Biochem. J. (1990) 269, 623–628 (Printed in Great Britain)

Molecular size of the 5-HT_3 receptor solubilized from NCB 20 cells

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The 5-HT_3 hydroxytryptamine receptor from NCB 20 cells was solubilized and the molecular and hydrodynamic properties of the receptor were investigated. The receptor was identified by binding of the radioligand 3-*NN'*-[^{3}H]dimethyl-8-azabicyclo[3.2.1]octanyl indol-3-yl carboxylate ester ([^{3}H]Q ICS 205-930) to NCB 20 membranes ($B_{max.}$ = 1.19 ± 0.31 pmol/mg of protein; K_d = 0.43 ± 0.076 nM) and was optimally solubilized with 0.5% deoxycholate. [^{3}H]Q ICS 205-930 labelled one population of sites in solution ($B_{max.}$ = 1.11 ± 0.4 pmol/mg of protein; K_d = 0.48 ± 0.06 nM; n = 4). The characteristics of [^{3}H]Q ICS 205-930 binding were essentially unchanged by solubilization, and competition for [^{3}H]Q ICS 205-930 binding by a series of 5-HT_3 agonists and antagonists was consistent with binding to a 5-HT_3 receptor site and was similar to that observed for 5-HT_3 receptors solubilized from rat brain [McKernan, Quirk, Jackson & Ragan (1990) J. Neurochem. **54**, 924–930]. Some physical properties of the solubilized receptor were investigated. The molecular size (Stokes radius) of the [^{3}H]Q ICS 205-930-binding site was measured by gel-exclusion chromatography in a buffer containing 0.2% Lubrol and 0.5 M-NaCl and was determined as 4.81 ± 0.15 nm (mean ± S.E.M.; n = 6). Sucrose-density-gradient centrifugation was also performed under the same detergent and salt conditions to determine the partial specific volume ($\bar{v}$) of the detergent–receptor site complex. This was found to be 0.794 ml·g^{-1}. Sucrose-density-gradient centrifugation was carried out in both ^1H_2O and ^2H_2O to allow correction for detergent binding to the receptor. The M_r of the 5-HT_3 receptor under these conditions was calculated as 249000 ± 18000 (n = 3). The size and physical properties of the 5-HT_3 receptor are similar to those observed for members of the family of ligand-gated ion channels.

INTRODUCTION

5-Hydroxytryptamine (5-HT) receptors have been separated into three main classes (Bradley *et al.*, 1986; Richardson & Engel, 1986; Peroutka, 1988*a*) most of which are linked to G-(guanine-nucleotide-binding) proteins. However, the 5-HT_3 subtype, whose mechanism of action has only relatively recently been demonstrated (Derkach *et al.*, 1989; Suprenant & Crist, 1988; Yakel & Jackson, 1988) is an exception. This receptor functions as a ligand-gated ion channel which is permeable to Na^+ and K^+ (Derkach *et al.*, 1989; Peters & Lambert, 1989) and as such is, as far as we are aware, the first mammalian monoamine neurotransmitter receptor of this type. Other monoamine receptors, with the exception of the invertebrate histamine receptor, which directly gates a chloride channel (McClintock & Ache, 1989), activate G proteins (for a review, see Strange, 1988). The 5-HT_3 receptor may be anticipated therefore to resemble other better-characterized ligand-gated ion channels, such as the glycine receptor (Pfeiffer *et al.*, 1982), the $GABA_A$ receptor (Stephenson, 1988) or the nicotinic acetylcholine receptor (Conti-Tronconi & Raftery, 1982; Whiting & Lindstrom, 1986). Although the binding site of this receptor has been identified with several radioligands (Kilpatrick *et al.*, 1987; Barnes *et al.*, 1988; Peroutka & Hamik, 1988; Watling *et al.*, 1988), little is known about its molecular size or properties. The 5-HT_3 receptor is widely distributed in the periphery, where it mediates neurotransmitter release (Richardson & Engel, 1986) and centrally, where it is reported to mediate emesis (Fozard, 1987; Higgins *et al.*, 1989) and anxiety (Jones *et al.*, 1988; Papp, 1988).

