OD) and centrifuged at 40,000 *g* for 20 min. The supernatant fraction was discarded and the pellet was washed twice by suspension in 40 vol. of Tris buffer followed by centrifugation. The resulting pellet was gently homogenized in 40 vol. of Tris buffer and incubated for 10 min at 37° (see Ref. 6). Membranes were then collected by centrifugation, washed once as above and finally suspended in 40–50 vol. of 50 mM Tris–HCl, pH 7.4. [^{3}H]5-HT binding was measured using 1 ml aliquots of this preparation.

Subcellular fractionation

The subcellular fractionation was carried out essentially as described by Laduron *et al.* [16]. Briefly, the tissue was homogenized in 5 vol. of 0.25 M sucrose using a Potter–Elvehjem apparatus fitted with a teflon pestle. Successive centrifugations of this homogenate at 600 *g* for 10 min, 10,000 *g* for 15 min, 20,000 *g* for 15 min and 82,000 *g* for 100 min produced the sedimentation of the nuclear (N), heavy mitochondrial (M), light mitochondrial (L) and microsomal (P) fractions, respectively. The 82,000 g supernatant fraction was called the soluble (S) fraction. All of the sedimented fractions (N, M, L, P) were finally suspended in either 0.05 M Tris–HCl, pH 7.4, for use in the binding assays or in a solution containing 300 mM sucrose, 2 mM EGTA and 2 mM Tris-maleate, pH 7.2, for the adenylate cyclase assay.

Measurement of [^{3}H]5-HT binding

The binding of [^{3}H]5-HT was measured according to the method of Bennett and Snyder [4] using the modifications previously described [6]. In brief, this consisted of incubating the membranes (0.30–1.10 mg protein) for 7 min at 37° in 2 ml of 50 mM Tris–HCl containing 5.7 mM ascorbic acid, 10 μM pargyline, 4 mM $CaCl_2$ and 0.5–8.0 nM of [^{3}H]5-HT, final pH 7.4. Membranes were collected by filtration on Whatman GF/B filters. Specific binding is the difference between total radioactivity entrapped by the filter minus that found with similar samples containing 10 μM unlabelled 5-HT.

Measurement of adenylate cyclase activity

Adenylate cyclase activity was measured as described by Enjalbert *et al.* [3]. Tissues were homogenized using a Dounce homogenizer and then filtered through a silk screen before being used. The final assay mixture (40 μl) contained 25 mM Tris-maleate (pH 7.2), 0.5 mM unlabelled ATP, 1 mM $MgSO_4$, 0.5 mM EGTA, 0.2 mg/ml creatine kinase, 20 mM creatine phosphate, 10 mM theophylline, 2.0 μCi [α-^{32}P] ATP, 1.0 nCi cyclic [^{3}H]AMP (added as the recovery standard) and 10 μl of a 1:20 homogenate. Samples were incubated for 5 min at 30° in the presence or the absence of various concentrations of 5-HT (usually 10 μM). The reaction was stopped by adding 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. Isolation of cyclic [^{32}P] and [^{3}H]AMP was accomplished using Dowex AG 50 W-X8 and alumina columns [17]. The activity of 5-HT-sensitive adenylate cyclase is expressed as the increase in [^{32}P]cyclic AMP production (in pmoles/mg protein/min) due to the addition of 5-HT to the assay mixture.

Protein concentrations were determined using bovine serum albumin as the standard [18].

Statistical determinations were performed according to Snedecor and Cochran [19] with $P = 0.05$ chosen as the maximum level of significance.

RESULTS

Regional localizations of 5-HT-sensitive adenylate cyclase and [^{3}H]5-HT binding sites. When the bind-

