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Solubilization and characterization of kainate receptors from goldfish brain

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The binding of [^{3}H]kainate to goldfish brain membrane fragments was investigated. Scatchard analysis revealed a single class of binding sites in Tris-HCl buffer with a K_d of 352 nM and a B_{max} of 3.1 pmol/mg wet weight. In Ringer's saline, [^{3}H]kainate bound with a B_{max} of 1.8 pmol/mg wet weight and a K_d of 214 nM. Binding in Ringer's saline, but not Tris-HCl buffer, displayed positive cooperativity with a Hill coefficient of 1.15. The [^{3}H]kainate binding sites were solubilized in Ringer's saline using the nonionic detergent *n*-octyl-β-D-glucopyranoside. Approximately 30–50% of the total number of membrane-bound binding sites were recovered on solubilization. The K_d of [^{3}H]kainate for solubilized binding sites was approximately 200 nM. The rank order of potency for glutamatergic ligands at inhibiting [^{3}H]kainate binding was identical and the competitive ligands had similar K_i values in both membranes and solubilized extracts. In membrane preparations, [^{3}H]kainate displayed a two component off-rate with k_{off} values of 0.97 min^{-1} and 0.07 min^{-1}; in solubilized extracts, however, only a single off-rate ($k_{off} = 0.52$ min^{-1}) was observed. The hydrodynamic properties of *n*-octyl-β-D-glucopyranoside solubilized [^{3}H]kainate binding sites was investigated by sucrose density centrifugation. A single well defined peak was detected which yielded a sedimentation coefficient of 8.3 S. The results presented in this report suggest that goldfish brain may provide an ideal system in which to study kainate receptor biochemistry.

Introduction

The excitatory amino acid L-glutamate is a major neurotransmitter in the vertebrate central nervous system [1,2]. The receptors which mediate the actions of L-glutamate have been investigated using a variety of experimental approaches including electrophysiological, autoradiographic, and ligand binding techniques. There is now considerable evidence to indicate that glutamate receptors can be classified into at least three distinct subtypes which are selectively activated by the amino acid analogues *N*-methyl-D-aspartate (NMDA), quisqualate or kainate [1,3,4,5]. Despite recent advances in the understanding of the molecular function and pharmacology of glutamate receptors, virtually nothing is known about their biochemistry. A crucial first step towards the biochemical characterization of glutamate receptors will be the removal of the protein(s) from the plasma membrane by solubilization to allow subsequent purification and reconstitution of these receptors.

Kainate is a cyclic analogue of glutamate that

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisooxazole-4-proprionic acid; ABP, 2-amino-4-phosphonobutyric acid.

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has been shown to exert potent neuroexcitatory and neurotoxic effects [6]. Radioligand binding studies with [^{3}H]kainate have demonstrated specific, saturable, high-affinity binding to rat brain membrane preparations [1,6]. The pharmacological specificity of [^{3}H]kainate binding in rat brain membranes differs markedly from that of L-glutamate, NMDA and AMPA. The most potent displacer of kainate appears to be kainate, with APB, AMPA and the NMDA agonists and antagonists exerting only low potency as inhibitors of [^{3}H]kainate binding [1].

Structure activity studies with kainate analogues have suggested that the isopropylene side chain of the molecule is important for receptor recognition [7]. The isopropylene side chain, however, has no structural homology to glutamate or aspartate molecules. Several alternative theories have been proposed to reconcile these observations including the suggestions that the side chain may be involved in interactions with a lipophilic membrane region [8], that π electrons associated with the side chain may be important in receptor binding [9], or that the side chain may be involved in conformational alterations of the kainate molecule in the microenvironment of the receptor [10].

In goldfish brain membranes, kainate receptors have been demonstrated to be present in far greater numbers than in mammalian brain but are similar to the values reported for pigeon cerebellar membranes [11–13]. The kainate receptors appear to be concentrated in the synaptosomal membrane fraction where the density of kainate binding sites is approximately two orders of magnitude greater than that present in rat brain or pigeon optic tectum membranes [13].

