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MULTIPLE HIGH-AFFINITY BINDING SITES FOR [^{3}H]SEROTONIN IN THE BRAIN OF A TELEOST FISH, THE ARCTIC CHARR (*SALVELINUS ALPINUS*)

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Summary

Binding of [^{3}H]serotonin (5-HT) to membranes prepared from Arctic charr brain homogenates was most consistent with a one-site model for [^{3}H]5-HT binding, with K_D and B_{max} values of 5.7±0.3 nmol l^{-1} and 60.7±7.3 fmol mg^{-1} protein, respectively. Similarly, 5-HT displacement of [^{3}H]5-HT was best explained by a monophasic model with an apparent K_i of 4.3±0.7 nmol l^{-1}. The ability of a number of synthetic 5-HT receptor ligands to displace [^{3}H]5-HT was studied. 8OH-DPAT was found to interact with three [^{3}H]5-HT binding sites, whereas buspirone, TFMPP, spiperone and mianserin all distinguish two sites. In the presence of 300 nmol l^{-1} buspirone, 8OH-DPAT and mianserin distinguished two [^{3}H]5-HT binding sites, whereas spiperone interacted with only one. Moreover, 8OH-DPAT differentiated three [^{3}H]5-HT binding sites even in the presence of 0.5 mmol l^{-1} GTP, making it unlikely that these sites represent different affinity states of G-protein-coupled receptors. GTP had no effect on apparent K_i values for 8OH-DPAT, but reduced the B_{max} value of the high-affinity site by 60 %. GTP had a similar effect on the saturation binding curve for [^{3}H]5-HT, reducing B_{max} by approximately 50 %, whereas K_D was unaffected. The results provide evidence for at least three different high-affinity [^{3}H]5-HT binding sites, one of them showing a pharmacological profile strikingly similar to that of the mammalian 5-HT$_{1A}$ receptor.

Key words: brain, serotonin, 5-hydroxytryptamine, serotonin receptor, radioligand studies, Arctic charr, *Salvelinus alpinus*.

Introduction

The morphological organization of the central serotonergic system, which has been examined in all major vertebrate classes, appears to have remained remarkably stable through evolution (Parent, 1981; Parent *et al.* 1984). The serotonergic system probably constitutes the most extensive and complex anatomical/neurochemical system in the vertebrate brain, displaying an extremely divergent projection pattern (Jacobs and Azmitia, 1992).

The serotonergic system of the mammalian brain displays a considerable receptor divergence (for recent reviews, see Peroutka, 1990; Zifa and Fillion, 1992; Martin and Humphrey, 1994; Boess and Martin, 1994). On the basis of their pharmacological characteristics, molecular structure and transduction mechanisms, mammalian 5-HT (serotonin) receptors have been categorized into as many as seven different classes, of which the 5-HT$_1$, 5-HT$_2$ and 5-HT$_3$ receptor classes are well defined (Martin and Humphrey, 1994). Some of these receptor classes have been subdivided into different receptor subtypes. The 5-HT$_1$ class is now believed to consist of at least five different subtypes, 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$. The 5-HT$_1$ receptors are all G-protein-coupled receptors that exert a negative control on adenylyl cyclase activity. By contrast, 5-HT$_2$ receptors, which are also G-protein-coupled receptors, act through stimulation of phosphoinositide turnover. So far, three 5-HT$_2$ receptor subtypes, 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ (originally classified as a 5-HT$_1$-like site, the 5-HT$_{1C}$ receptor), have been identified in the mammalian brain. 5-HT$_3$ receptors, however, differ from other 5-HT receptor classes by being ligand-gated cation channels.

In sharp contrast to the rapidly growing literature on mammalian 5-HT receptors, very little is known about brain 5-HT receptors in non-mammalian vertebrates. As stated above, the organization of the 5-HT system seems to be phylogenetically old, and similarities in brain 5-HT functions between teleosts and mammals have been identified (reviewed by Winberg and Nilsson, 1993). However, information on 5-HT receptor subtypes in non-mammalian vertebrates is urgently needed if we are to understand the function of this neurotransmitter system in these animals.

In an autoradiographic study, Dietl and Palacios (1988) presented evidence for the presence of 5-HT$_{1A}$ receptors in fish, amphibians, reptiles and birds, suggesting that this receptor occurred early in vertebrate evolution. However, binding of

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[^{3}H]5-HT and [^{3}H]mesulergine to putative 5-HT$_{1B}$ and 5-HT1$_{2C}$ sites was not observed in fish, amphibians or reptiles, but was detected in the avian brain (Dietl and Palacios, 1988).