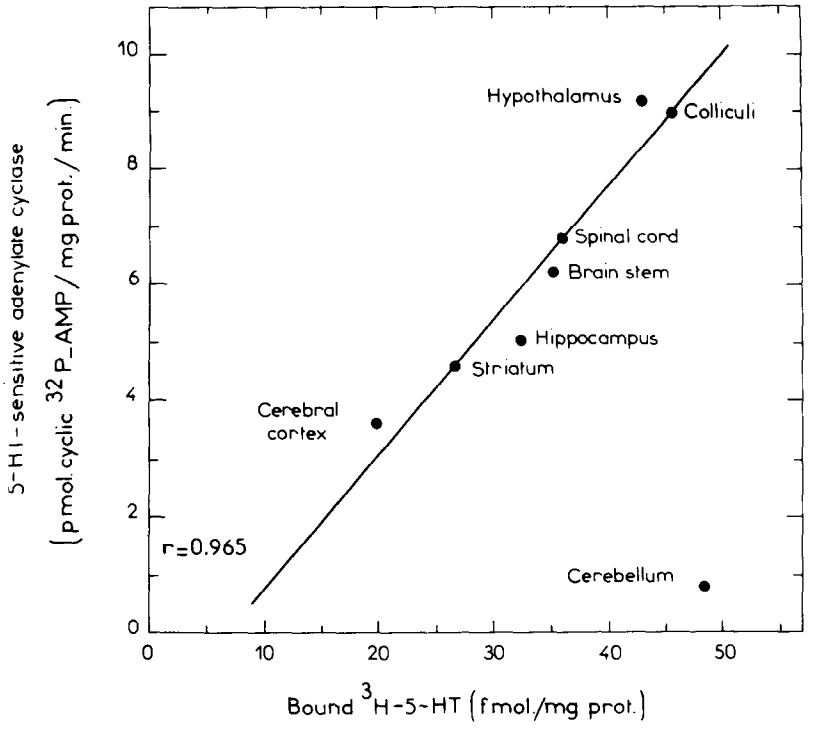

Fig. 1. Comparison of the regional distributions of [^{3}H]5-HT binding and 5-HT-sensitive adenylate cyclase in the CNS of newborn rats. The binding of [^{3}H]5-HT was measured in lysed P_2 fractions from various regions of the CNS of 5-day-old rats with 1.12 nM of the labelled ligand. The data for the distribution of 5-HT-sensitive adenylate cyclase is taken from Ref. 3 and correspond to pmoles cyclic-[^{32}P]AMP formed per mg protein and per min in the presence of 10 μM 5-HT. Comparison of the distributions of [^{3}H]5-HT binding and 5-HT-sensitive adenylate cyclase using linear regression analysis gave a coefficient of correlation (r) of 0.965 ($P < 0.001$) for all the structures examined, excluding the cerebellum.

ing of [^{3}H]5-HT was measured in various regions of the CNS of newborn rats and compared to the 5-HT-sensitive adenylate cyclase activity, a good correlation was found, with the exception of the cerebellum (Fig. 1).

Previous studies indicated that: (1) the topographical distribution of the 5-HT-sensitive adenylate cyclase in the CNS of newborn rats is significantly correlated with that of endogenous 5-HT in adult animals [3]; (2) no correlation exists between the regional distributions of endogenous 5-HT levels and [^{3}H]5-HT binding in the CNS of adult rats [6]. Therefore, it was of interest to compare the distributions of [^{3}H]5-HT binding in the CNS of adult and newborn rats. As expected of these two observations just mentioned, large differences were noted between the repartitions of [^{3}H]5-HT binding sites in the CNS of adult and newborn rats (Fig. 2). For anterior areas such as the cerebral cortex, the hippocampus and the striatum, [^{3}H]5-HT binding was markedly higher in adults than in newborns. Conversely, in the spinal cord and the cerebellum, a reduction of [^{3}H]5-HT binding was noted in adults as compared to newborns (Fig. 2).

It should be noted here that for the comparison of binding between adult and newborn rats, the results are expressed on a per gram wet weight basis (Fig. 2, see also Ref. 4). Differences in the protein content of the tissues and in the recovery of protein in the P_2 fraction between the two age groups preclude the expression of data on a per mg protein basis for valuable comparison.

Subcellular distributions of [^{3}H]*5-HT binding and 5-HT-sensitive adenylate cyclase.* For these experiments, pooled colliculi, hippocampi and striata were used because they correspond to well defined areas with relatively high [^{3}H]5-HT binding capacities and 5-HT-sensitive adenylate cyclase activities (Figs. 1 and 2). All fractions, except the 82,000 g supernatant fraction (S), obtained by differential centrifugations [16] of sucrose homogenates from newborn tissues had the capacity to synthesize cyclic[^{32}P]AMP in the presence of 5-HT and to specifically bind [^{3}H]5-HT (Table 1). When expressed on a per mg protein basis, the [^{3}H]5-HT binding and the cyclase activity in the newborn were greatest in fraction M and lowest in fraction N. In fact, the comparison of [^{3}H]5-HT binding and 5-HT-sensitive adenylate cyclase in all the fractions revealed that the respective subcellular distributions of these two markers of 5-HT receptors were significantly correlated ($r = 0.802$) in tissues of newborn rats (Table 1).