However, the density of binding sites in the rat brain is low (Kilpatrick *et al.*, 1987; Milburn & Peroutka, 1988; Peroutka, 1988*b*; Watling *et al.*, 1988; McKernan *et al.*, 1990), and an enriched source of receptor would provide a better starting material for biochemical studies. Radioligand binding (Hoyer & Neijt, 1988; Neijt *et al.*, 1988) and electrophysiological studies (Yakel & Jackson, 1988; Peters & Lambert, 1989) have shown that NG 108-15 and NIE 115 cell lines also express 5-HT_3 receptors. However, the reported densities on these cell lines are not much greater than those observed in rat brain membranes (Hoyer & Neijt, 1988; Neijt *et al.*, 1988). Electrophysiological responses mediated through 5-HT_3 receptors have also been recorded in another cell line, NCB 20 (Lambert *et al.*, 1989). We therefore investigated the density of receptors expressed on this cell line using the radioligand 3-*NN'*-[^{3}H]dimethyl-8-azabicyclo [3.2.1.]octanyl indol-3-yl carboxylate ester ([^{3}H]Q ICS 205-930) and have used this as an enriched source of receptor in which to study its molecular properties. The 5-HT_3 receptor has been successfully solubilized from rat brain membranes (McKernan *et al.*, 1990) and we have used a similar methodology for solubilizing the 5-HT_3 receptor from NCB 20 cells.

EXPERIMENTAL

Materials

[^{3}H]Quaternized ICS 205-930 ([^{3}H]Q ICS 205-930) was prepared by methylation of the *N*-desmethyl precursor using [^{3}H]methyl iodide (Amersham International; sp. radioactivity

Abbreviations used: 5-HT, 5-hydroxytryptamine; [^{3}H]Q ICS 205-930, 3-*NN'*-[^{3}H]dimethyl-8-azabicyclo[3.2.1]octanyl indol-3-yl carboxylate ester; L-680,652, [2'-(1-methyl-1*H*-indol-3-yl)]spiro(1-azabicyclo[2.2.2]octane-3-5'(4'*H*)-oxazole dihydrochloride hydrate; GR 38032F, 1,2,3,9-tetrahydro-*g*-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4-one; PMSF, phenylmethanesulphonyl fluoride; GABA, γ-aminobutyric acid; G protein, guanine-nucleotide-binding protein.

55 Ci/mmol). Sodium deoxycholate was from Calbiochem, M_r markers were from Pharmacia and ^2H_2O was from Aldrich. Desalting columns (PD-10) and M_r markers were from Pharmacia. Drugs were from the following sources: MDL 72222 from Research Biologicals, quipazine from Miles Laboratories, BRL 24924 from Beechams Pharmaceuticals, 5-hydroxytryptamine (5-HT) from Sigma, and ketanserin from Janssen, ICS 205-930, Q ICS 205-930, L680,652 and 2-methyl-5-HT were synthesized in the Chemistry Department at Merck, Sharp and Dohme Research laboratories (1,2,3,9-tetrahydro-*g*-methyl-3-[(2-methyl-1*H*-imidazozol-1-yl)methyl]-4*H*-carbazol-4-one (GR 38032 F) was from Glaxo. All other materials were from Sigma.

Cell culture

NCB 20 cells (Minna *et al.*, 1975) were grown in Dulbecco's modified Eagle's medium containing Hepes (25 mM), the antibiotics penicillin (100 i.u. $\cdot$ ml^{-1}) and streptomycin (100 μg $\cdot$ ml^{-1}), glutamine (2 mM) and foetal bovine serum (9%). Cells were grown in flat Falcon 850 ml flasks and were subcultured every 2 or 3 days. Cells were used from passage 39–60.

Membrane preparation

Cells were harvested by scraping and were centrifuged at 100 *g* for 5 min. The supernatant medium was discarded and the pellet resuspended in 5 mM-Tris/HCl/1 mM-EDTA, pH 7.5, at 4 °C. The preparation was homogenized using a Semat Ultra-Turrax homogenizer (3 × 5 s bursts at setting 5), and then centrifuged at 48000 *g* for 20 min at 4 °C. The pellets were washed twice in the same buffer by centrifugation and resuspension and were stored as pellets at −20 °C for up to 4 weeks with no detectable loss in binding activity.