In a study on the effect of *p*-chlorophenylalanine treatment on [^{3}H]5-HT binding in the brain of rainbow trout (*Oncorhynchus mykiss*), Johnston and Glanville (1994) reported that addition of 10 μmol l^{-1} (±)8-hydroxy-2-(di-*n*-propylamino)-tetralin (8OH-DPAT) displaced 85 % of specific [^{3}H]5-HT binding. However, the interaction of 8OH-DPAT with [^{3}H]5-HT binding was tested only at a single concentration. Lima *et al.* (1992) studied [^{3}H]5-HT binding in goldfish (*Carassius auratus*) retinal membranes. Their results indicated the presence of two 5-HT binding sites in the goldfish retina, a high-affinity, low-capacity site, possibly a 5-HT$_1$ receptor, and a low-affinity, high-capacity site, probably representing a 5-HT transporter.

In the present study, we have examined the effect of several 5-HT receptor ligands, over wide concentration ranges, on [^{3}H]5-HT binding to whole-brain homogenates from Arctic charr. The results provide evidence for at least three subtypes of high-affinity [^{3}H]5-HT binding sites in the Arctic charr brain.

Materials and methods

Animals

The fish (weighing 30–60 g) were 2-year-old juvenile Arctic charr (*Salvelinus alpinus* L.). They were kept indoors at the Department of Limnology, in a holding tank continuously supplied with aerated Uppsala tap water (8–11 °C). The light/dark regime was continuously and automatically adjusted to conditions at a latitude of 51°N. The fish were fed daily with commercial trout pellets (EWOS ST40, Astra-EWOS Sweden) at 2 % of their body mass.

Receptor binding assay

Receptor binding assays were performed according to the method of Bennett and Snyder (1976). Briefly, fish were killed by decapitation, between 10:00 and 11:00 h, and their brains were rapidly removed. Individual whole brains, with a tissue mass of approximately 200 mg, were immediately homogenized in 10 ml of 50 mmol l^{-1} Tris–HCl (pH 7.4) using a Potter–Elvehjem homogenizer and then centrifuged at 45 000 ***g*** for 12 min. The supernatant was discarded, and the pellet was resuspended in 10 ml of Tris–HCl buffer and incubated at 25 °C for 30 min before a second centrifugation at 45 000 ***g*** for 12 min. Once again the pellet was resuspended in 10 ml of Tris–HCl and centrifuged at 45 000 ***g*** for 12 min, whereupon the final pellet was resuspended in 10 ml of Tris–HCl buffer (pH 7.4) containing 10 μmol l^{-1} pargyline, 4 mmol l^{-1} $CaCl_2$ and 0.1 % ascorbic acid. Suspensions from 6–12 brains were mixed and used immediately in the binding assay.

Binding assays consisted of 0.1 ml of a solution of [^{3}H]5-HT (0.8–120.0 nmol l^{-1} in saturation experiments and 26.0±3.1 nmol l^{-1} in competition experiments), 0.1 ml of buffer or displacing ligand and 0.8 ml of tissue suspension (1.2±0.2 mg protein ml^{-1}). Following incubation at 25 °C for 45 min, the assay mixture was rapidly filtered under vacuum through Whatman GF/B glass fibre filters using a Brandel cell harvester, and rinsed four times in 5 ml of ice-cold Tris–HCl buffer (pH 7.4). Radioactivity was measured by liquid scintillation spectroscopy in 5 ml of Aquasafe (Zinser Analytic GmbH, Frankfurt, Germany) at 50 % efficiency. Specific binding was defined using 100 μmol l^{-1} 5-HT in all experiments and represented 30–50 % of total binding.

All drugs except spiperone were dissolved and diluted in 50 mmol l^{-1} Tris–HCl buffer (pH 7.4) containing 0.1 % ascorbic acid, 10 μmol l^{-1} pargyline and 4 mmol l^{-1} $CaCl_2$; spiperone was first dissolved in 0.2 ml of glacial acetic acid and then diluted in assay buffer (final concentration 1 mmol l^{-1}). Drugs were obtained from the following sources: [^{3}H]5-HT (939.8 GBq mmol^{-1}) from NEN Research Products, Du Pont de Nemours (GmbH, NEN Division, Dreiech, Germany); 5-HT, mianserin, 8OH-DPAT and pargyline from Sigma Chemical Co. (St Louis, MO, USA); buspirone, spiperone and 1-(*m*-trifluoromethylphenyl)piperazine (TFMPP) from Research Biochemicals International (Waltham, MA, USA); methysergide was a gift from Sandoz AB (Täby, Sweden).

Saturation as well as competition studies were analyzed using the non-linear curve-fitting program LIGAND (included in the RADLIG program package Version 4, Biosoft, UK; originally designed by Munson and Rodbard, 1980). In drug-displacement studies, drug concentrations ranged from 10^{-11} to 10^{-4} mol l^{-1}, except for spiperone for which the highest concentration used was 4.6×10^{-6} mol l^{-1} (owing to its lower solubility). All data were initially analyzed on the basis of the assumption of a one-site model of [^{3}H]5-HT binding. The data were then analyzed according to two-site and three-site models, and the curve-fitting results were compared statistically using an *F*-test. A more complex model was accepted if the observed 'fit' was significantly better ($P<0.05$) than that observed using a simpler model of ligand binding.