The distributions of [^{3}H]5-HT binding were different between newborns and adults because fraction P was found to have the greatest capacity (on a per mg protein basis) to bind specifically the labelled ligand in older animals (Table 1). Consequently, the subcellular repartition of [^{3}H]5-HT binding in tissues

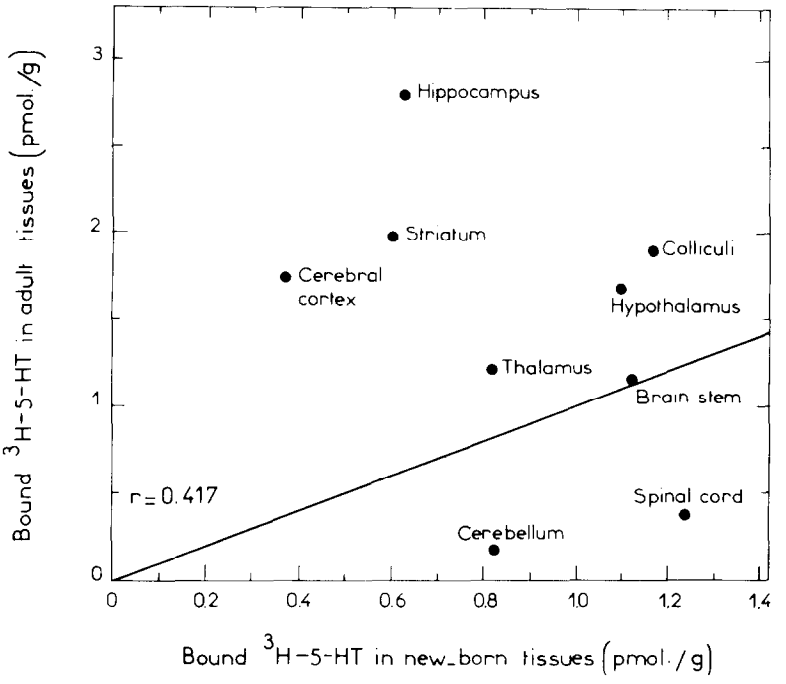

Fig. 2. Comparison of the regional distributions of the binding of [^{3}H]5-HT in the CNS of adult and newborn rats. The specific binding of [^{3}H]5-HT was measured in lysed P_2 fractions prepared from various brain regions of newborn (5-day-old) and adult rats as described in Materials and Methods. The concentration of [^{3}H]5-HT was 1.12 nM. Data are expressed as pmoles of [^{3}H]5-HT specifically bound to membranes prepared from 1 g of fresh tissue. Linear regression analysis indicates that no significant correlation ($r = 0.417$, $P > 0.05$) existed between respective adult and newborn values. The slope of the straight line is equal to 1.0 so that points situated above indicate that [^{3}H]5-HT binding was higher in adults than in newborns for the corresponding region. Reciprocally, [^{3}H]5-HT binding in the spinal cord and the cerebellum was greater in newborn than in adult rats.

from adults was correlated neither with that of the cyclase nor with that of the binding in newborns ($r \leqslant 0.327$, $P > 0.05$).

As shown in Table 1, the distribution of proteins in the various fractions differed markedly between newborn and adult rats. Thus for instance, 65 per cent of the total protein content of the sedimented material (N+M+L+P) were found in the 'microsomal fraction P' from newborn tissues whereas this proportion was reduced to 43 per cent with tissues from adult animals (Table 1). Therefore, in addition to the determination of the [^{3}H]5-HT binding capacity and the adenylate cyclase activity per mg protein in each fraction, it was also of interest to consider the total binding and cyclase activity per fraction. As indicated in Table 1, these calculations led to good correlations between the subcellular distributions of [^{3}H]5-HT binding in newborns and in adults on the one hand ($r = 0.993$) and between these two repartitions and that of the 5-HT-sensitive adenylate cyclase on the other hand ($r \geqslant 0.995$). The fraction containing the highest proportions of 5-HT-sensitive adenylate cyclase and [^{3}H]5-HT binding capacity was the microsomal (P) fraction (Table 1). Other fractions were at least three times less active in regard to these two markers of 5-HT receptors (Table 1).

Changes in [^{3}H]5-HT binding and 5-HT-sensitive adenylate cyclase during ontogenesis in discrete areas of the rat brain. When the binding of [^{3}H]5-HT was examined at various times after birth, it was noted that the binding capacity per g of fresh tissue increased steadily from birth to adulthood in both the hippocampus and cortex (Table 2). Binding also increased greatly during development in the striatum, although the capacity found in adults was slightly lower than that found at 3 weeks of age (Table 2).