Preparation of solubilized receptor

5-HT$_3$ receptors were solubilized from NCB 20 cells using the method described for the solubilization of 5-HT$_3$ receptors from rat brain (McKernan *et al.*, 1990). Briefly NCB-20-cell membranes (2–5 mg of protein/ml) in 10 mM-Tris/HCl/1 mM-EDTA/10% (v/v) glycerol/proteinase inhibitors [phenylmethanesulphonyl fluoride (PMSF) 0.1 mM, soybean trypsin inhibitor (10 μg $\cdot$ ml^{-1}), chymostatin (10 μg $\cdot$ ml^{-1}) and bacitracin (10 μg $\cdot$ ml^{-1})]/NaN_3 (10 μg $\cdot$ ml^{-1}) at pH 7.5 and 4 °C were solubilized by the dropwise addition of deoxycholate to a final concentration of 0.5%. After homogenization for 2 × 5 s, using the Semat Ultra-Turrax homogenizer (setting 5) the preparation was centrifuged at 200000*g* for 1 h to pellet any remaining particulate material. The supernatant was immediately exchanged by gel filtration into buffer (10 mM-Tris/HCl/1 mM-EDTA/10% glycerol/0.1% Triton X-100, pH 7.5 at 4 °C) because the receptors were not stable in deoxycholate (up to 60% of receptor activity was lost after incubation overnight at 4 °C in 0.5% deoxycholate). 5-HT$_3$ receptors were detected by radioligand binding with [^{3}H]Q ICS 205-930.

Binding of [^{3}H]Q ICS 205-930 to soluble and membrane preparations

Membrane binding was performed in a total volume of 0.5 ml containing 50–100 μg of membrane protein in 5 mM-Tris/HCl/1 mM-EDTA, pH 7.5 at 4 °C, and 0.10–5 nM-[^{3}H]Q ICS 205-930. Non-specific binding was defined as that not displaced by 10^{-5}M-[2′-(1-methyl-1*H*-indol-3-yl)]spiro(1-azabicyclo[2.2.2]octane-3-5′(4′*H*)-oxadiazole dihydrochloride hydrate (L-680,652) or 10^{-5}M-quipazine, which gave identical results. Binding was carried out for 30 min at 4 °C and the incubations were terminated by filtration through GF/B filters followed by two 3 ml washes with ice-cold 5 mM-Tris/HCl/1 mM-EDTA pH 7.5.

Binding to solubilized receptors was usually carried out in a total volume of 0.5 ml, containing a maximum of 50 μl of solubilized receptor preparation, with the exception that fractions from gel-filtration chromatography were assayed in a total volume of 0.6 ml. Most detergents except Lubrol inhibit binding of [^{3}H]Q ICS 205-930 to solubilized receptors (McKernan *et al.*, 1990). However, it was found that Triton X-100 at less than 0.01% had a negligible effect on binding, and hence up to 50 μl of soluble receptor could be added to the binding assay. Binding was carried out at 4 °C for 30 min and the incubations were terminated by filtration through GF/B filters which had been presoaked for at least 3 h in 1% polyethyleneimine. Non-specific binding at a K_d concentration of ligand was generally less than 10% under these conditions.

Gel-exclusion chromatography

Gel-exclusion chromatography was carried out on a Superose 6 column (1 cm × 31 cm) using a Pharmacia f.p.l.c. system. The column was equilibrated with 60–100 ml of buffer (10 mM-Tris/HCl/1 mM-EDTA/10% glycerol/0.2% Lubrol/500 mM-NaCl, pH 7.5 at 4 °C). Gel-exclusion chromatography was carried out in Lubrol because this detergent was the least inhibitory to receptor binding (McKernan *et al.*, 1990). NaCl, (500 mM) was included because this has been shown to prevent aggregation of receptor protein (Mamalaki *et al.*, 1989). Solubilized receptor was exchanged into Triton buffer (0.1%) before application on to the column, since in preliminary experiments this was observed to reduce the amount of protein eluted in the void volume. A volume of 200 μl was loaded on to the column and 1 ml fractions were collected at a constant flow rate of 0.2 ml/min at room temperature. Aliquots (500 μl) of each fraction were assayed for [^{3}H]Q ICS 205-930 binding.

The column was calibrated with the marker proteins RNAase A (Stokes radius, a, = 1.64 nm), chymotrypsinogen A (a = 2.09 nm), haemoglobin (a = 3.4 nm), catalase (a = 5.2 nm) and ferritin (a = 6.1 nm). Blue Dextran 2000 and [^{3}H]inositol were used to measure the void volume and the total included volume of the column respectively.

Sucrose-density-gradient centrifugation

Discontinuous sucrose gradients (20 ml) were prepared by layering 5 ml each of 20%, 15%, 10% and 5% (w/v) sucrose all in buffer (5 mM-Tris/HCl/1 mM-EDTA/500 mM-NaCl/0.2% Lubrol, pH 7.5 at 4 °C). The gradients were equilibrated at 4 °C for 4 h until they became continuous (Martin & Ames, 1961). The linearity of the gradients was verified by removing and weighing sequential 500 μl volumes. Receptor or marker proteins of known sedimentation coefficient were layered on top of the gradient in 1 ml of Lubrol buffer as described above and the gradients were centrifuged for 17 h at 90400 *g* in a Beckman SW 28 rotor. After centrifugation, 0.5 ml fractions were collected from the bottom of the gradient and were assayed for [^{3}H]Q ICS 205-930 binding. Sucrose gradients were calibrated with the following marker proteins ($s_{20,w}$): ferritin, (15.9 S), catalase (11.3 S), alcohol dehydrogenase (7.4 S), bovine serum albumin (4.9 S), haemoglobin (4.5 S).