The physiological action mediated by kainate receptors is unclear, but it has been suggested that they may be important in a mechanism of synaptic transmission or modulation and could be involved in eliciting the release of a variety of neurotransmitters [14,15]. More recent evidence to suggest that kainate receptors may be important in synaptic transmission in goldfish has been reported by Langdon and Freeman [16,17]. These workers, using electrophysiological techniques, report that a variety of antagonists selective for glutamatergic receptor subtypes can block optic tract evoked postsynaptic field potentials in isolated optic tectum and that the rank order of potency of the ligands that inhibit postsynaptic field potentials suggests that synaptic transmission in this system may be mediated via kainate receptors.

In this study the binding of [^{3}H]kainate to goldfish brain membranes and solubilized extracts was characterized. The pharmacology of the receptors in membranes and solubilized extracts was compared. We provide evidence to suggest that goldfish brain is a rich source of kainate binding sites which can be successfully solubilized and that this tissue may be a convenient model system in which to study the biochemistry of kainate receptors.

Methods

Tissue preparation. Whole brains were removed from ice anesthetized goldfish and either used immediately or rapidly frozen in liquid N_2 until use. Brains were homogenized in a cooled glass homogenizer in 4–6 volumes of buffer containing 320 mM sucrose. Several different buffers were investigated to determine the optimum binding conditions for [^{3}H]kainate: (1) 50 mM Mops-NaOH, 1 mM EGTA (pH 7.4); (2) 50 mM Tris-HCl (pH 7.4); (3) Ringer's saline, 80 mM NaCl, 3.5 mM KCl, 10.6 mM sodium phosphate, 1.2 mM $MgCl_2$, 1.3 mM $CaCl_2$ (pH 7.4). For some experiments, protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM 1,10-phenanthrolene, 1 mM iodoacetamide, 1 μM pepstatin A, 1 mg/l antipain, 1 mg/l leupeptin) were included in the buffers. The homogenate was centrifuged for 10 min at $1000 \times g$ at 0 °C. The supernatant was collected and centrifuged for 30 min at $48\,000 \times g$ at 0 °C, and the resultant pellet resuspended in 2 parts buffer, 1 part distilled water. The suspension was centrifuged for 20 min at $18\,000 \times g$ and the pellet collected. For membrane experiments the pellet was resuspended as appropriate in ice-cold buffer and used immediatley.

Detergent solubilization. Brain membranes were prepared in Ringer's saline as described and the washed P2 pellet resuspended in Ringer's saline containing a final concentration of 1% detergent (routinely, *n*-octyl-β-D-glucopyranoside). The detergent-tissue suspension was incubated for 60–90

min at 4° C with gentle agitation and then centrifuged for 1 h at 100 000 × *g* in a Beckman Ti50 rotor at 4° C to separate solubilized proteins from nonsolubilized tissue. The clear supernatant was collected and used as appropriate.

Membrane binding assays. Specific [^{3}H]kainate binding was determined using a filtration assay. [^{3}H]kainate, typically at a final concentration of 10 nM, was added to tubes containing membranes (approximately 20 mg wet weight per tube) in either the presence or absence of 100 μM cold kainate. Following incubation on ice for 1 h, the reaction mixtures were filtered under negative pressure on polyethylenimine (PEI) wetted (0.05%) Schleicher and Schuell No. 32 glass filters [18]. The filters were rapidly washed three times with 3 ml of ice-cold buffer and dried under suction. The filters were then transferred to scintillation vials and the bound radioactivity measured.

Solubilized extract binding assays. Solubilized extracts (approximately 20 mg wet weight per tube) were incubated with [^{3}H]kainate under exactly the same conditions as described for membrane preparations. For solubilized receptor assays two protocols were investigated: a polyethylenimine (PEI) method [18] and a polyethylene glycol (PEG) method [19]. For the PEG assay technique, the solubilized binding sites were incubated with 10 nM [^{3}H]kainate for 1 h at 0° C. An equal volume of 20% PEG was then added and the mixture incubated a further 20 min on ice. The mixture was filtered on Schleicher and Schuell No. 32 glass filters under negative pressure and the filters washed three times with ice-cold Ringer's saline containing 10% PEG. For the PEI assay technique, the procedure was essentially the same as described for membrane fragments except that a higher concentration of PEI was used. The reaction mixtures were filtered on Schleicher and Schuell No. 32 glass filters that had been wetted with 0.3% PEI. The filters were washed three times with ice-cold Ringer's saline, dried and the bound radioactivity measured.