Adenylate cyclase activity presented a somewhat different pattern of development. In the case of the striatum, the ontogenetic evolution of the 5-HT-sensitive adenylate cyclase closely resembled that of [^{3}H]5-HT binding. As indicated in Table 2, a significant correlation ($r = 0.968$) was found between the developments of [^{3}H]5-HT binding and 5-HT-sensitive adenylate cyclase in this structure. In the hippocampus, there was only a slight increase in 5-HT-sensitive cyclase activity during the first two weeks after birth followed by a decrease so that the activity was lower in adults than at birth. In the cerebral cortex, there was little change in the cyclase during development (Table 2). Thus, there was no correlation between developmental patterns of [^{3}H]5-HT binding and 5-HT-sensitive adenylate cyclase activity in either the hippocampus or the cerebral cortex (Table 2).

Effects of the local injection of kainic acid on [^{3}H]5-HT binding and DA-and 5-HT-sensitive adenylate cyclases in the striatum of newborn rats. The injection of 2 μg kainic acid into the right striatum of 6-day-

Table 1. Comparison of the subcellular distributions of 5-HT-sensitive adenylate cyclase and [^{3}H]5-HT binding*

Fraction	Newborn					Adult		
	Proteins (mg/g)	5-HT-sensitive adenylate cyclase (pmoles.min.$^{-1}$.mg protein^{-1})	(%)	Bound [^{3}H]5-HT (fmoles/mg protein)	(%)	Proteins (mg/g)	Bound [^{3}H]5-HT (fmoles/mg protein)	(%)
N	3.71 ± 0.41	1.74 ± 0.47	(3.0)	5.86 ± 0.73	(7.3)	10.05 ± 1.27	7.92 ± 1.03	(3.9)
M	4.04 ± 0.48	8.05 ± 0.39	(14.9)	13.20 ± 1.01	(17.8)	19.46 ± 2.32	11.65 ± 1.47	(11.1)
L	5.14 ± 0.35	5.40 ± 0.80	(12.7)	10.18 ± 1.28	(17.4)	14.21 ± 1.25	17.35 ± 2.04	(12.0)
P	24.19 ± 2.07	6.26 ± 0.62	(69.4)	7.11 ± 0.56	(57.5)	33.42 ± 3.16	44.71 ± 1.43	(73.0)

* The subcellular fractionation was carried out as described in Materials and Methods using pooled colliculi, hippocampi and striata from newborn (5-day-old) or adult (~90-day-old) rats. The total protein content recovered in each fraction obtained from 1 g of fresh tissue is expressed in mg. The activity of 5-HT-sensitive adenylate cyclase corresponds to the production of [^{32}P]cyclic AMP (in pmoles.min.$^{-1}$mg protein^{-1}) due to the addition of 10 μM 5-HT to the assay mixture. The specific binding of [^{3}H]5-HT (in fmoles/mg protein) was measured with 0.52 nM of the labelled ligand. Each value represents the mean ± S.E.M. of quadruplicate determinations from two separate experiments for the newborn animals and four separate experiments for the adult animals. The percentage in parentheses is equal to the total cyclic[^{32}P]AMP production or [^{3}H]5-HT specific binding found in a particular fraction divided by the total cyclic[^{32}P]AMP production of [^{3}H]5-HT binding in the sum of all the fractions (N+M+L+P) × 100. It corresponds to the recovery percentage of 5-HT-sensitive adenylate cyclase activity or of [^{3}H]5-HT specific binding in each fraction as compared to whole homogenates. No specific [^{3}H]5-HT binding and 5-HT-sensitive adenylate cyclase activity was detected in the soluble fraction S.

Table 2. Comparison between changes in [^{3}H]5-HT binding and 5-HT-sensitive adenylate cyclase in three different areas of the rat brain during ontogenesis*

Age	Hippocampus				Striatum				Cerebral cortex			
	[^{3}H]5-HT Bound	R	5-HT-Sensitive adenylate cyclase	R	[^{3}H]5-HT Bound	R	5-HT-sensitive adenylate cyclase	R	[^{3}H]5-HT Bound	R	5-HT-Sensitive adenylate cyclase	R
NB	0.43 ± 0.04		0.29 ± 0.02		0.26 ± 0.04		0.21 ± 0.02		0.17 ± 0.02		0.17 ± 0.01	
1 week	0.78 ± 0.03	1.80	0.42 ± 0.02	1.46	0.91 ± 0.13	3.54	0.32 ± 0.06	1.50	0.37 ± 0.02	2.18	0.18 ± 0.02	1.06
2 weeks	1.41 ± 0.03	3.25	0.48 ± 0.08	1.69	1.60 ± 0.07	6.21	0.88 ± 0.08	4.17	0.56 ± 0.04	3.32	0.22 ± 0.13	1.28
3 weeks	2.16 ± 0.09	5.00	0.17 ± 0.04	0.61	2.50 ± 0.10	9.69	1.59 ± 0.14	7.51	0.99 ± 0.06	5.81	0.28 ± 0.13	1.63
Adults	2.79 ± 0.12	6.46	0.13 ± 0.02	0.46	2.15 ± 0.07	8.32	1.09 ± 0.41	5.15	1.31 ± 0.05	7.71	0.10 ± 0.02	0.59
		$r = -0.671$ (NS)				$r = 0.968$ ($P < 0.01$)					$r = -0.155$ (NS)	

