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Rat Brain 5-HT_{1C} Receptors Are Encoded by a 5–6 kbase mRNA Size Class and Are Functionally Expressed in Injected *Xenopus* Oocytes

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Injection of rat brain RNA into *Xenopus laevis* oocytes induces synthesis of receptors that show an electrophysiological response to bath application of serotonin. While there are at least 4 pharmacologically distinct subtypes of 5-HT binding sites in the rat brain, we find that the pharmacological characteristics of the predominant electrophysiologically active receptor synthesized in *Xenopus* oocytes are most consistent with those of the 5-HT_{1C} subtype. Additional electrophysiologically active 5-HT receptor types could not be detected. Injection of mRNA isolated from a number of rat brain regions shows that the choroid plexus is particularly enriched for 5-HT_{1C} mRNA. Oocytes injected with RNA isolated from this region respond 16 or 8 times more strongly to serotonin than do oocytes injected with RNA isolated from cortex or substantia nigra, respectively. In addition, by fractionation of rat brain mRNA through agarose gels, we have identified a single RNA size class of about 5–6 kbase that encodes this serotonin receptor.

Serotonergic systems function to modulate the activity of numerous brain regions and are involved in a large number of physiological, psychological, and developmental processes (Green, 1985). Originally, the central nervous system receptors were divided into 5-HT_1 and 5-HT_2 receptors, with nanomolar and micromolar affinities to 5-HT, respectively (Peroutka and Snyder, 1979). Recently, 3 different kinds of 5-HT_1 binding sites, denoted 1A, 1B, and 1C, have been distinguished by pharmacological criteria in rat and mouse brain (Pedigo et al., 1981; Middlemiss and Fozard, 1983; Pazos et al., 1984; Yagaloff and Hartig, 1985). Only 2 types, resembling the rat 1A and 1C, were found in chicken, turtle, pig, bovine, dog, guinea pig, frog, and human brain (Hoyer, 1985; Heuring et al., 1986).

It has been reported that injection of RNA isolated from rat (Gundersen et al., 1983) or human (Gundersen et al., 1984a) brain into *Xenopus laevis* oocytes results in the synthesis of functional serotonin receptors. Stimulation of these receptors by application of serotonin leads to an increased membrane conductance for chloride ions, which can be recorded electrophysiologically (Gundersen et al., 1983). It has been suggested that this response is mediated by a rise in cytoplasmic IP3 and Ca concentrations (Dascal et al., 1987).

In this report, we have examined the question of whether the observed electrophysiological response to serotonin in *Xenopus* oocytes injected with rat brain mRNA is due to the activation of several 5-HT receptor subtypes or whether a single receptor subtype dominates. We find that the predominant 5-HT receptor that is functional in *Xenopus* oocytes after injection of 14–17 d rat brain mRNA resembles the 5-HT_{1C} class. Fractionation of the mRNA coding for this receptor shows that a single mRNA size class of about 5–6 kbase is sufficient to direct the synthesis of functional receptors.

Materials and Methods

RNA isolation. RNA was isolated from rat brains by the lithium chloride–urea–SDS procedure (Dierks et al., 1981) or by a modification of the procedure of Chirgwin et al. (1979) using guanidine hydrochloride. Poly(A) RNA was isolated by chromatography on oligo(dT) cellulose type III (Collaborative Research) by the usual binding and elution protocol (Maniatis et al., 1982). However, we have found that, in order to obtain the greatest yield of active high-molecular-weight RNA, it is helpful to pretreat the column with an available RNA sample (for example, the poly(A^-) fraction from a previous run) at 1 mg/ml in binding buffer, followed by a wash in elution buffer. Columns are stored in 0.02% sodium azide and never rewashed with NaOH.

RNA injection into oocytes and electrophysiological procedures. RNA injection into *Xenopus* oocytes and electrophysiology were carried out as described (Dascal et al., 1986). After the removal of follicle cells through incubation of the oocytes for 3 hr in Ca^{2+}-free OR-2 solution (Wallace et al., 1973) containing 2 mg/ml collagenase (Sigma, type IA), approximately 50 nl of RNA solution was injected into the cytoplasm using a device similar to that described by Contreras et al. (1981). The oocytes were maintained in ND 96 (Dascal et al., 1986) supplemented with 550 mg/liter Na pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.5 mM theophylline for 2–3 d at room temperature.

A standard 2-electrode voltage clamp (model 8500, Dagan Instruments) in the virtual ground mode was used to test the oocytes in a recording chamber continuously perfused with ND 96. The electrodes were filled with 3 M potassium chloride and had resistances of about 1 MΩ. Holding potential for the response to transmitters and drugs was −70 mV unless otherwise stated, and −80 mV for the voltage-activated sodium channel. Computerized stimulation and analysis procedures have been described (Kegel et al., 1985).