[^{3}H]Kainate dissociation rate assay. Goldfish brain membrane fragments or solubilized extracts were incubated with 50 nM [^{3}H]kainate for 1 h on ice. At time zero, 1 M unlabeled kainate was added to a final concentration of 1 mM and the suspension mixed thoroughly. Samples (0.5 ml) were taken at appropriate intervals and the amount of bound [^{3}H]kainate assayed as described above.

Sucrose density gradients. Continuous 5% to 20% sucrose density gradients (34 ml) were prepared as described previously [19] in Ringer's saline containing 1% *n*-octyl-β-D-glucopyranoside. Solubilized goldfish brain extract (4 ml) containing 1% sucrose and catalase and alkaline phosphatase (approx. 1 mg/ml) were layered on top of 34 ml sucrose gradient and the tubes filled with buffer. The tubes were sealed and centrifuged for 2 h at 200 000 × *g* in a Beckman VTi50 vertical rotor (slow acceleration and no brake). Fractions (35 drops, approx. 0.5 ml) were collected from the bottom of the tube at 4° C. Aliquots (200 μl from each fraction were added to 300 μl Ringer's saline containing 50 nM [^{3}H]kainate and the amount of binding protein present determined using PEI filtration assays. Catalase activity was assessed by measuring the hydrolysis of H_2O_2 as described by Beers and Sizer [20]. Human placental alkaline phosphatase activity was monitored using a *p*-nitrophenyl phosphate (PNPP) assay [21].

Data analysis. Saturation isotherms were analysed with a nonlinear least-squares fit (Marquart-Levenberg algorithm in the VMS version of PLOT obtained from New Unit, Ithaca, NY) to a one component model with or without cooperativity (Hill equation). Dissociation rates were determined by a nonlinear least squares fit of the normalized data to a two exponential decay. All calculations were performed on a Digital VAXStation II minicomputer.

Materials

Goldfish (*Carassius auratus*) were from Grassyfork fisheries, Martinsville IN. [^{3}H]Kainate (3.6 Ci/mmol) was from New England Nuclear, Boston, MA. *n*-Octyl-β-D-glucopyranoside, kainate and all other reagents were obtained from Sigma, St. Louis, MO.

Results

[^{3}H]Kainate binding to membranes

The binding of [^{3}H]kainate to membrane preparations was investigated in three different buffers. No reproducible [^{3}H]kainate binding was