RESULTS

Binding of [^{3}H]Q ICS 205-930 to membrane and solubilized receptor sites

The 5-HT$_3$ receptor has been identified in rat brain using [^{3}H]Q ICS 205-930, and this site has been solubilized by using the

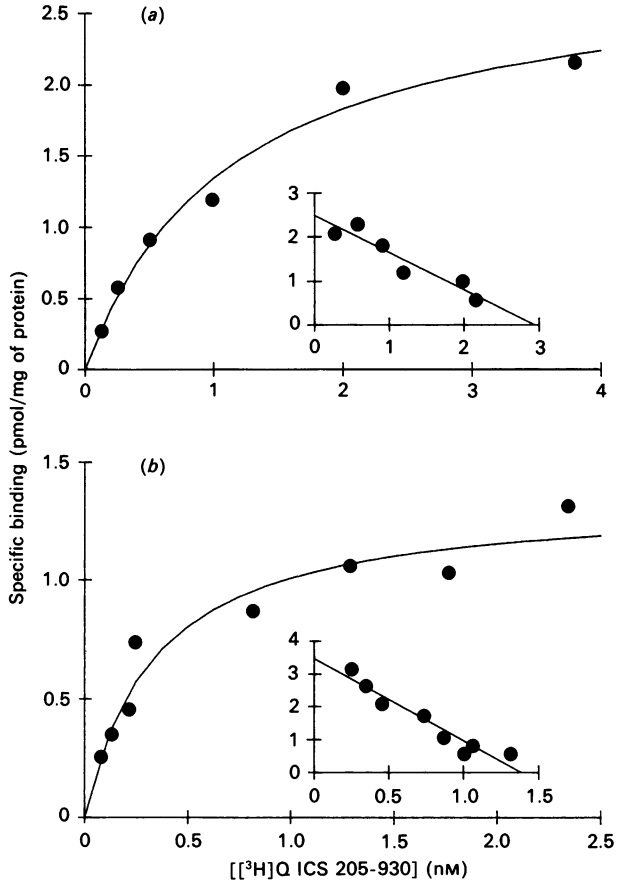

Fig. 1. Saturation isotherms and Scatchard analysis of [^{3}H]Q ICS 205-930 binding to 5-HT$_3$-receptor-binding sites on NCB-20-cell membranes (*a*) and to a preparation solubilized from NCB-20-cell membranes with 0.5% deoxycholate (*b*)

Results are representative of four and three experiments respectively. Curves were fitted by using a computer-generated curve-fitting procedure using RS1 (BBN Software Products, Cambridge, MA, U.S.A.). Scatchard plots were fitted by linear regression.

detergent deoxycholate (McKernan *et al.*, 1990). The density of receptors in brain is low (approx. 50 fmol/mg of protein); therefore it was important to identify a more abundant source of receptors for further biochemical studies. Membranes prepared from NCB 20 cells were found to have a greater density of these sites than rat brain. [^{3}H]Q ICS 205-930 binding was 1.19±0.31 pmol/mg of protein, with a K_d of 0.4±0.07 nM (mean±S.D., n = 4). As shown in Fig. 1, [^{3}H]Q ICS 205-930 labelled one population of sites in membranes prepared from NCB 20 cells. In this particular experiment [^{3}H]Q ICS 205-930 binding was 2.8 pmol/mg of protein and with a K_d of 0.73 nM. Some variation was observed in the apparent density of receptors on NCB 20 cells. The density appeared to decline with passage number, and therefore cells were discarded after passage number 60, when their density was approx. 0.5 pmol/mg of protein. Receptor sites were solubilized with 0.5% deoxycholate, essentially as described by McKernan *et al.* (1990) and binding of [^{3}H]Q ICS 205-930 was preserved in the solubilized preparation when binding assays were conducted in 0.1% Triton X-100. A $B_{max.}$ value for binding of 1.11±0.4 pmol/mg of protein and a K_d of 0.48±0.06 nM (n = 3) were observed in the solubilized preparation (see Fig. 1). Binding of [^{3}H]Q ICS 205-930 to both the membrane and solubilized preparations was consistent with binding to a 5-HT$_3$ receptor as described by the rank order of

Table 1. K_i values of various antagonists and agonists for the 5-HT$_3$-receptor-binding site in membrane and solubilised preparations of NCB20 cells

K_i values were obtained from the Cheng & Prussof (1973) equation, using IC_{50} values obtained from a computer-generated iterative curve-fitting procedure using RS1 (BBN Software Products). Data was best fitted by a one-site model with Hill slope of 1, and there was no evidence for more than one population of binding sites. Results shown are means±S.E.M. for three to six determinations.