Rat brain dissections. Sprague-Dawley rats were decapitated and the fresh brains dissected at 4°C under a dissecting microscope according to the rat brain atlas by Paxinos and Watson (1982). Cortex, hippocampus, and choroid plexus could be isolated unambiguously. The substantia nigra preparation contained some surrounding tissue.

RNA fractionation. Poly(A) RNA (70 μg) was separated in a 1% ultralow-melting agarose gel (Fourcroy, 1984). Before loading, RNA was heated in 80% formamide, 0.1% SDS, 1 mM EDTA to 65°C for 1 min to dissociate aggregates. The gel buffer was 15 mM sodium phosphate,

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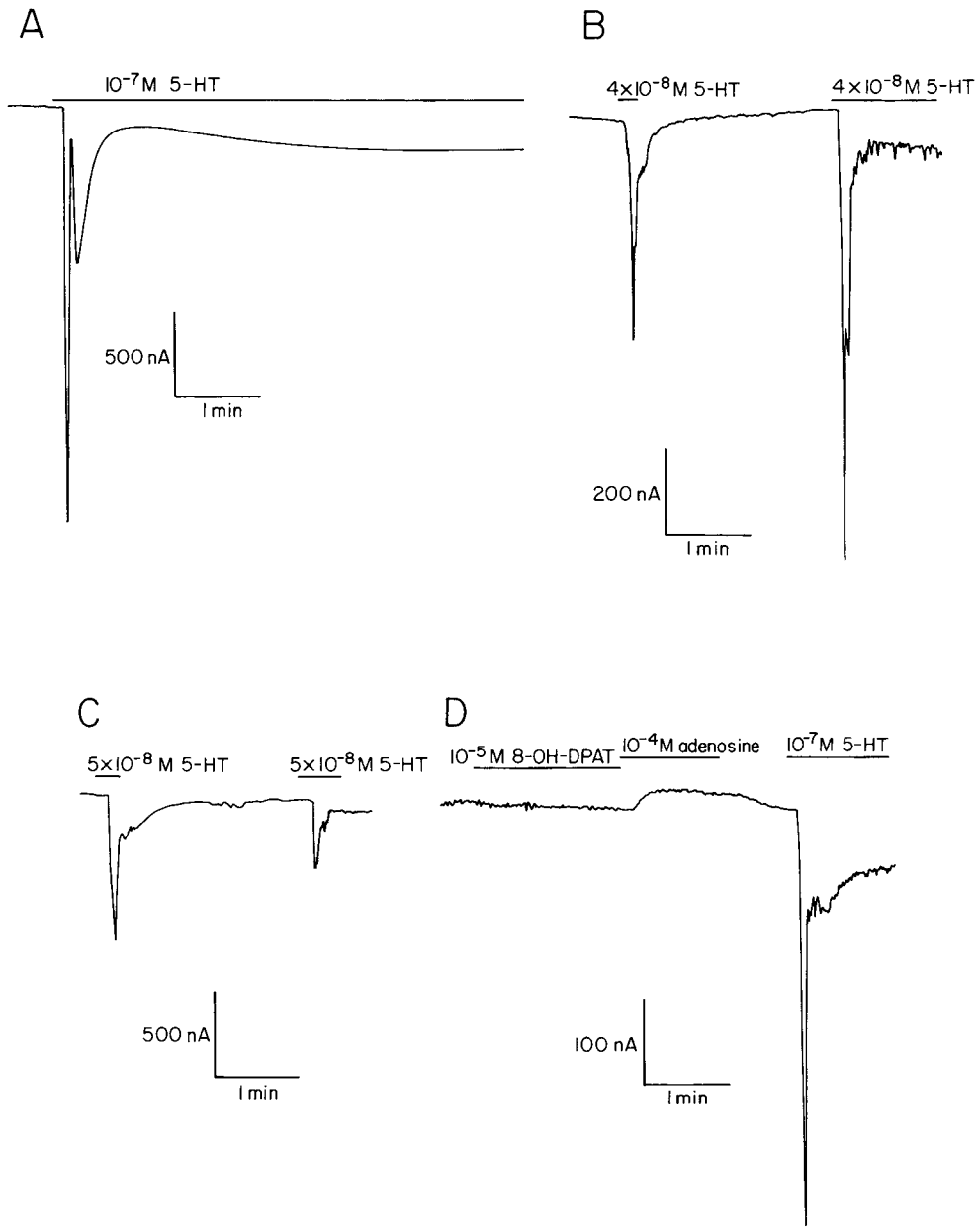

Figure 1. Voltage-clamp currents in *Xenopus* oocytes injected with rat brain mRNA. *Traces* were recorded with a 2-electrode voltage clamp at a holding potential of −70 mV. *Bars* indicate the duration of agonist application. *A,* Serotonin response of a denuded oocyte injected with 50 ng poly(A) RNA. *B,* Effects of sequential application of serotonin in 1 injected oocyte. The denuded oocyte was injected with 10 ng poly(A) RNA in order to obtain a facilitation of the second response. *C,* After injection of 50 ng or more RNA and/or application of higher serotonin concentrations, desensitization was usually observed. *D,* Effect of 8-OH-DPAT on an undenuded oocyte injected with 50 ng poly(A) RNA. No response to 8-OH-DPAT could be observed, although the usual serotonin, as well as the endogenous adenosine response, was present in this oocyte.