* The specific binding of [^{3}H]5-HT was measured in lysed P_2 fractions in the presence of 1.10 nM of the labelled ligand. Data are the means ± S.E.M. of triplicate determinations (in two to five separate experiments) of pmoles[^{3}H]5-HT specifically bound in lysed P_2 fraction prepared from 1 g of fresh tissue. The production of cyclic [^{32}P]AMP due to 10 μM 5-HT is expressed as nmoles per min and per g tissue wet wt (means ± S.E.M. of triplicate determinations in three or four independent experiments). NB (newborn) animals were between 3 and 24 hours old when killed and adult animals were 90 days old. R is the ratio of [^{3}H]5-HT binding or cyclic [^{32}P]AMP production found at a given age to that found in newborn (NB) rats. The correlations between the changes in [^{3}H]5-HT binding and cyclic [^{32}P]AMP production during ontogenesis in the three structures examined were determined using linear regression analysis; the coefficients of correlation are given as r. NS indicates a statistically nonsignificant value.

Table 3. Effects of local injection of kainic acid with or without L-glutamic acid on the activity of 5-HT-sensitive adenylate cyclase in the striatum*

		5-HT-Sensitive adenylate cyclase		
Injection	Death	Control	Kainic acid	Kainic acid + L-glutamic acid
6th day	15th day	9.13 ± 0.88	7.15 ± 0.76 (78)	3.66 ± 1.39† (40)
10th day	15th day	9.02 ± 0.78	—	1.14 ± 0.64† (13)

* Kainic acid (2 μg) was injected with or without L-glutamic acid in the right striatum of 6- or 10-day-old rats. Animals were killed when 15 days old and the cyclic [^{32}P]AMP production due to 10 μM 5-HT was measured in homogenates of their right striatum as described in Materials and Methods. The control 5-HT-sensitive adenylate cyclase activity was determined in striatal homogenates from 15-day-old untreated rats. Each value (in pmoles cyclic[^{32}P]AMP formed per mg protein and per min) is the mean ± S.E.M. of four to seven separate determinations. Figures in parentheses are percentages of respective control values.

† $P < 0.05$ when compared to control values.

old rats resulted in a slight decrease (22 per cent) in 5-HT-sensitive adenylate cyclase as observed on the ninth following day (Table 3). The co-injection of L-glutamic acid significantly improved the neurotoxicity of kainic acid because the decrease in 5-HT-sensitive adenylate cyclase activity thus reached 60 per cent (Table 3). As already reported by Campochiaro and Coyle [15], the effect of kainic acid was markedly age-dependent because the administration of this neurotoxic + L-glutamic acid in 10-day-old rats produced a greater reduction in the cyclase activity (87 per cent) than that observed in rats treated on the sixth postnatal day (Table 3). The examination of the time-course of changes produced by kainic acid + L-glutamic acid injection revealed that the decrease in the 5-HT-sensitive enzymic activity was very rapid. As shown in Fig. 3, the lowest activity level of the 5-HT-sensitive adenylate cyclase was observed as soon as the second day after the treatment. Measurements of the 5-HT-sensitive adenylate cyclase activity on the fifteenth day after the administration of kainic acid + L-glutamic acid in the right striatum indicated that the reduction in this enzymic activity was long-lasting (Table 4). However, the decrease observed on the fifteenth day was regularly less than that seen on the second day following the kainic acid + L-glutamic acid administration (Table 4). In contrast, the basal adenylate cyclase activity in the right striatum was decreased to the same extent (20–25 per cent) on the second (Fig. 4) and the fifteenth day (not shown) after the local administration of kainic acid + L-glutamic acid.