Antagonist/ agonist	K_i (nM)	
	Membranes	Soluble
Zacopride	0.17±0.03	0.25±0.05
Q ICS 205-930	0.64±0.2	0.43±0.05
ICS 205-930	0.77±0.2	0.46±0.1
Quipazine	1.02±0.3	0.83±0.1
L-680,652	1.09±0.19	1.19±0.2
GR 38032F	4.35±0.4	5.1±1.0
BRL 24924	8.66±1.9	11.2±1
MDL 72222	62.3±8	51.3±11
Ketanserin	> 10000	> 10000
5-HT	50.2±13	38.3±4.4

potency for a series of agonists and antagonists (see Table 1). There was no suggestion either in the saturation analysis or in the competition curves that [^{3}H]Q ICS 205-930 was labelling more than one site. This is of importance, since it has been reported that an additional type of 5-HT receptor exists, and that ICS 205-930 is an antagonist at this receptor (Dumuis *et al.*, 1988*a*).

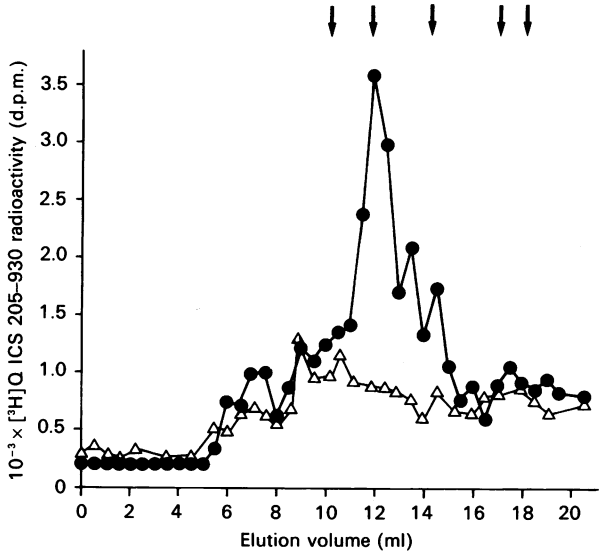

Fig. 2. Gel-exclusion chromatography of the solubilized 5-HT$_3$ receptor

Sodium deoxycholate-solubilized extract of NCB 20 cells was exchanged into buffer containing 0.1% Triton X-100 as described in the Experimental section and then chromatographed on a Sepharose 6B column equilibrated and eluted with buffer containing 5 mM-Tris/HCl, 1 mM-EDTA, 10% glycerol, 500 mM-NaCl and 0.2% Lubrol pH 7.5, at room temperature. Each fraction was assayed with [^{3}H]Q ICS 205-930 (0.7 nM) in the presence or absence of 10^{-5} M-L-680,652. The elution profile shows total (●) and non-specific (△) binding and is representative of six similar experiments. The Sepharose 6B column was calibrated as indicated by the arrows with (from right) ribonuclease A, chymotrysinogen A, haemoglobin, catalase and ferritin.

Gel-exclusion chromatography

In all gel-exclusion-chromatographic experiments, binding of [^{3}H]Q ICS 205-930 was predominantly present in a single broad peak with a mean elution volume of 12.25 ml ($K_{av.} = 0.36$). This peak represented specific binding of [^{3}H]Q ICS 205-930, since it was competed for by 10^{-5}M-L-680,652. A typical experiment is shown in Fig. 2, and is representative of six similar experiments. In some experiments a small peak (less than 20% of the specific binding) was present in the void volume, and this presumably represented aggregated material. Extrapolation from the calibration curve gave a mean Stokes radius for the [^{3}H]Q ICS 205-930 binding site of 4.81 ± 0.15 nm ($n = 6$).