Protein concentrations were determined by the method of Lowry *et al.* (1951).

Results

[^{3}H]5-HT saturation binding experiments

Incubation of whole-brain homogenates in the presence of increasing concentrations of [^{3}H]5-HT (from 0.08 to 12 nmol l^{-1}) yielded a binding curve that was most consistent with a one-site model with a dissociation constant (K_D) of 5.7±0.3 nmol l^{-1} and a B_{max} value of 60.7±7.3 fmol mg^{-1} protein (Fig. 1A; Table 1). Moreover, the slope of the corresponding Scatchard plot showed no deviation from linearity (Fig. 1B). Addition of 0.5 mmol l^{-1} GTP had a striking effect on the saturation curve, reducing B_{max} by approximately 50 % without significantly affecting K_D for [^{3}H]5-HT (Fig. 1A,B; Table 1).

5-HT receptor–ligand interactions with [^{3}H]5-HT binding

All the 5-HT receptor ligands tested interacted with [^{3}H]5-HT binding in characteristic ways (Fig. 2).

The Hill constant for 5-HT displacement of [3H]5-HT was close to 1 and the competition curve was most consistent with a monophasic model with an apparent inhibitor constant (K_i) of 4.3±0.7 (Table 2). Specific [3H]5-HT binding plateaued at about 10^{-6} mol l^{-1}, remaining constant up to 10^{-4} mol l^{-1} (Fig. 2A). By contrast, 8OH-DPAT started to displace [3H]5-HT at a concentration of 10^{-10} mol l^{-1}, with 50 % displacement occurring at approximately 30nmol l^{-1} 8OH-DPAT (Fig. 2B). The Hill constant for 8OH-DPAT displacement of [3H]5-HT was 0.42±0.03 and the competition curve agreed best (P<0.001) with a three-site model, giving apparent K_i values of 1.7±0.8, 82±51 and 9000±6000nmol l^{-1}, the corresponding B_{max} values being 21.5±3.5, 16.6±3.2 and 8.5±1.4fmol mg^{-1} protein. Similarly, shallow competition curves were observed with buspirone, TFMPP, spiperone and mianserin, with the Hill constants ranging from 0.34 to 0.66 (Fig. 2C–F; Table 2). These competition curves were all significantly more consistent with two-site models (P<0.001). However, the density of low- and high-affinity binding sites differed among drugs (Table 2). Buspirone distinguished two populations of 5-HT binding sites, one with an apparent K_i value of 32±7nmol l^{-1} and another with an apparent K_i value of 8900±4000nmol l^{-1}, B_{max} being 28.3±1.8 and 19.3±2.0fmol mg^{-1} protein, respectively (Table 2). Similarly, TFMPP recognized two 5-HT binding sites with apparent K_i

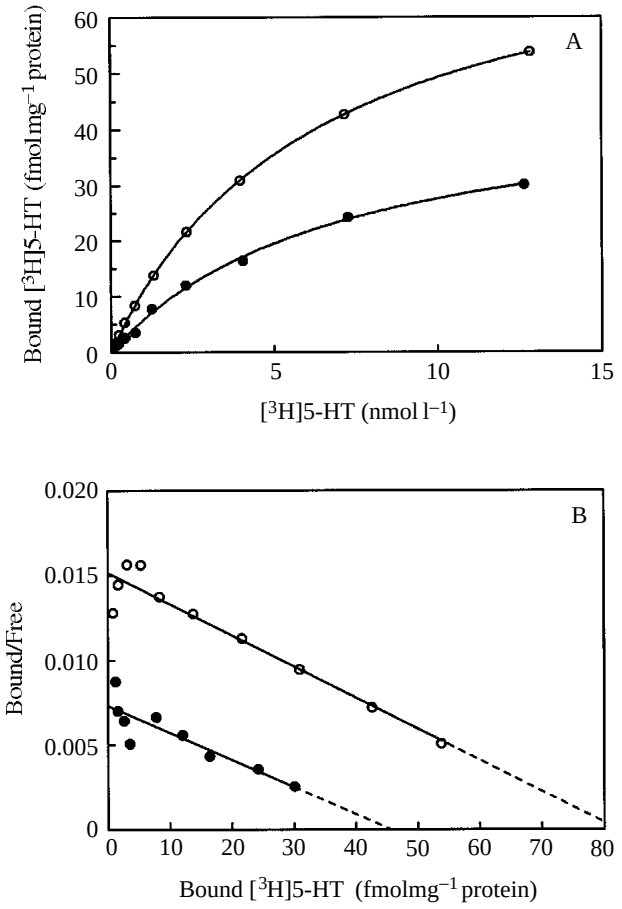

Fig. 1. Specific binding of [3H]5-HT, in the presence (●) and absence (○) of 0.5mmol l^{-1} GTP, to membranes prepared from Arctic charr whole-brain homogenates. The binding assay was performed as described in Materials and methods with 10 concentrations of [3H]5-HT ranging from 0.08 to 12nmol l^{-1}, assayed in triplicate. (A) Typical binding curves generated by non-linear curve-fitting using LIGAND (see Materials and methods) and a one-site model. (B) Scatchard analysis of the same experiments.