15 mM iodoacetic acid, and 1 mM EDTA adjusted to pH 6.5. We found that a preliminary boiling of the agarose in the presence of iodoacetate reduced RNA degradation (Locker, 1979). Electrophoresis was performed at 6.5 V/cm at 4°C for 4 hr.

Following electrophoresis, the gel was cut into 3-mm slices. To each slice was added 3 ml of prewarmed standard binding buffer for oligo-dT cellulose (Maniatis et al., 1982), and the agarose was melted by heating at 65°C for 4 min. After cooling to room temperature, 0.6 ml 5× binding buffer was added and the solution was passed over an oligo-dT cellulose column 3 times. The column was washed and the RNA eluted as described above.

After 2 ethanol precipitations, the RNA from each fraction was dissolved in 100 μl H_2O. One microliter of each fraction was separated on a standard formaldehyde gel and blotted to nitrocellulose, as described by Thomas (1980). One-quarter of each fraction was precipitated and dissolved in 3 μl H_2O, and 50 nl was injected into *Xenopus* oocytes.

Preparation of a poly(dT) probe and hybridization conditions. For the probe preparation, usually 3 pmol of oligo$(dT)_{15}$ was incubated with 100 μCi α-^{32}P-dTTP and 100 units of terminal transferase (Ratliff Biochemicals, La Cueva, NM) under standard buffer conditions at 37°C for 30 min. Then the mixture was adjusted to 1 mM dTTP, 50 units of terminal transferase were added, and the incubation was continued for another 10 min. This resulted in an average length of labeled poly(dT) of about 1000 nucleotides. The reaction mixture was made 0.02 M in EDTA and passed twice over Sephadex G50-spun columns.

Prehybridization and hybridization conditions for this probe were 0.01 M Na acetate, pH 6.5, 0.09 M NaCl, 0.001 M EDTA, 0.1% Na pyrophosphate, 0.2% SDS, and 500 μg/ml heparin at 42°C. Prehybridization was usually performed for 3–4 hr, hybridization for 18–20 hr. The use of heparin as a blocking agent (Singh and Jones, 1984) resulted in the lowest background with this particular probe. The filters were washed twice in 0.05 M NaCl, 0.1% SDS, and twice in 0.025 M NaCl, 0.1% SDS at 42°C, and then autoradiographed on Kodak x-ray films.

This method was designed to analyze the size distribution of poly(A) RNA after electrophoresis and blotting, and to determine the amount of poly(A) RNA on dot blots. On the average, the poly(dT) probe is longer than mRNA poly(A) tails; therefore, every mRNA molecule binds only 1 probe molecule. Thus, the intensity of an autoradiographic signal is expected to be directly proportional to the molar amount of poly(A) RNA. We found that 0.05 fmol poly(A) RNA could easily be detected with this method (data not shown).

Drugs. Bufotenine, cyproheptadine, yohimbine, and 5-methoxytryptamine were obtained from Sigma; quipazine, spiperone, mianserin, and 5-methoxy-*N,N*-dimethyltryptamine from Research Biochemicals (Wayland, MA). Ritanserin was kindly provided by Janssen Pharmaceutica.

Table 1. Dissimilar effects of various amounts of injected mRNA on the serotonin response versus the activity of the voltage-sensitive sodium channel

Poly(A+) RNA per oocyte (ng)	Na current	5-HT response
50	950 ± 200	2000 ± 400
10	100 ± 50	1500 ± 300
2.5	25 ± 10	400 ± 100

Oocytes were injected with the indicated amounts of mRNA. The serotonin response was induced by bath application of 10^{-7} M serotonin, and voltage-sensitive sodium currents were elicited by voltage jumps from a holding potential of −80 mV to a test potential of −20 mV. Values are means ± SE of at least 7 oocytes each.

Results

Affinity to 5-HT of the rat brain serotonin receptor functionally expressed in Xenopus *oocytes*

Serotonin-induced voltage-clamp currents in an oocyte injected with rat brain RNA are shown in Figure 1*A*. Typically, the response consists of 2 main components: an initial, large transient phase followed by a delayed, long-lasting component, which in some oocytes is superimposed with current fluctuations (described in detail by Dascal et al., 1987). An endogenous response to serotonin in control oocytes that were either uninjected or injected with water or yeast RNA (Sigma, type III) was not detected.