Sucrose-density-gradient centrifugation

The binding of [^{3}H]Q ICS 205-930 was present in a single peak after sucrose-density-gradient centrifugation located 26.5 ± 0.90 mm from the meniscus ($n = 3$). This was in approximately the same position as the marker protein, catalase (see Fig. 3*a*). When sucrose-density-gradient-centrifugation experiments were carried out in ^2H_2O, [^{3}H]Q ICS 205-930 binding was associated with a peak which was slightly retarded compared with catalase, having travelled 19.2 ± 0.64 mm ($n = 4$) through the sucrose gradient (see Fig. 3*b*). The observation that the peak of binding travelled further, relative to catalase, in ^1H_2O than in ^2H_2O indicates that the receptor site has a higher partial specific volume than the calibrating proteins. This is most probably due to detergent binding to the hydrophobic portions of the protein, and this anomalous behaviour is not uncommon with membrane receptors (e.g. Haga, 1980). Calibration of sucrose gradients in both ^1H_2O and ^2H_2O using globular proteins with known sedimentation coefficients yielded an approximately linear relationship between sedimentation coefficient(s) and the distance travelled through the gradient (mm), as shown in Fig. 3(*c*).

Determination of M_r

Gel-exclusion chromatography and sucrose-density-gradient centrifugation are two techniques which, together, can be used for estimating the M_r of an integral membrane protein (Mamalaki *et al.*, 1989). Calculation of the molecular mass of the receptor was done as follows.

(1) From density-gradient centrifugation, the distance (r) travelled through the gradient is given by the equation:

$$r = Ks_{20,w}(1 - \bar{v}\rho) \qquad \text{(Martin \& Ames, 1961)}$$

where K is a constant in a medium of a given average density, ρ, for molecules with the same partial specific volume ($\bar{v}$). The average density (i.e. density at $r/2$) was measured as 1.0075 and 1.1184 g·m^{-1} in ^1H_2O and ^2H_2O respectively. Since the sedimentation coefficient ($s_{20,w}$) and the average density of the solution (ρ) had been measured, and the partial specific volume ($\bar{v}$) is a known published value for each of the calibrating proteins, the parameter $s_{20,w}(1 - \bar{v}\rho)$ for each of these was calculated and plotted against the mean distance moved (r) (Fig. 3*c*). A straight line was fitted by linear regression to the data obtained for both ^1H_2O and ^2H_2O. The value of $s_{20,w}(1 - \bar{v}\rho)$ for the receptor in both ^1H_2O and ^2H_2O was obtained from these two curves and the following two simultaneous equations were generated:

$$2.679 = s_{20,w}[1 - (\bar{v} \cdot 1.0075)] \qquad \text{(A) } (^1H_2O)$$
$$1.5058 = s_{20,w}[1 - (\bar{v} \cdot 1.1184)] \qquad \text{(B) } (^2H_2O)$$

The solution of these simultaneous equations yielded a sedimentation coefficient, $s_{20,w}$, for the receptor–detergent complex of 12.77 S and a partial specific volume ($\bar{v}$) of 0.794 ml·g^{-1}.