Table 1. *Results for [3H]5-HT binding to Arctic charr whole brain measured on membranes prepared from a tissue homogenate*

	B_{max} (fmol mg^{-1} protein)	K_D (nmol l^{-1})
Without GTP	60.7±7.3	5.7±0.3
With 0.5mmol l^{-1} GTP	28.8±3.8	5.5±0.7

Measurements were made in the presence (N=3) or absence (N=5) of GTP, as described in Materials and methods.

Values are means ± S.E.M. calculated by non-linear curve-fitting using LIGAND (see Materials and methods) and a one-site model.

[3H]5-HT was tested over the concentration range 0.08–12.0nmol l^{-1}.

Table 2. *Results from drug inhibition studies of [3H]5-HT binding to membranes prepared from a tissue homogenate of Arctic charr whole brain*

Drug	n_H	K_{i1} (nmol l^{-1})	B_{max1} (fmol mg^{-1} protein)	K_{i2} (nmol l^{-1})	B_{max2} (fmol mg^{-1} protein)	K_{i3} (nmol l^{-1})	B_{max3} (fmol mg^{-1} protein)
5-HT	0.80±0.07	4.3±0.7	56.8±5.2				
8-OHDPAT	0.42±0.03	1.7±0.8	21.5±3.5	82±51	16.6±3.2	9000±6000	8.5±1.4
Buspirone	0.49±0.05	32±7	28.3±1.8	8900±4000	19.3±2.0		
TFMPP	0.66±0.08	57±24	38.5±6.7	1900±1800	17.4±6.3		
Spiperone	0.34±0.11	13±7	22.5±3.5	2300±1700	52.3±13.7		
Mianserin	0.54±0.07	62±30	23.7±4.0	4000±2100	24.5±3.7		
Methysergide	0.82±0.09	57±12	45.3±2.6				

Data (means ± S.E.M.) are from the curves in Fig. 2 as determined by non-linear curve-fitting using LIGAND (see Materials and methods) and a one-site (inhibition by 5-HT and methysergide), a two-site (inhibition by buspirone, TFMPP, spiperone and mianserin) or a three-site (inhibition by 8OH-DPAT) model.

5-HT, serotonin; 8-OHDPAT, (±)8-hydroxy-2-(di-*n*-propylamino)-tetralin; TFMPP, 1-(*m*-trifluoromethylphenyl)piperazine; n_H, Hill constant.

values of 57±24 and 1900±1800nmol l^{-1}, B_{max} being 38.5±6.7 and 17.4±6.3fmolmg^{-1} protein, respectively (Table 2). Spiperone distinguished one high-affinity site with an apparent K_i value of 13±7nmol l^{-1} and a B_{max} of 22.5±3.5fmolmg^{-1} protein, and one low-affinity site with an apparent K_i value of 2300±1700nmol l^{-1} and a B_{max} of 52.3±13.7fmolmg^{-1} protein (Table 2). Finally, mianserin also interacted with two binding sites with apparent K_i values of 62±30 and 4000±2100nmol l^{-1}, the corresponding B_{max} values being 23.7±4.0 and 24.5±3.7fmolmg^{-1} protein (Table 2). Displacement of [^{3}H]5-HT by methysergide resulted in a competition curve with a Hill constant of 0.82±0.09 that was most consistent with a one-site model giving an apparent K_i value of 57±12nmol l^{-1} (Table 2).

5-HT receptor–ligand interactions with [^{3}H]5-HT binding in the presence of 300nmol l^{-1} buspirone

In an attempt to characterize the component of [^{3}H]5-HT binding showing low affinity for buspirone, the interactions of 8OH-DPAT, spiperone and mianserin with [^{3}H]5-HT binding were analyzed in the presence of 300nmol l^{-1} buspirone.