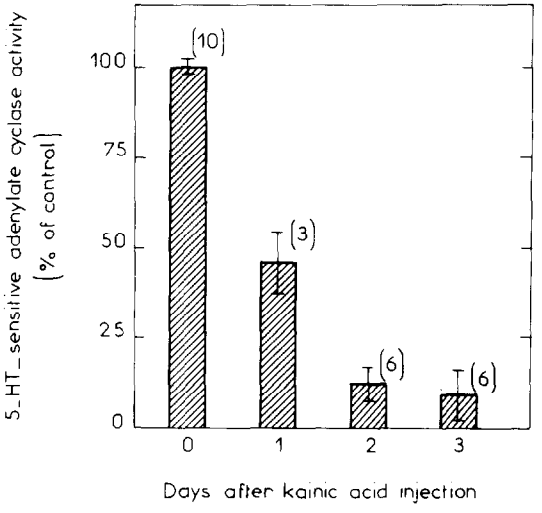

Fig. 3. Time course changes in 5-HT-sensitive adenylate cyclase activity in the striatum after local injection of kainic acid. Kainic acid (2 μg in 0.25 μl of saline saturated with L-glutamic acid) was injected in the right striatum of 10-day-old rats (see Materials and Methods). The animals were killed 1, 2 or 3 days after the injection, and cyclic-[^{32}P]AMP production due to 5-HT (10 μM) was measured in the lesioned striata. Control values were taken from the striata of non-lesioned age-paired rats. Results (means ± S.E.M.) are expressed as per cent of control values. The range for cyclic-[^{32}P]AMP production due to 10 μM 5-HT in the controls was 6.05–12.65 pmoles.min.$^{-1}$.mg protein^{-1}. The numbers in parentheses refer to the number of individual animals at each time point.

Kinetic analyses of the 5-HT-sensitive adenylate cyclase clearly indicated that the reduction produced by kainic acid treatment involved the maximal enzymic activity (Fig. 4). No conclusion could be drawn about the $K_a$app (concentration of 5-HT producing half maximal stimulation) because the activity was too low for its accurate determination.

Because the DA-sensitive adenylate cyclase is considered as a good marker of neuronal degeneration following intrastriatal kainic acid administration (see Ref. 20), it was of interest to measure its activity under the present experimental conditions. As already described in adult rats [20], a marked decrease in the DA-sensitive adenylate cyclase activity was noted in the right striatum of young animals treated with kainic acid + L-glutamic acid. As shown in Fig. 4, this kainic acid-induced effect was due to a marked reduction in the maximal enzymic activity with no change in the $K_a$app (Fig. 4).

The binding of [^{3}H]5-HT was also decreased in the striatum after the injection of kainic acid. This

Table 4. Effects of local injection of kainic acid+L-glutamic acid on 5-HT-sensitive adenylate cyclase and [^{3}H]5-HT binding in the striatum*

Injection	Death	5-HT-Sensitive adenylate cyclase		[^{3}H]5-HT Binding			
				K_d		B_{max}	
		Control	Lesioned	Control	Lesioned	Control	Lesioned
10th day	12th day	9.53 ± 0.74	1.19 ± 0.49† (−87.5%)	2.12 ± 0.12	2.02 ± 0.08	179.1 ± 7.2	127.7 ± 10.6† (−28.7%)
10th day	25th day	8.13 ± 0.45	2.58 ± 0.40† (−68.3%)	2.40 ± 0.62	1.98 ± 0.64	249.1 ± 43.4	145.7 ± 25.1† (−41.5%)

* Kainic acid was injected into the right striatum of 10-day-old rats (see the legend to Fig. 3), and the animals were killed 2 or 15 days later. The 5-HT-sensitive adenylate cyclase activity is expressed as pmoles of [^{32}P]cyclic AMP produced per min and per mg protein in the presence of 10 μM 5-HT. [^{3}H]5-HT binding was measured with 0.40–3.50 nM of the labelled ligand. B_{max} (total number of binding sites calculated from Scatchard plots) is expressed as fmoles bound/mg protein and K_d as nM. Each value represents the mean ± S.E.M. of four to six separate determinations.

† $P < 0.05$ when compared to respective control values. The percentage decrease produced by kainic acid-induced lesion is indicated in parentheses.

change was due to a decrease in the number of binding sites with no change in the apparent affinity of the receptor for its ligand (Table 4). However, the reduction in [^{3}H]5-HT binding did not reach the magnitude of that of the 5-HT-sensitive adenylate cyclase activity. Thus, two days after the injection of kainic acid, this enzymic activity was decreased by 88 per cent whereas [^{3}H]5-HT binding was only reduced by 29 per cent (Table 4). Although the reduction in [^{3}H]5-HT binding increased slightly on the fifteenth day following the administration of the neurotoxin, it was still markedly less than that of the 5-HT-sensitive adenylate cyclase activity (Table 4).