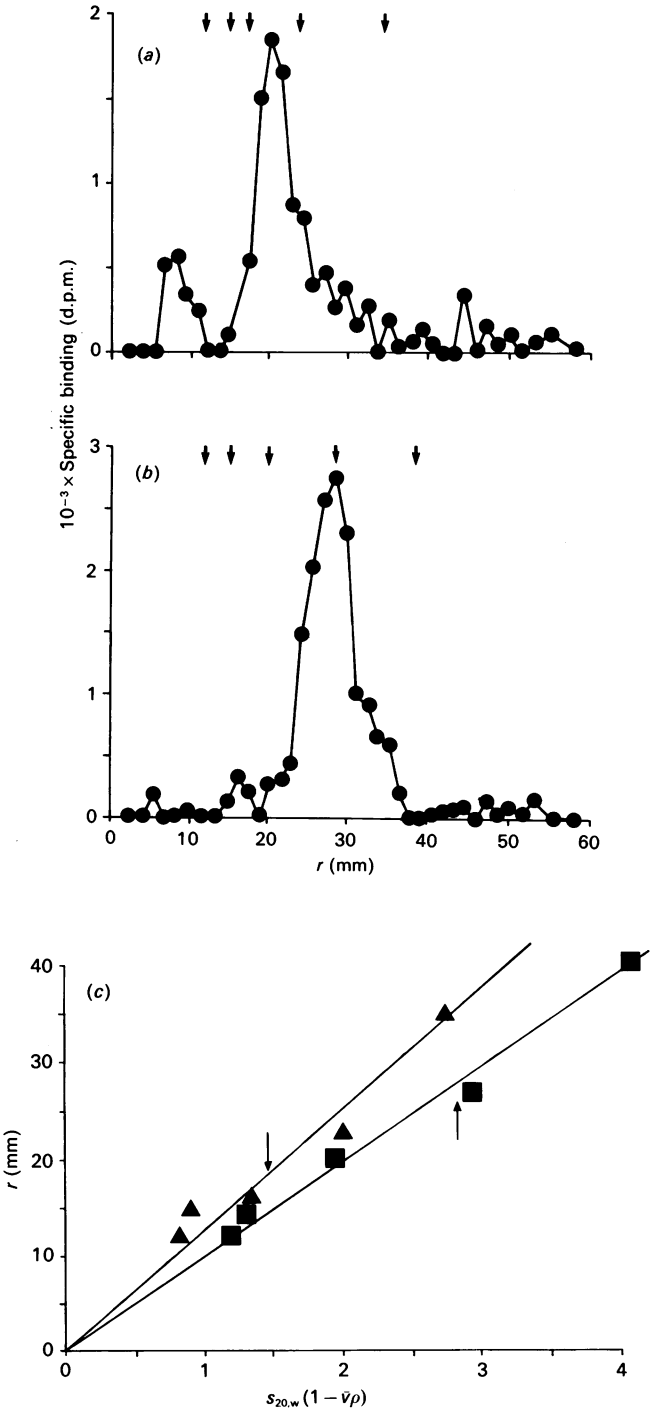

Fig. 3. Sucrose-density-gradient centrifugation of the solubilized 5-HT$_3$ receptor

Sodium deoxycholate-solubilized extract of NCB 20 cells was separated on 5–20% (w/v) sucrose gradients in buffer containing 5 mM-Tris/HCl, 1 mM-EDTA, 500 mM-NaCl and 0.2% Lubrol, pH 7.5, at 4 °C as described in the Experimental section. Each fraction was assayed with [^{3}H]Q ICS 205-930 in the presence or absence of L-680,652. Sucrose-density-gradient centrifugation was carried out using ^2H_2O (*a*) and ^1H_2O (*b*). The sucrose gradients were calibrated with markers of known sedimentation coefficient. These were (from left to right) haemoglobin, BSA, alcohol dehydrogenese, calatase and ferritin. (*c*) Calibration data for calculation of the sedimentation coefficient, $s_{20,w}$ and the partial specific volume, $\bar{v}$, of the 5-HT$_3$ receptor carried out in ^2H_2O (▲) and ^1H_2O (■). The arrows show the respective $s_{20,w}(1 - \bar{v}\rho)$ values for the receptor in ^2H_2O and ^1H_2O. r is defined in the text.

Table 2. Molecular size of the 5-HT_3 receptor

Parameter	Value
Stokes radius, a (nm)	4.811 ± 0.15 (mean $\pm$ S.E.M., $n = 6$)
Partial specific volume, $\bar{v}$ ($ml \cdot g^{-1}$)	0.794 ± 0.008*
Sedimentation coefficient $s_{20,w}$ (S)	12.77 ± 1.11*
M_r of detergent–receptor complex	338280 ± 27060†
Lubrol bound ($g \cdot g^{-1}$)	0.264
M_r of receptor protein	248975 ± 18090†

* Mean $\pm$ S.D. for three pairs of determinations carried out in ^1H_2O and ^2H_2O.

† Mean $\pm$ S.D., based on error of measurement of $\bar{v}$ and $s_{20,w}$ ($n = 3$) and using the mean value obtained for Stokes radius ($a = 4.811$ nm).

(2) With these values the molecular mass of the detergent receptor complex was calculated using the equation:

$$M_r = \frac{6\pi \eta_{20,w} N \cdot s_{20,w} a}{1 - \bar{v}\rho_{20,w}}$$

where $\eta_{20,w}$ is the viscosity of water at 20 °C (0.010002), N is Avogadro's number (6.022×10^{23}), $s_{20,w}$ is the experimentally determined sedimentation coefficient for the receptor–detergent complex (12.77 S), a is the experimentally determined Stokes radius (4.81 nm), $\bar{v}$ is the partial specific volume determined from the sucrose-density-gradient-centrifugation experiments ($0.794\ ml \cdot g^{-1}$) and $\rho_{20,w}$ is the density of ^1H_2O at 20 °C ($0.9998\ g \cdot cm^{-2}$). The mean M_r for the receptor–detergent complex was determined to be 338280.