In these experiments, shallow displacement of [^{3}H]5-HT binding was observed with 8OH-DPAT and mianserin (Fig. 3A,C), with Hill constants of 0.43±0.07 and 0.47±0.10,

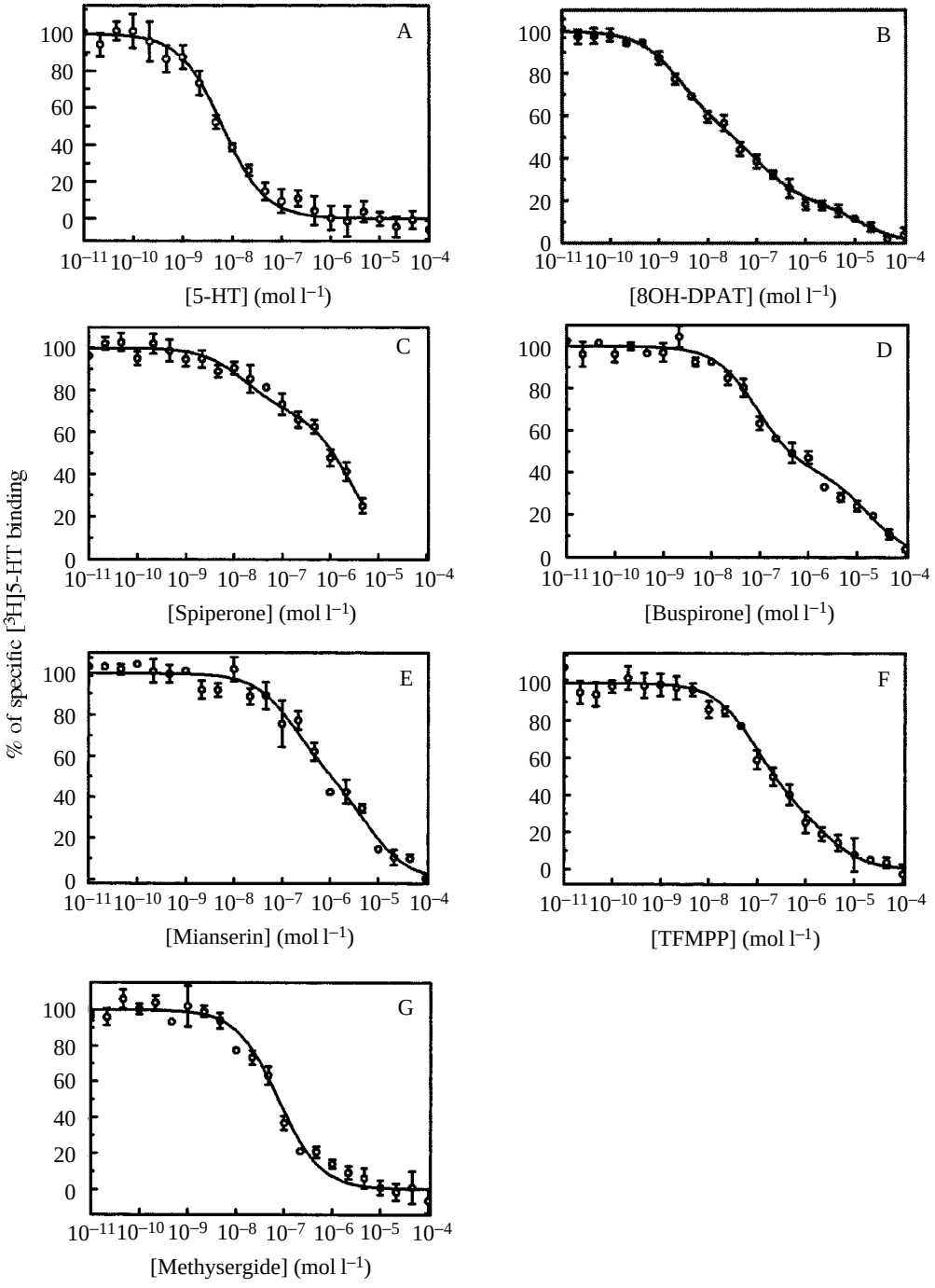

Fig. 2. Inhibition of specific [^{3}H]5-HT binding to membranes prepared from Arctic charr whole-brain homogenates by 5-HT (A), (±)8-hydroxy-2-(di-*n*-propylamino)-tetralin (8OH-DPAT) (B), spiperone (C), buspirone (D), mianserin (E), 1-(*m*-trifluoromethyl-phenyl)piperazine (TFMPP) (F) and methysergide (G). Each data point represents the mean ± S.E.M. from 3–5 experiments performed in triplicate, using 2 nmol l^{-1} [^{3}H]5-HT, as described in Materials and methods. The curves were generated by non-linear curve-fitting using LIGAND (see Materials and methods) and a one-site (A, G), two-site (C–F) or three-site (B) model.

respectively (Table 3). 8OH-DPAT began to displace [3H]5-HT at a concentration of approximately 5nmol l^{-1}, with 50 % displacement occurring at 2500nmol l^{-1} 8OH-DPAT (Fig. 3A). The curve was most consistent with a two-site model (P<0.001) with apparent K_i values of 65±18 and 19000±11000nmol l^{-1}, the corresponding B_{max} values being 16.1±1.2 and 16.2±2.4fmol mg^{-1} protein (Table 3). Similarly, displacement of [3H]5-HT by mianserin was most consistent with a two-site model with apparent K_i values of 200±90 and 43000±12000nmol l^{-1}, with the corresponding B_{max} values being 19.1±2.0 and 23.6±2.0fmol mg^{-1} protein (Table 3). By contrast, spiperone displaced [3H]5-HT in a monophasic manner, starting at 100nmol l^{-1} and reaching 50 % displacement at 3000nmol l^{-1} (Fig. 3B).