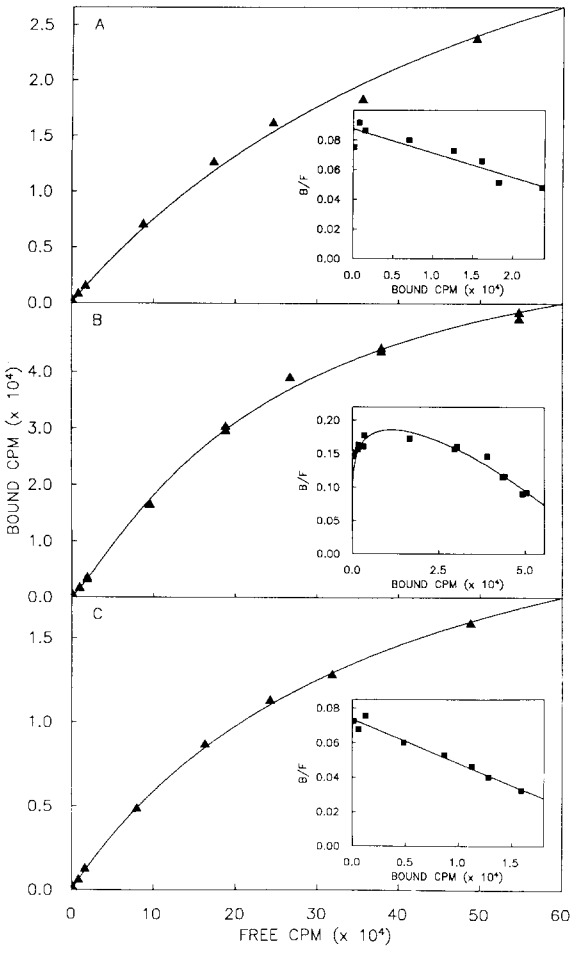

Fig. 1. Saturation binding curves and Scatchard plot of [^{3}H]kainate binding to membrane fragments and *n*-octyl-β-D-glucopyranoside solubilized extracts from goldfish brain. (A) Membrane fragments in Tris-HCl buffer. Samples of tissue (10–20 mg wet weight) were incubated in Tris-HCl buffer with various concentrations of [^{3}H]kainate in either the presence (nonspecific) or absence (total binding) of 100 μM unlabeled kainate. Specific binding was determined by subtracting the total bound from the expected value for nonspecific binding calculated from a linear least-squares fit to the [^{3}H]kainate bound in the presence of excess nonradioactive kainate. [^{3}H]Kainate bound to goldfish brain membranes in Tris-HCl buffer with a K_d of 352 ± 36 nM and a B_{max} of 3.1 ± 0.3 pmol/mg wet weight of tissue (mean and S.E. of three experiments each performed in duplicate). (B) Membrane fragments in Ringer's saline. Experiments were performed exactly as described above except Ringer's saline was substituted for Tris-HCl. The K_d was 214 ± 28 nM and the B_{max} was 1.83 ± 0.11 pmol/mg wet weight of tissue (mean and S.E. of eight experiments each performed in duplicate). There was positive cooperativity under these conditions with a Hill coefficient of 1.15 ± 0.01. (C) Solubilized extracts. Exactly the same incubation conditions were used as described for membrane fragment experiments in Ringer's saline. The K_d for [^{3}H]kainate binding to detergent extract was 199 ± 17 nM and the B_{max} of 0.98 ± 0.06 pmol/mg wet weight of tissue (mean and S.E. of five experiments each performed in duplicate). In all cases the solid line through the points represents a nonlinear least-squares fit to the data as described in Methods.

observed in Mops/EGTA buffer (data not shown) but binding was obtained in Tris-HCl (Fig. 1A) and Ringer's saline (Fig. 1B). The binding characteristics were slightly different in the two buffers, the K_d and B_{max} being 352 ± 36 nM and 3.1 ± 0.3 pmol/mg wet weight of tissue, respectively, in Tris-HCl (n = 3) and 214 ± 29 nM and 1.83 ± 0.11 pmol/mg wet weight of tissue, respectively, in Ringer's saline (n = 8). In Ringer's saline, but not in Tris-HCl buffer, positive cooperativity with a Hill coefficient of 1.15 ± 0.01 was observed. An alternative explanation for the curved Scatchard plots in Ringer's saline is that the tissue was not incubated with [^{3}H]kainate for sufficient time to reach equilibrium (usually 90 min on ice). This possibility, however, was ruled out by experiments in which membranes were incubated with [^{3}H]kainate at 0 °C for 7 h. Identical results were obtained (data not shown). Similar results suggesting positive cooperativity of [^{3}H]kainate binding sites in pigeon cerebellar membranes and goldfish brain membranes have been reported previously [22,13]. The presence or absence of protease inhibitors (see Methods) did not alter [^{3}H]kainate binding to membrane preparations in either buffer. Despite the more complex binding characteristics, [^{3}H]kainate bound with a higher affinity in Ringer's saline than in the other buffers and was therefore routinely used for solubilization experiments.