Since the amplitude of the initial transient peak reflects the amount of mRNA injected into the oocyte or the serotonin concentration used, this component was always measured in the following study.

In order to study the pharmacology of the serotonin receptors, the optimal amount of injected rat brain mRNA was determined. Table 1 shows that, while the agonist-induced current increased linearly over the range 2.5–10 ng RNA, injection of 50 ng per oocyte did not significantly increase the response. Apparently, saturation of the oocyte's membrane-bound polysome translational capacity did not cause this result, since there was a large increase in voltage-dependent sodium currents after injection of 50 ng RNA. Although other explanations are conceivable, we think it most likely that, since serotonin responses are mediated by second messenger pathways (Peroutka et al., 1981), we were saturating components of those pathways at amounts of RNA greater than about 10–15 ng per oocyte.

In oocytes injected with 10 ng poly(A) RNA, the response to serotonin was half-maximal at $5 \pm 1 \times 10^{-8}$ M and maximal at about 10^{-6} M (Fig. 2). This affinity to serotonin suggests that the functional receptor in the oocytes is of the 5-HT_1 receptor class. However, since 10^{-7} M serotonin induced nearly maximal size signals in oocytes injected with 10 ng poly(A) RNA, it is possible that a small number of lower-affinity 5-HT_2 receptors, if present, could be masked by the 5-HT_1 receptor activation. To address this question, we injected only 2 ng poly(A) RNA into oocytes. In these oocytes, again, no difference was detectable between the response to 10^{-7} and 10^{-5} M serotonin (data not shown). Thus, no functional 5-HT_2 receptor could be detected in the oocytes.

Pharmacological characterization of the receptor

A first application of serotonin to an injected oocyte can either desensitize or facilitate a second response in the same oocyte.

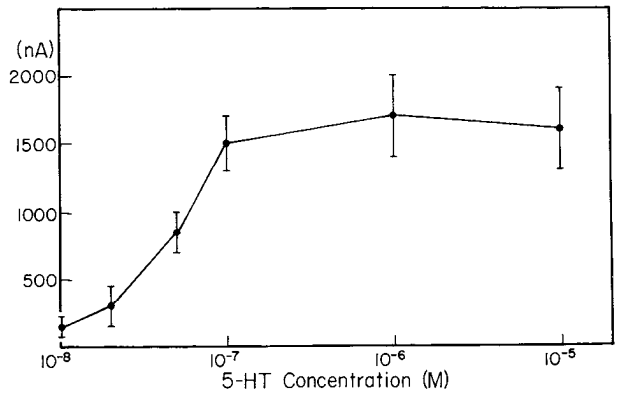

Figure 2. Dose–response relation for serotonin-induced currents in mRNA-injected *Xenopus* oocytes. The oocytes were injected with 10 ng rat brain poly(A) RNA. Serotonin was applied at the indicated concentrations and the effects on membrane conductance were measured. *Values* are means ± SEM from 3 to 6 independent experiments.

As reported recently by Dascal et al. (1987), a first response to 10^{-7} or 10^{-5} M serotonin leads to a decrease in the signal size of a second response in the same oocyte (Fig. 1*C*). We found that oocytes injected with smaller amounts of RNA—10 ng or less—displayed increased agonist-induced currents during a second response if the serotonin concentration used was smaller than 5×10^{-8} M (Fig. 1*B*).

The second response is often twice as large as the first response. The differences between oocytes that show desensitization or facilitation could not solely be attributed to the absolute signal size, but were also correlated with the amount of injected rat brain poly(A) RNA, which suggests that a rat brain gene product is involved in the desensitization process. Significantly, desensitization was not observed in oocytes injected with RNA isolated from choroid plexus or with gel-fractionated RNA (see below).

The presence of facilitation and desensitization, coupled with the variation in responses among identically injected oocytes, limits the extent and precision of dose–response studies. For these reasons, for every concentration of each drug several oocytes were tested using the protocol described in the legend to Table 2.

Table 2 shows the observed affinities of the antagonists examined. Affinities of these drugs to every receptor class determined by other authors in membrane-binding studies (Leysen et al., 1981; Hoyer, 1985; Peroutka, 1986), or by measurements of the increase in the cytoplasmic inositol 1-phosphate concentration after activation of 5-HT_{1C} receptors (Conn et al., 1986) are shown for comparison.