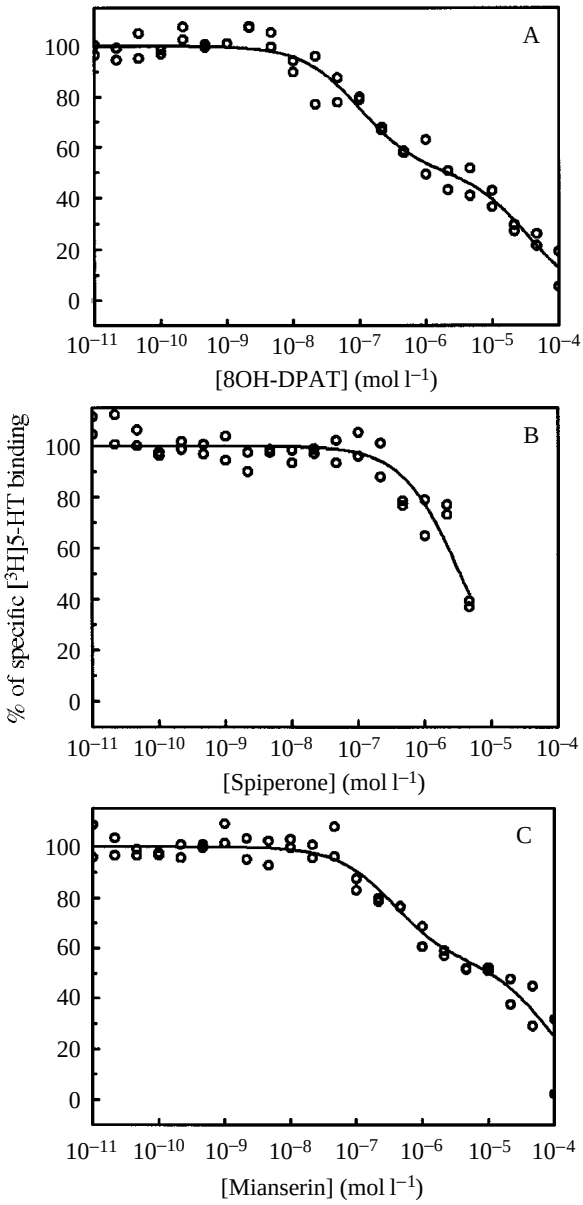

Fig. 3. Inhibition of specific [3H]5-HT binding to membranes prepared from Arctic charr whole-brain homogenates by 8OH-DPAT (A), spiperone (B) and mianserin (C) in the presence of 300nmol l^{-1} buspirone. Each data point represents the mean value from one experiment performed in triplicate, using 2nmol l^{-1} [3H]5-HT, as described in Materials and methods. The curves were generated by non-linear curve-fitting using LIGAND (see Materials and methods) and a one-site (B) or a two-site (A,C) model.

Effect of GTP on 8OH-DPAT displacement of [3H]5-HT binding

8OH-DPAT displacement of [3H]5-HT in the presence of 0.5mmol l^{-1} GTP was studied in a competition experiment (Fig. 4). Addition of GTP had no significant effect on the Hill constant (0.40±0.06 compared with 0.42±0.03 in the absence of GTP; Table 4). The displacement of [3H]5-HT with 8OH-DPAT was still most consistent with a three-site model (P<0.05), with apparent K_i values of 1.4±1.0, 49±32 and 13800±11400nmol l^{-1}, which is in good agreement with the K_i values obtained for 8OH-DPAT in the absence of GTP (Table 4). However, B_{max} values for the high-, low- and medium-affinity sites were 9.0±4.1, 17.1±3.8 and 11.0±2.2fmol mg^{-1} protein, respectively. Thus, the number of high-affinity sites was reduced by the addition of 0.5mmol l^{-1} GTP, an effect that was not seen on the B_{max} values for the medium- and low-affinity sites (Table 4).