Kainate receptor solubilization

(i) Detergents. Several different detergents and two different assay techniques were used to determine the optimum conditions for solubilization of the [^{3}H]kainate binding sites from goldfish brain (Table I). The two different assay techniques detected markedly different amounts of bound [^{3}H]kainate. The most probable reason for these differences is that the addition of PEG to the reaction mixture (20%) and to the wash buffer (10%) caused a substantial increase in the viscosity

TABLE I

RELATIVE EFFICIENCY OF VARIOUS DETERGENTS AT SOLUBILIZING [^{3}H]KAINATE BINDING SITES FROM GOLDFISH BRAIN

Membrane fragments were incubated at 4°C for 90 min in Ringer's saline containing protease inhibitors and a final concentration of 1% of one of a variety of different detergents. The resultant suspension was centrifuged at 100000 × *g* for 1 h to pellet all nonsolubilized material. The supernatant representing solubilized extract was collected and incubated with 50 nM [^{3}H]kainate for 1 h on ice in either the presence or absence of 100 μM unlabeled kainate. The number of [^{3}H]kainate binding sites were determined using two different filter assay techniques (see Methods). The results are expressed as the number of [^{3}H]kainate binding sites that were detectable in the solubilized extract as a percentage of the total number of sites detected in membrane fragments prior to solubilization. It is possible that the absolute fraction of binding sites solubilized by any detergent may be greater than the fraction detected since the binding assays used for solubilized receptors are likely to be less efficient than the assay used to measure membrane-bound receptors.

Detergent	Percentage of [^{3}H]kainate radioactivity bound to membrane fragments	
	PEI assay	PEG assay
Lubrol	6.3	4.3
Triton X-100	9.1	6.8
Cholic acid	12.2	5.7
Chaps	14.8	6.0
Digitonin	22.0	10.2
n-Octyl-β-D-glucopyranoside	31.2	13.2

of the Ringer's saline, increasing the time required to filter each sample. Because the dissociation of [^{3}H]kainate from its receptors is fairly rapid, a greater percentage of the bound radioactivity would be expected to dissociate during the PEG assay procedure than during the PEI assay procedure.

n-Octyl-β-D-glucopyranoside was the most efficient detergent investigated for solubilizing kainate binding sites, with a yield of between 30% and 50% of the total number of sites present in the membrane preparation. The reasons for the loss of the majority of kainate binding sites on detergent extraction is unclear, but may be due to either incomplete solubilization of the binding sites under the conditions used or to detergent modification and consequent inactivation of a fraction of the [^{3}H]kainate binding sites. Qualitatively similar losses have been observed on solubilization of neuronal nicotinic acetylcholine receptors from goldfish brain with the nonionic detergent Triton X-100 [24].

(ii) Binding to solubilized extracts. Saturation binding and Scatchard plots for the binding of [^{3}H]kainate to solubilized goldfish brain extracts are shown in Fig. 1C. The binding characteristics of [^{3}H]kainate were similar to those observed in membranes. The K_d slightly decreased from 214 nM to 200 nM and the B_{max} decreased from 1.8 pmol/mg wet weight of tissue to approx. 1 pmol/mg wet weight of tissue on solubilization. The major difference between [^{3}H]kainate binding to membranes and solubilized extract was that no positive cooperativity was observed in the solubilized tissue preparations.

Competitive binding studies

To determine the pharmacological characteris-

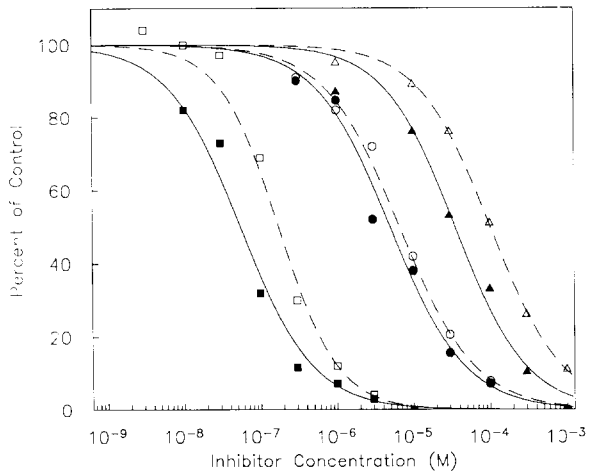

Fig. 2. [^{3}H]Kainate binding inhibition curves in membrane fragments and solubilized extracts. The rank order of potency and K_i values of kainate, kynurenic acid and L-glutamate for inhibition of [^{3}H]kainate binding are shown. The K_i values were calculated from the IC_{50} values using the equations derived by Cheng and Prusoff [38]. The K_i values for membrane fragments and solubilized extracts respectively were: kainate, 168 nM and 53 nM; kynurenic acid, 6.47 μM and 4.83 μM; L-glutamate, 99.2 μM and 34.4 μM. The symbols represent: □, kainate on membranes; ■, kainate on soluble extract; ○, kynurenic acid on membranes; ●, kynurenic acid on soluble extract; △, L-glutamate on membranes; ▲, L-glutamate on soluble extract.