As is described in detail in the legend to Table 2, we adjusted the antagonist concentrations to cause roughly 50% inhibition of the response to serotonin. Apparent K_i values were then calculated from observed IC_{50} values using the equation $K_i = IC_{50}/(1 + [5\text{-HT}]/EC_{50})$, where [5-HT] is the serotonin concentration used to determine the IC_{50}, and EC_{50} is the concentration of serotonin producing half-maximal stimulation (50 nM). The use of this equation (Cheng and Prusoff, 1973) assumes that the EC_{50} of serotonin roughly equals its K_i.

5-HT_{1A} receptors have nanomolar affinities to 8-OH-DPAT and spiperone. The agonist 8-OH-DPAT had no effect at the highest concentration used (10^{-5} M), and the apparent K_i of the antagonist spiperone was only about 4 μM (Table 2). This indicated that the receptor seen in the oocytes was not of the

Table 2. Affinity of serotonin antagonists for the receptor expressed in oocytes

Drug	Apparent K_i in oocytes (nM)	5-HT$_{1A}$[a]	5-HT$_{1B}$[a]	5-HT$_{1C}$[a]	5-HT$_{1C}$[b]
Spiperone	4000 ± 1000	130	47,000	4800	6200
Mesulergine	250 ± 100	630	12,600	2.5	–
Mianserin	40 ± 15	800	10,000	110	12
Ketanserin	35 ± 20	>10,000	>10,000	–	130
Ritanserin	30 ± 20	–	–	–	–
Cyproheptadine	300 ± 100	110	840	2600	–
Yohimbine	>7000	–	–	–	–

To determine the effect of a drug, a response to 2×10^{-8} M serotonin was recorded in oocytes injected with 10 ng poly(A) RNA isolated from rat brains without the cerebellum, or with 20 ng total RNA isolated from choroid plexus. Immediately after the transient phase of the response, serotonin was washed out and a drug was applied for 2 min. Serotonin was then applied in the presence of the drug. The induced current was compared to the first response. The amplitude ratio for 2 sequential responses without the action of an antagonist was determined separately in several oocytes. Drug concentrations were adjusted to give roughly 50% inhibition of the second stimulation. Apparent K_i values were then estimated from these IC_{50} values, adapting the Cheng-Prusoff (1973) equation, $K_i = IC_{50}/(1 + [5\text{-HT}]/EC_{50})$, where [5-HT] is the serotonin concentration used and EC_{50} the concentration of serotonin producing half-maximal stimulation (= 50 nM). Each value is the mean ± SEM of at least 3 independent determinations. If available, the affinities of the antagonists to 5-HT$_1$ receptors found by other authors are shown for comparison. These data were derived from membrane-binding experiments (*a*) (Leysen et al., 1981; Hoyer, 1985; Peroutka, 1986) or by a determination of antagonist effects on the serotonin-stimulated phosphoinositol (PI) turnover in rat choroid plexus (*b*) (Conn et al., 1986).

Table 3. Relative amounts of effective mRNAs in various brain regions

Brain region	Na current	5-HT (2×10^{-8} M)	ACh (10^{-5} M)
Cortex	500 ± 150	120 ± 50	390 ± 100
Substantia nigra	430 ± 100	230 ± 60	350 ± 100
Choroid plexus	5 ± 2	1900 ± 300	0

Total RNA was isolated from cortex, substantia nigra, and choroid plexus and 100 ng was injected per oocyte. The voltage-activated sodium current and the responses to serotonin (2×10^{-8} M) and acetylcholine (10^{-5} M) were tested. The low serotonin concentration was chosen to avoid saturation of the response. The data presented are mean values ± SE of at least 10 oocytes each.

5-HT$_{1A}$ class. The 5-HT$_{1A}$ receptor may be coupled to adenylate cyclase in the vertebrate brain (Fillion et al., 1979; Schenker et al., 1985). *Xenopus* oocytes have an endogenous purinergic response, which is mediated by cAMP and acts on a K channel (Dascal et al., 1985). This endogenous response is usually destroyed during collagenase treatment of the oocytes. To optimize the chance for the expression of functional 5-HT$_{1A}$ receptors, we injected poly(A) RNA isolated from hippocampi of 16-d-old rats into oocytes that had not been subject to the defolliculation procedure. Hippocampus is the highest source of 5-HT$_{1A}$ receptors (Pazos and Palacios, 1985). We applied 10^{-5} M 8-OH-DPAT in order to test whether a 5-HT$_{1A}$ receptor would activate a K^+ channel in these follicles (Fig. 1*D*). Again, we did not find a response to 8-OH-DPAT, although the expected outward current induced by 10^{-5} M adenosine, as well as the usual serotonin-induced inward current, was indeed present.