Discussion

The results from the present study shows that [3H]5-HT binding to a preparation of Arctic charr brain membrane is reversible and saturable with a K_D of 5.7nmol l^{-1}, within the

Table 3. *Results from drug inhibition studies of [3H]5-HT binding to membranes prepared from a tissue homogenate of Arctic charr whole brain in the presence of 300 nmol l^{-1} buspirone*

Drug	n_H	K_{i1} (nmol l^{-1})	B_{max1} (fmol mg^{-1} protein)	K_{i2} (nmol l^{-1})	B_{max2} (fmol mg^{-1} protein)
8-OHDPAT	0.43±0.07	65±18	16.1±1.2	19000±11000	16.2±2.4
Spiperone	0.60±0.36	2900±1600	42.6±12.7		
Mianserin	0.47±0.10	200±90	19.1±2.0	43000±12000	23.6±2.0

Data (means ± S.E.M.) are from the curves in Fig. 3 as determined by non-linear curve-fitting using LIGAND (see Materials and methods) and a one-site (inhibition by spiperone) or a two-site (inhibition by 8OH-DPAT and mianserin) model.

8-OHDPAT, (±)8-hydroxy-2-(di-*n*-propylamino)-tetralin; n_H, Hill constant.

K_D range (2.1–10.8nmol l^{-1}) reported for mammalian brains (Pedigo *et al.* 1981; Pazos *et al.* 1985; Bennett and Snyder, 1976; Peroutka, 1986) and also similar to the value of 3.4–3.7nmol l^{-1} given for rainbow trout brain by Johnston and Glanville (1994). Furthermore, the competition experiments with 5-HT receptor ligands suggest that there are at least three subtypes of high-affinity 5-HT binding sites in the Arctic charr brain, all showing high affinities for the endogenous ligand but differing affinities for the synthetic ligands, best illustrated by the three-site model found with 8OH-DPAT.

The effects of six different 5-HT receptor ligands on [^{3}H]5-HT binding were individually analyzed by competition studies, using computer-assisted curve-fitting. Of the ligands tested, 8OH-DPAT was the only agent that could discriminate three [^{3}H]5-HT binding sites. This is probably related to the fact that curve-fitting analysis has a limited ability to differentiate between sites that have a less than 10-fold difference in their affinity for a given ligand (DeLean *et al.* 1982). Complex competition curves could be caused by the ability of guanine nucleotides to modulate agonist binding to G-protein-linked receptors (Sills *et al.* 1984). However, in the present study, 8OH-DPAT differentiated three [^{3}H]5-HT binding sites even in the presence of 0.5mmol l^{-1} GTP, making it unlikely that these binding sites represent different affinity states of G-protein-coupled receptors.

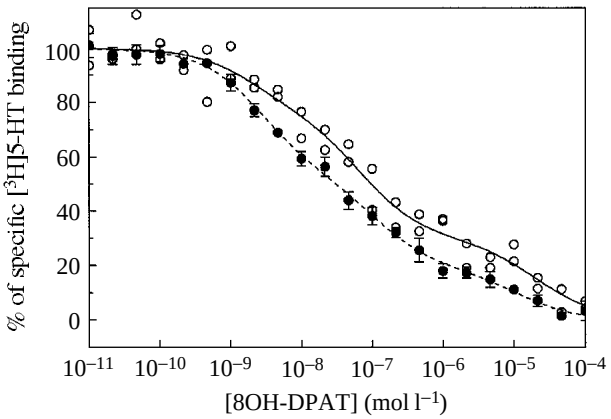

Fig. 4. Inhibition of specific [^{3}H]5-HT binding to membranes prepared from Arctic charr whole-brain homogenates by 8OH-DPAT in the absence (●) or presence (○) of 0.5mmol l^{-1} GTP. Each data point represents the mean value from one experiment performed in triplicate (in the presence of 0.5mmol l^{-1} GTP), or the mean ± S.E.M. from four experiments performed in triplicate (in the absence of GTP, data from Fig. 2), using 2nmol l^{-1} [^{3}H]5-HT, as described in Materials and methods. The curve was generated by non-linear curve-fitting using LIGAND (see Materials and methods) and a three-site model

The [^{3}H]5-HT binding site showing highest affinity for 8OH-DPAT (apparent K_i of 1.7nmol l^{-1}) was blocked by the addition of 300nmol l^{-1} buspirone, without giving noticeable effects on apparent K_i and B_{max} values for sites with a moderate and low affinity for 8OH-DPAT. Furthermore, 300nmol l^{-1} buspirone blocked the binding site displaying nanomolar affinity for spiperone, while mianserin still recognized two [^{3}H]5-HT binding sites under these conditions. Consequently, the [^{3}H]5-HT binding site displaying high affinity for 8OH-DPAT (<10nmol l^{-1}) also shows high affinity for spiperone, intermediate affinity (<100nmol l^{-1}) for buspirone and methysergide, and low affinity (>1000nmol l^{-1}) for mianserin, a pharmacological profile strikingly similar to that of the mammalian 5-HT$_{1A}$ receptor (Zifa and Fillion, 1992). For the mammalian 5-HT$_{1A}$ receptor, the following K_i values have been reported; 8OH-DPAT, 0.4–3.0nmol l^{-1}; spiperone, 23–320nmol l^{-1}; buspirone, 14–29.5nmol l^{-1}; mianserin, 800–1150nmol l^{-1}; and methysergide, 25–70.8nmol l^{-1} (reviewed by Zifa and Fillion, 1992).