tics of the solubilized kainate binding site, a series of displacement studies were carried out on both solubilized extracts and membrane fragments (see Fig. 2). The inhibition profiles of kainate, kynurenic acid and L-glutamate on [^{3}H]kainate binding to solubilized receptors and membranes were very similar. The order of potency is identical in membrane and solubilized extracts and agrees well with previously reported values in both goldfish brain membranes [13] and rat brain membranes [1]. The data are also in close agreement with previous results obtained in this laboratory using an extensively washed 'glutamate free' goldfish brain membrane preparation and Tris-HCl buffer [39].

[^{3}H]Kainate dissociation rates from membranes and solubilized extracts

To characterize [^{3}H]kainate binding further, the association rates to solubilized extracts (data not shown) and the dissociation rates from membranes and solubilized extracts (Fig. 3) were investigated. The association rate at 0°C was very rapid, with the major portion (80–90% of total bound) of [^{3}H]kainate binding being complete

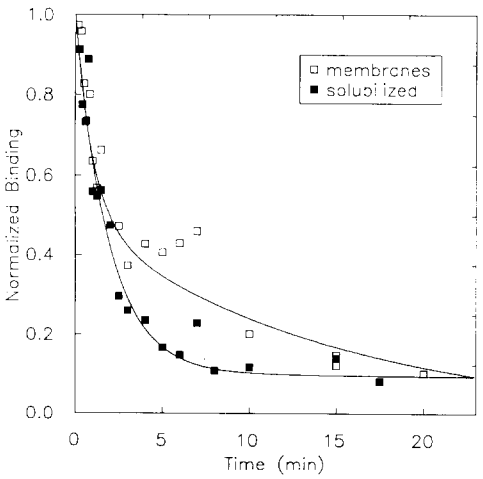

Fig. 3. Dissociation rates of [^{3}H]kainate from membrane fragments and solubilized extracts. Membrane fragments or solubilized extract were incubated with [^{3}H]kainate for 1 h on ice. Excess unlabeled kainate (1 mM) was then added and samples taken at the indicated time points and immediately filtered and washed on PEI-treated glass filters. The off-rates were 0.972 min^{-1} and 0.071 min^{-1} from membranes and 0.515 min^{-1} from solubilized extracts.

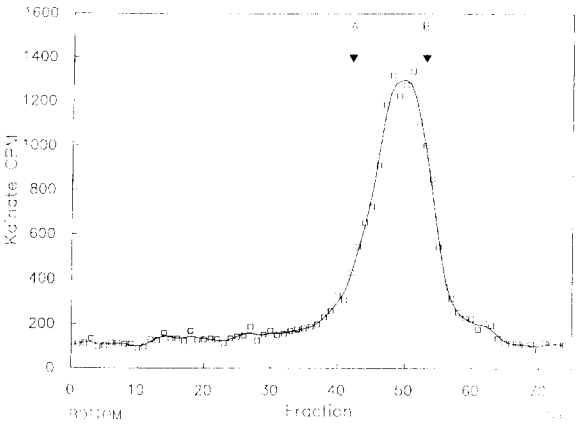

Fig. 4. Hydrodynamic characateristics of [^{3}H]kainate binding protein in sucrose density gradients. Solubilized extract (4 ml) containing the size markers catalase (A) and alkaline phosphatase (B) was layered on top of a 34 ml 5–20% sucrose gradient. The gradients were centrifuged for 2 h at 200000 × *g* and 35 drop fractions collected from the bottom of the tube. Aliquots (0.2 ml) were taken from each fraction, diluted to 0.5 ml in Ringer's saline and incubated for 1 h on ice with 50 nM [^{3}H]kainate. The number of [^{3}H]kainate binding sites in each fraction was measured using a PEI filtration assay.

before the first samples were taken (15 s). [^{3}H]Kainate had a rapid off-rate from both membranes and solubilized extracts. The dissociation rate (k_{off}) of [^{3}H]kainate from membranes was composed of two components (0.972 min^{-1} and 0.07 min^{-1}) each representing approx. 50% of the binding sites at 10 nM. For solubilized binding sites, the dissociation consisted of essentially a single off-rate (0.515 min^{-1}), with only a small component (less than 10%) with a much slower dissociation.