Only 5-HT$_{1C}$ and 5-HT$_2$ receptors have high affinities for mesulergine, mianserin, ritanserin, and ketanserin. We found relatively high affinities for all these drugs. 5-HT$_2$ receptors have a micromolar affinity to 5-HT and a nanomolar affinity to spiperone (Peroutka and Snyder, 1979), neither of which was the case for the receptor expressed in the oocytes (see above). Therefore, the pharmacological profile of the functional receptors in the oocytes is most consistent with the 5-HT$_{1C}$ receptor type. The K_i of mesulergine was determined to be 250 nM, which is higher than that described previously for the rat brain 5-HT$_{1C}$ receptor, but lower than that described for the 1A or the 1B site. In contrast to Peroutka (1986), we found a relatively high affinity of the receptor to cyproheptadine (300 nM). Yohimbine had no effect on the oocyte's serotonin response at the highest concentration tested (10^{-5} M).

We also tested several serotonin agonists on the receptor expressed in the oocytes. Of the agonists tested, only 5-methoxytryptamine activated the receptor efficiently at low concentrations. The response was half-maximal at 80 ± 20 nM. Agonists without the free amino group, such as TFMPP (*m*-trifluoromethylphenyl piperazine), RU24969, bufotenine, quipazine, and 5-methoxy-*N,N*-dimethyltryptamine (5-OMeDMT) induced rather small currents at low concentrations, although some of them have been shown to have relatively high affinities to the 5-HT$_{1C}$ receptor in membrane-binding experiments (Peroutka, 1986). However, to various extents they stimulated signals with the typical waveform of the serotonin response at concentrations of 10^{-5} M or higher.

Effects of RNAs isolated from regions of the rat brain

It has been shown that several rat brain regions are enriched in certain receptor types (Pazos and Palacios, 1985). We were interested in 3 specific regions: cortex, substantia nigra, and the choroid plexus of the fourth ventricle. On the basis of autoradiography of brain slices, Pazos and Palacios (1985) reported that the cortex has about 1100–1300 fmol 5-HT$_1$ receptor per milligram of membrane-bound protein. The relative proportions of 1A, 1B, and 1C receptors are roughly 1:1:1. Substantia nigra has 2750 fmol receptor per milligram protein, with a distribution of about 1:3:0.5. Choroid plexus is the richest source for the 5-HT$_{1C}$ receptor, with 4265 fmol receptor per milligram protein and a distribution of 1:4.5:13.5 (Pazos and Palacios, 1985). Yagaloff and Hartig (1985, 1986) reported that the 5-HT$_{1C}$ site was the only identified serotonin-binding site in choroid plexus. We isolated RNA from each of these regions from 16 d-old-rats for injection into oocytes. At this age serotonin receptors accumulate in most brain regions (Zilles et al., 1985, 1986).

After a 2-d incubation, the oocytes were tested for the presence of voltage-activated sodium channels, serotonin, and muscarinic acetylcholine receptors. Since voltage-dependent sodium channels are encoded by very large RNAs (Goldin et al., 1986; Noda et al., 1986), we generally used sodium currents as an indicator for the quality of an RNA preparation. By injection of 50 ng total RNA isolated from cortex and substantia nigra, we observed voltage-activated sodium currents of 500 and 430 nA, respectively. On the basis of previous experience in our laboratory, this indicated that the RNA was not degraded. We observed very small sodium currents with the choroid plexus RNA, consistent with the presumed physiology of this predominantly epithelial and vascular tissue.

As shown in Table 3, oocytes injected with RNA from cortex, substantia nigra, and choroid plexus had serotonin responses with a ratio of 1:1.9:16. According to the data of Pazos and Palacios (1985) mentioned above, the expected ratio for a