DISCUSSION

We have already presented evidence suggesting that, in the rat CNS, the 5-HT receptor linked to adenylate cyclase is not identical to the high affinity [^{3}H]5-HT binding site [1]. Indeed, no relationship could be found between the pharmacological sensitivities of these two markers of 5-HT receptors. In particular, direct acting 5-HT agonists of the piperazine series displaced [^{3}H]5-HT from its high affinity binding sites, but had no effect on the cyclase, affecting neither the basal nor the 5-HT-stimulated activity [1]. In the present paper, additional facts indicate that the 5-HT receptor linked to adenylate cyclase is different from the [^{3}H]5-HT binding site. However, these two entities could not be distinguished by subcellular fractionation or by their regional distributions in the CNS of newborn rats.

The examination of the subcellular distributions of [^{3}H]5-HT binding and 5-HT-sensitive adenylate cyclase showed, within the limits of the technique used, no significant difference in their localizations suggesting that the membranes containing these two receptors have some similar properties.

Another point of convergence between the 5-HT-sensitive adenylate cyclase and the specific binding of [^{3}H]5-HT concerned their regional distributions in newborn animals. With the exception of the cerebellum which exhibited a surprisingly high capacity to specifically bind [^{3}H]5-HT, there was a good correlation between the levels of 5-HT-sensitive adenylate cyclase activity and the amount of [^{3}H]5-HT binding (Fig. 1). However, except in the striatum, this correlation did not persist during ontogenesis. For example, in the hippocampus and the cerebral cortex, there were large increases in [^{3}H]5-HT binding during development while little change occurred in the activity of the 5-HT-sensitive adenylate cyclase (Table 2).

Because both the 5-HT-sensitive adenylate cyclase and the [^{3}H]5-HT binding sites appear to be postsynaptic [3–6, 21, 22], kainic acid was injected into the striatum of young rats to see if their position could be further localized to intrinsic neurons or to nerve terminals of afferent fibres. Campochiaro and Coyle [15] have previously observed that kainic acid is less efficacious in destroying neurons in the striatum of young as compared to adult rats, presumably due to the lack of glutamatergic corticostriatal innervation in the young animals. This problem was overcome by injecting kainic acid in the presence of L-glutamic acid: as previously shown in adult rats by

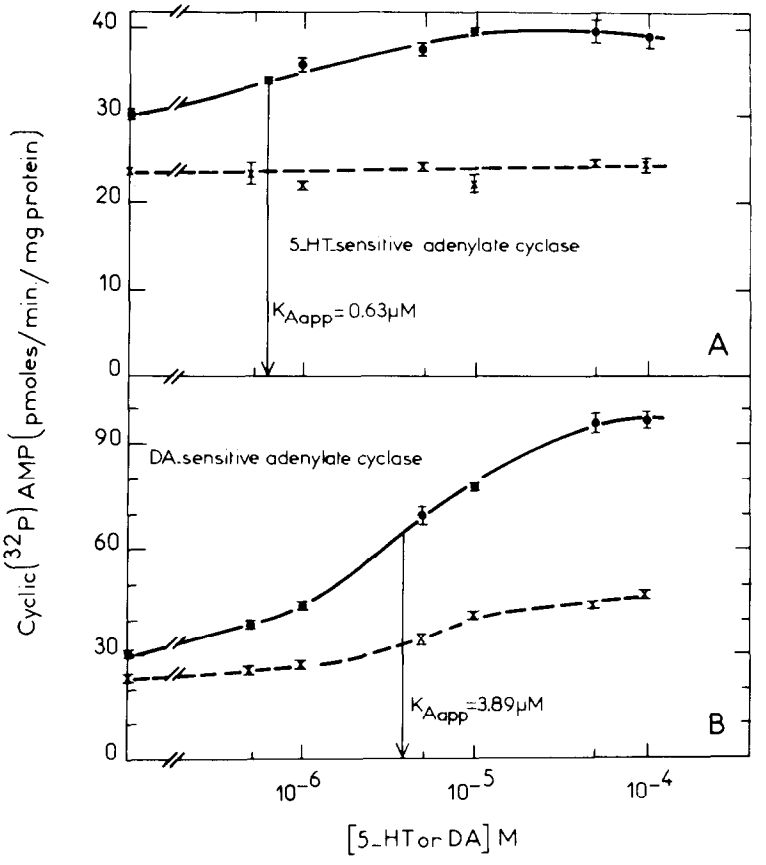

Fig. 4. Effects of kainic acid injection into the striatum on 5-HT- and DA-sensitive adenylate cyclases. Kainic acid was injected into the right striatum of 10-day-old rats as described in the legend to Fig. 3. Animals were killed two days later. 5-HT-sensitive (A) and DA-sensitive (B) adenylate cyclase activities were measured in homogenates of lesioned striata (pooled from 13 rats) or of striata from 12-day-old control animals (7 rats). Each point is the mean ± S.E.M. of triplicate determinations in two independent experiments. $K_a$app corresponds to the concentration of 5-HT (A) or DA (B) producing half maximal stimulation of cyclic-[^{32}P]AMP formation. ●: Control rats; ×: kainic acid-treated rats.