The lack of specific 5-HT receptor ligands makes it hard to speculate on the identity of the [^{3}H]5-HT binding sites showing moderate and low affinity for 8OH-DPAT. However, one of these sites apparently displays a moderate affinity for mianserin, while the other site only shows micromolar affinity for this ligand. Mammalian 5-HT$_2$ receptors typically show high affinity for mianserin (Peroutka, 1990), and one of these receptors, the 5-HT$_{2C}$ receptor (previously known as 5-HT$_{1C}$), also shows high affinity for 5-HT (Pazos *et al.* 1985). Thus, a [^{3}H]5-HT binding site displaying moderate or low affinity for 8OH-DPAT and also moderate affinity for mianserin could represent a salmonid variant of the mammalian 5-HT$_{2C}$ receptor.

Molecular biology studies have revealed a very low level of homology between 5-HT$_1$ and 5-HT$_2$ receptors, suggesting that these two 5-HT receptor classes diverged early in evolution, perhaps even before the separation of vertebrates and invertebrates (Hen, 1992). The results from the present study suggest that representatives of both these receptor classes are present in the teleost brain. By contrast, Dietl and Palacios (1988) could not detect any 5-HT$_{2C}$ receptors in the teleost

Table 4. *Results from inhibition of [^{3}H]5-HT binding to membranes prepared from a tissue homogenate of Arctic charr whole brain by 8OH-DPAT in the presence or absence of GTP*

	n_H	K_{i1} (nmol l^{-1})	B_{max1} (fmol mg^{-1} protein)	K_{i2} (nmol l^{-1})	B_{max2} (fmol mg^{-1} protein)	K_{i3} (nmol l^{-1})	B_{max3} (fmol mg^{-1} protein)
Without GTP	0.42±0.03	1.7±0.8	21.5±3.5	82±51	16.6±3.2	9000±6000	8.5±1.4
With 0.5mmol l^{-1} GTP	0.40±0.06	1.4±1.0	9.0±4.1	49±32	17.1±3.8	13800±11400	11.0±2.2

Data (means ± S.E.M.) are from the curve in Fig. 4 as determined by non-linear curve-fitting using LIGAND (see Materials and methods) and a three-site model.

n_H, Hill constant.

brain, as determined by [3H]mesulergine binding using an autoradiographic technique. However, teleosts separated from other vertebrates during the Devonian period, 400 million years ago, so differences in binding characteristics between mammalian and teleost 5-HT receptors are to be expected. In fact, even though the human 5-$HT_{1Dß}$ receptor and the rat 5-HT_{1B} receptor display 93% amino acid sequence identity, they show distinct differences in their pharmacology (Boess and Martin, 1994).

In the present study, GTP had a drastic effect on the saturation binding curve, reducing B_{max} values by approximately 50%, whereas K_D values for [3H]5-HT were not affected. Similarly, GTP had no effect on apparent K_i values for 8OH-DPAT but reduced the B_{max} value for the binding site displaying high affinity for 8OH-DPAT by 58%, whereas B_{max} values for sites showing medium and low affinity for this drug were unaffected by GTP. Hydrolyzable guanine nucleotides, such as GTP and GDP, are known to alter agonist binding to G-protein-linked receptors by increasing the receptor affinity for agonists (Peroutka *et al.* 1979; Mallat and Hamon, 1982; Sills *et al.* 1984). Moreover, there are some reports on GTP-induced reductions in B_{max} of putative 5-HT receptors. For instance, Robaut *et al.* (1985) found that 0.1mmol l^{-1} GTP reduced B_{max} values of putative 5-HT_1 receptors in synaptosomal fractions prepared from rat brain cortex by approximately 80%. Mallat and Hamon (1982) reported that GTP reduced specific [3H]5-HT binding to rat hippocampal membranes, an effect that was mediated by a decrease in B_{max} as well as by an increase in apparent affinity, while in the study by Robaut *et al.* (1985), GTP reduced B_{max} without affecting the apparent K_D for [3H]5-HT.

In conclusion, the results of the present study show that there are at least three different high-affinity [3H]5-HT binding sites in the Arctic charr brain. One of these sites has a pharmacological profile similar to that of the mammalian 5-HT_{1A} receptor, is sensitive to GTP (suggesting G-protein coupling) and is thus probably a teleost version of this receptor. The identities of the remaining two [3H]5-HT binding sites are not as clear, although one of them may represent a teleost 5-HT_{2C} receptor.

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