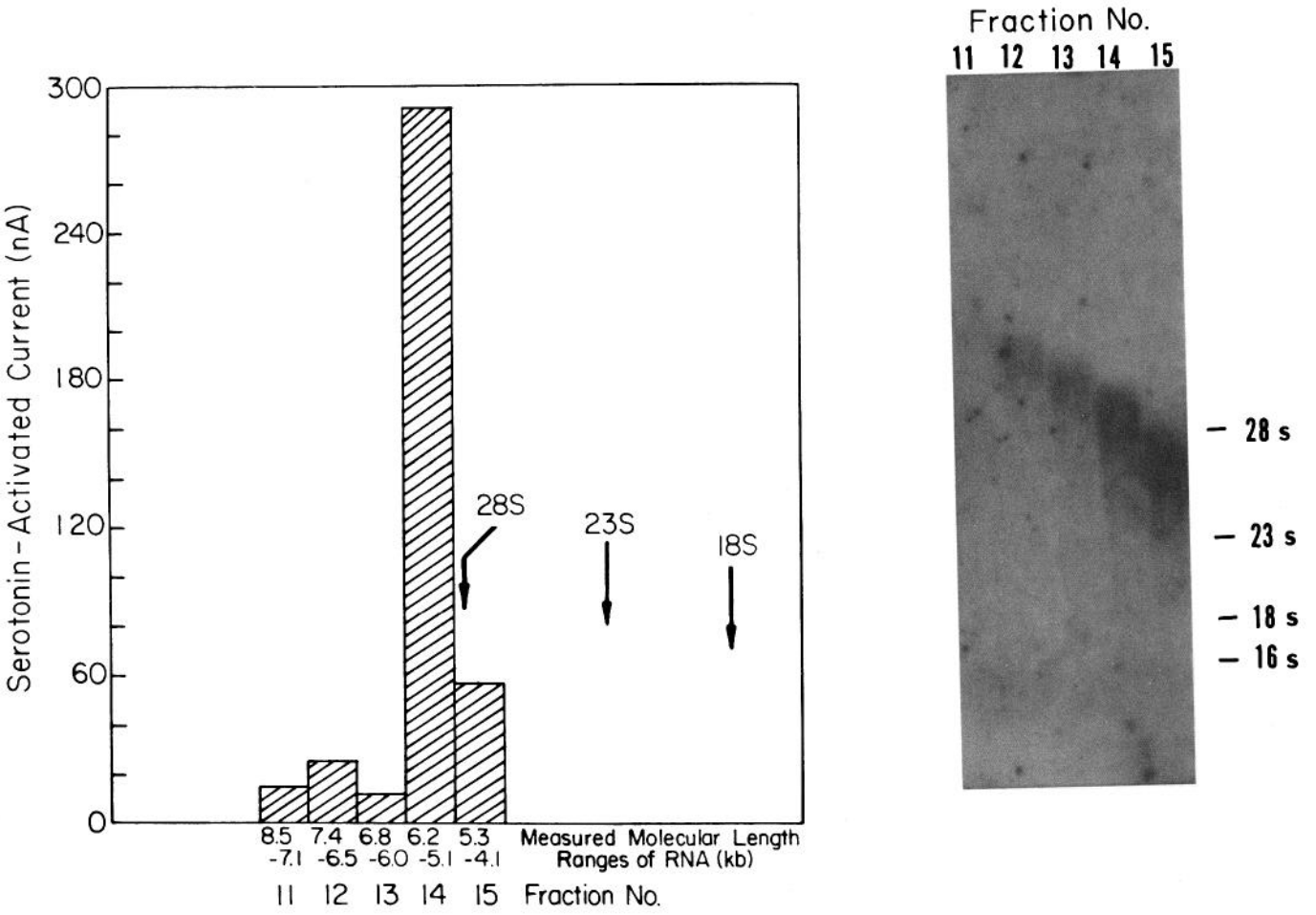

Figure 3. Serotonin response in oocytes injected with size-fractionated poly(A) RNA. *Left,* RNA was size-fractionated by gel electrophoresis as described. RNA from each fraction was injected individually into *Xenopus* oocytes and the amplitude of the response to application of 10^{-5} M serotonin was measured at a holding potential of −80 mV. *Right,* RNA from several fractions was separated on an agarose gel containing formaldehyde, blotted to nitrocellulose, and hybridized with a ^{32}P-labeled poly(dT) probe to localize poly(A) RNA.

5-HT_{1A} receptor would be 1:1.5:0.6; for 1B, 1:5:2.5; and for 1C, 1:0.5:7.5. Clearly, our comparison of the 3 RNAs correlated well with the expected ratio for a 1C receptor, confirming our proposed interpretation of the pharmacological data. Using oocytes injected with RNA isolated from choroid plexus, we determined the K_is of the antagonists listed in Table 2 and obtained results very similar to those with RNA from whole brain.

RNA size fractionation

In order to study the specific RNA(s) coding for the rat brain serotonin receptor, we size fractioned poly(A) RNA by gel electrophoresis, recovered the RNA from individual gel fractions, and injected it into *Xenopus* oocytes (see Materials and Methods). The sizes of RNA in each fraction were determined by electrophoresing a portion of each sample through a denaturing gel, transferring the RNA to nitrocellulose, and then hybridizing it with a ^{32}P-labeled poly(dT) probe. Figure 3 shows the results of injecting each oocyte with about 0.4% of the RNA from each fraction, and testing for the serotonin response. It is apparent that while the serotonin sensitivity was correlated with several fractions, the majority of the response was found in RNA with a length distribution of between 5 and 6 kbase. Pooling of all fractions from across the gel showed that the 5–6 kbase RNA size class was sufficient to give the maximal serotonin response; other RNAs did not augment the response.