(3) The molecular mass of the receptor was corrected for detergent binding using the equation:

$$v_{\text{receptor–detergent complex}} = X\bar{v}_{\text{detergent}} + (1 - X)\bar{v}_{\text{receptor protein}}$$

where the partial specific volume for the detergent Lubrol PX is known ($0.958\ ml \cdot g^{-1}$) and X is the fraction by weight of the bound detergent. $\bar{v}_{\text{receptor protein}}$ was assumed to be $0.73\ ml \cdot g^{-1}$, as is commonly assumed for membrane proteins (e.g. Mamalaki *et al.*, 1989). The fraction of the M_r which was derived from the detergent was calculated to be 0.264 (89305). Therefore the M_r of the 5-HT_3 receptor protein, corrected for binding of detergent, is 248965. Results are summarized in Table 2.

DISCUSSION

The present study demonstrates specific binding of [^{3}H]Q ICS 205-930 in membranes and solubilized preparations of NCB 20 cells. The density of receptor sites in these cells is 20–30 times that observed in rat brain (McKernan *et al.*, 1990) and this has proved a valuable and enriched source of receptor for biochemical characterization of this novel neurotransmitter receptor. It was noted during the course of these experiments that the density of 5-HT_3 receptors on NCB 20 cells is subject to fluctuation and declined with passage number. After subculturing the cells 25 times, the density of receptors was reduced by approx. 50% (results not shown). The mechanisms and regulatory factors involved are not known, and this presents an interesting area for future study.

The rank order of potency of a series of agonists to compete for [^{3}H]Q ICS 205-930 binding confirmed that this was a 5-HT_3-receptor-binding site, and there were no striking differences between the affinity of compounds for the 5-HT_3 receptor in NCB 20 cells compared with rat brain or other cell lines. Since the NCB 20 cell is derived from a Chinese-hamster ovary cell and a mouse superior-cervical-ganglion cell (Minna *et al.*, 1975), the binding site for [^{3}H]Q ICS 205-930 would appear to be well conserved between these species and the rat.

ICS 205-930 has also been demonstrated to bind to an additional 5-HT receptor, the 5-HT_4 receptor, with a 700-fold lower affinity for the receptor (Dumuis *et al.*, 1988*a*,*b*). We did not observe binding of [^{3}H]Q ICS 205-930 to any additional sites, but even if these receptors are present on NCB 20 cells, it is unlikely that they would be detected using the filtration assay employed here because of their low affinity. There were no significant changes in affinity of any compounds on solubilization of the receptor, and this contrasts with other ligand-gated ion channels, such as the $GABA_A$ receptor, where solubilization results in a reduced affinity of several compounds for the ligand-binding site (Stephenson, 1988). This effect is dependent on the detergent used, and the maintained high affinity of the 5-HT_3 receptor for [^{3}H]Q ICS 205-930 and other ligands observed here is probably because the receptor was exchanged from deoxycholate into a relatively low concentration of Triton-X 100 immediately after solubilization.

It is difficult to compare the physical characteristics determined here for the 5-HT_3 receptor with those of other better-characterized ion channels because parameters such as the Stokes radius and the sedimentation coefficient are influenced by many other factors. These include the nature of the buffer in which the experiments are conducted, the concentration of detergent, the amount of detergent binding, and the conditions employed to solubilize the receptor (as exemplified by Mamalaki *et al.*, 1989). However, where M_r has been corrected for detergent binding and care has been taken to ensure that the receptor is not in an aggregated condition, it should be possible to compare the M_r values of different receptors.

The M_r determined here for the 5-HT_3 receptor (249000) is in the same range as that determined by similar methods for two other ligand-gated ion channels i.e. the $GABA_A$ receptor from bovine brain (230000–240000) (Stephenson, 1988; Mamalaki *et al.*, 1989) and the glycine receptor of rat spinal cord (246000) (Pfeiffer *et al.*, 1982). The nicotinic acetylcholine receptor is also of a similar size, with an M_r of 268000 as determined by cDNA cloning (Noda *et al.*, 1983). The technique of radiation inactivation is often used to estimate the M_r of membrane receptors. To date no information is available on this with regard to the 5-HT_3 receptor. This may be due in part to the low density of receptors in most tissues. Experiments of this type may be possible now that a suitably enriched source of the receptor has been identified.

This is the first estimation of the molecular size of the 5-HT_3 receptor and demonstrates its similarity with other ligand-gated ion channels. However, the subunit composition of the receptor has yet to be defined, and further information is unlikely to become available until either the development of photoaffinity or irreversible probes of the 5-HT_3 receptor, or alternatively, the receptor can be purified and the subunits observed on a polyacrylamide gel.

We thank Ms. Jill Gillard for maintaining the NCB 20 cells and Ms. Eleanor Brawn for typing the manuscript.

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Received 10 January 1990/2 March 1990; accepted 6 March 1990