Bizière and Coyle [14], we did observe a marked increment of the neurotoxicity of kainic acid when it was co-injected with L-glutamic acid in young animals. Under these conditions, DA-sensitive adenylate cyclase was decreased by 71 per cent two days after the treatment in 10-day-old animals. This effect closely resembles that previously noted in adult rats as Schwarcz and Coyle [20] mentioned a 85 per cent reduction in striatal DA-sensitive adenylate cyclase activity two days after the local administration of kainic acid.

5-HT-sensitive adenylate cyclase was also destroyed by injection of kainic acid + L-glutamic acid into the striatum of young rats. Very recently, Fillion *et al.* [23] also observed a marked decrease in the stimulating effect of 5-HT on striatal adenylate cyclase activity following the local injection of kainic acid in adult rats. Apparently, this 5-HT-sensitive enzyme is quite different from that presently studied because it was activated by nM concentrations of 5-HT whereas we failed to detect any significant stimulation with 5-HT concentrations lower than 0.1 μM under the experimental conditions presently reported. On the basis of experiments with various subcellular fractions, Fillion *et al.* [24] have proposed that the 5-HT-sensitive adenylate cyclase that is activated by μM concentrations of 5-HT might be localized on glial cells. The present findings strongly suggest that this hypothesis is not correct because the selective neuronal loss produced by kainic acid was associated with a marked reduction in this particular enzymic activity. Indeed, recent experiments using primary cultures of glial cells from colliculi (the brain area containing the highest activity of 5-HT-sensitive adenylate cyclase in newborn rats [2, 3]) confirmed that no 5-HT-sensitive adenylate cyclase was present on glial elements (Bockaert *et al.*, in preparation).

Although the 5-HT-sensitive adenylate cyclase was markedly affected by the intrastriatal injection of kainic acid, [^{3}H]5-HT binding was only decreased by about 29 per cent within two days and by 42 per cent on the fifteenth following day. In both cases, this was due solely to a reduction in the number of binding sites with no change in the K_d. The discrepancy between the respective reductions in [^{3}H]5-HT binding and in 5-HT-sensitive adenylate cyclase activity suggests that these two markers of 5-HT

receptors are not located on the same cells. According to Fillion and coworkers, [^{3}H]5-HT-high affinity binding sites that disappeared after intrastriatal kainic acid administration [23] would be associated with neuronal elements [23, 24] whereas the adenylate cyclase sensitive to μM concentrations of 5-HT which persisted (at least partly [23]) under these conditions would be a glial enzyme [24]. As discussed above, neither of these two markers—at least in young animals—is likely to be associated with glial cells. Accordingly, our findings suggest that most of the (μM) 5-HT-sensitive adenylate cyclase is located on neurons intrinsic to the striatum whereas [^{3}H]5-HT binding sites are preferentially distributed on the terminals of afferent fibres or on intrinsic neurons not destroyed by kainic acid. Previous data obtained in adult rats [25] also led to the conclusion that a great proportion (46 per cent) of [^{3}H]5-HT binding sites are not associated with intrinsic striatal neurons sensitive to the neurotoxic effect of kainic acid.

In conclusion, the 5-HT receptor which is coupled to adenylate cyclase in the rat CNS is not the same as that measured by the binding of [^{3}H]5-HT. This is shown by the differences in: (1) the pharmacological profiles of these receptors [1]; (2) the $K_a$app for the activation of the cyclase and the K_d for [^{3}H]5-HT binding which persist when the enzymic activity and the binding are measured under identical conditions [1]; (3) the developmental patterns of [^{3}H]5-HT binding and 5-HT-sensitive adenylate cyclase during ontogenesis as observed in the hippocampus and the cerebral cortex (this paper); (4) the locations of these two markers of 5-HT receptors in the striatum as revealed by their respective sensitivities to kainic acid (this paper). Thus, as already demonstrated in other tissues [26–28], our results strongly suggest that at least two different types of 5-HT receptors exist in the rat brain. Because neither of these two entities seems to correspond to that labelled with [^{3}H]spiroperidol [10] or to presynaptic autoreceptors [11, 29, 30], it can be concluded that the central effects of 5-HT very likely involve multiple specific receptors.

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