Discussion

Serotonin-induced currents in *Xenopus* oocytes injected with rat brain RNA have been described by Gundersen et al. (1983) and Dascal et al. (1987). Their results suggest that the serotonin receptor activates a GTP-binding protein, possibly coupled to a phosphatidylinositol pathway, which then acts to open a Ca-dependent chloride channel. These conclusions are based partly on the observed interaction of the muscarinic acetylcholine response and the serotonin response, as well as on the effects of injecting EGTA, IP3, cAMP, and Ca (Dascal et al., 1987). Both receptors appear to use the same second messenger pathway and to act on the same channels. These common components seem to be endogenous to the oocyte.

In this study we have characterized this serotonin receptor and the mRNA coding for it. Our data suggest that the major response seen in the oocytes is initiated by a 5-HT_{1C} receptor. Although our data are most consistent with the 5-HT_{1C} site, there are certain differences in the pharmacological profiles. These variations may be due to the difficulties involved in reconciling data obtained by receptor binding and by physiology. Furthermore, the oocyte membrane environment or oocyte-specific posttranslational modifications may alter receptor properties.

We were unable to detect any other active serotonin binding site, although we cannot rule out the possibility that a minor component of the oocyte's response to serotonin might be due to other receptor types.

Gundersen et al. (1984b) previously tested the effects of some antagonists on the oocyte serotonin response. Their data are consistent with ours. Some apparent discrepancies are due to the fact that their measurements were made at 5-HT and drug concentrations that do not permit straightforward inferences as to the relative K_is of the drugs.

We do not know why the 5-HT_{1C} receptor is the only electrophysiologically active rat brain serotonin receptor in the oocytes. Presumably, the RNA for every receptor type will be translated. Brain-specific posttranslational modifications may be necessary to transform the receptor to its active configuration. An alternative possibility is that only some receptors are able to couple to one of the oocyte's second messenger systems. In addition to the serotonin and the muscarinic acetylcholine receptor, several rat brain receptors, for example those for glutamate and noradrenalin, elicit the same type of electrophysiological response (Gundersen et al., 1984b; Sumikawa et al., 1984). Thus, if indeed the serotonin response observed here is mediated by an oocyte phosphoinositol (PI) pathway, the same may be true for these other neurotransmitters. On the other hand, 5-HT_2 receptors are believed to be coupled to IP3 turnover in rat cortex (Conn and Sanders-Bush, 1985) and we were unable to detect a response stimulated by this receptor subtype. It is also of interest that oocytes injected with mRNA isolated from chicken brain do not respond to serotonin (unpublished observations), although it has been shown that chicken has 5-HT_{1C} receptors (Heuring et al., 1986).

To our knowledge, there is no example in the literature of an exogenous receptor expressed in the oocytes that has been observed to be coupled to the oocyte's adenylate cyclase system and to act on the K channel linked to it. Either the rat brain receptors cannot couple or larger amounts of receptor are necessary to activate this pathway than are supplied by the RNA preparations.

It is clear from these several observations that a full picture of the second messenger pathways and coupling mechanisms operative in the responses of injected oocytes is not yet at hand.

It has been shown that the 5-HT_{1C} receptor of rat and pig choroid plexus is coupled to a phosphoinositol pathway (Conn et al., 1986; Hoffman et al., 1986). In excellent agreement with our results, these authors obtained half-maximal stimulation of PI turnover with 46 and 49 nM serotonin, respectively. As noted in Results (Table 2), their observed antagonist effects are consistent with those obtained here.

We found that a single RNA size fraction of about 5–6 kbase is sufficient to generate a serotonin response in the oocytes. This suggests, but obviously does not prove, that only 1 protein component is necessary. The molecular weight of this component can be expected to be somewhere between 120 and 160 kDa.

We assume that the active RNA fraction codes for the serotonin receptor and not for a component of the second messenger system or the chloride channel. Serotonin and acetylcholine receptors are, as mentioned above, possibly coupled to the same G proteins (Lübbert et al., 1985; Dascal et al., 1987) and the same mediators for their responses. Enhancement of a single, limiting step of signal processing would therefore lead to an increase in both responses. The results obtained with the RNA preparations from cortex and choroid plexus show very clearly that the serotonin and acetylcholine responses are independent. In addition, RNA fractionation experiments show that the acetylcholine receptor is encoded by RNA(s) with lower molecular weight than that of the RNA coding for the serotonin receptor (unpublished observations). We cannot rule out the rather unlikely possibility that the RNA component selectively stimulates the synthesis of a frog serotonin receptor. However, we could not detect an endogenous response to serotonin in the controls of the oocytes used in this study.

In conclusion, the present results show that rat brain 5-HT_{1C} receptors appear to be responsible for the serotonin-induced currents in *Xenopus* oocytes injected with rat brain RNA. A single RNA size class is sufficient for the synthesis of the receptor.

Note added during revision. Heuring and Peroutka (1986) have reported the characterization of a new 5-HT_1 receptor subtype. This 5-HT_{1D} receptor is pharmacologically different from the receptors expressed in oocytes.

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