Hydrodynamic properties of the kainate receptor

Some hydrodynamic properties of the solubilized kainate receptor were investigated using sucrose density centrifugation. As shown in Fig. 4, the solubilized [^{3}H]kainate binding protein migrated into the gradient with a peak occurring between bovine liver catalase (M_r 250000, sedimentation coefficient 11.4 S [20]) and human placental alkaline phosphatase (M_r 125000, sedimentation coefficient 6.8 S [21]). The sedimentation coefficient for the *n*-octyl-β-D-glucopyranoside-[^{3}H]kainate binding protein complex was 8.3 S.

Discussion

The main observations made in this study were: (1) [^{3}H]Kainate binds to a single class of high-affinity sites in goldfish brain membranes. (2) Up to 50% of the [^{3}H]kainate receptors were recoverable on solubilization with 1% *n*-octyl-β-D-glucopyranoside. (3) The K_d values were 214 ± 28 nM and 199 ± 17 nM for membrane bound and solubilized receptors, respectively, and the B_{max} values were 1.83 ± 0.11 pmol/mg wet weight of tissue and 0.98 ± 0.06 pmol/mg wet weight of tissue for membrane bound and solubilized receptors, respectively. (4) The pharmacological characteristics were similar for both membrane bound and solubilized receptors with an identical rank order of potency of displacement by competitive glutamatergic ligands. (5) The solubilized kainate receptor sedimented in sucrose density gradients at 8.3 S.

The effects of kainate on the vertebrate central nervous system are of interest because it exerts potent neuroexcitant, neurotoxic and convulsant effects. Evidence to suggest that the [^{3}H]kainate binding sites represent kainate receptors includes a good correlation between the regional distribution of [^{3}H]kainate binding sites and the ability of kainate to cause lesions in rat brain [25–29]. In addition, because kainate does not appear to be actively taken up by neuronal tissue [30], the concentration of kainate applied to tissue in vitro should be the concentration present at the receptor, providing equilibrium between the tissue and the bathing medium is achieved. Kainate concentrations in the order of 10–100 μM are necessary to half maximally activate increases in cGMP levels or $^{22}Na^+$ fluxes in rat brain slices [31,32]. Localized application of 0.1–1 μM kainate to CA3 pyramidal cells in the hypocampus, however, produces excitatory responses at concentrations similar to the K_i for kainate binding.

The characteristics of [^{3}H]kainate binding to goldfish brain membrane fragments presented in this study differ slightly from the results reported by Migani et al. [13] using similar conditions. These workers detected [^{3}H]kainate binding sites in the P2 fraction which displayed positive cooperactivity ($n_H = 1.29$) and had a K_d of about 50 nM and a B_{max} of approx. 140 pmol/mg protein. Similarly, in this study [^{3}H]kainate binding sites in Ringer's saline displayed positive cooperativity ($n_H = 1.15$) but the apparent K_d was markedly different (214 nM). The reasons for the difference in K_d is unclear. The rank order of potency of various glutamatergic ligands at inhibiting [^{3}H]-kainate binding and the concentration at which they were half maximally effective, however, agreed well between the two studies. Also, a rapid biphasic dissociation of [^{3}H]kainate from its binding sites in membranes was also observed in both reports.

The presence of positive cooperativity in Ringer's saline but not in Tris-HCl buffer suggest that [^{3}H]kainate binding to goldfish brain membranes may be modulated by the presence or absence of ions. Detailed investigation of possible ion effects was not pursued in this study, but in rat brain membranes, both mono- and divalent cations have been shown to depress [^{3}H]kainate binding while anions appear to be without effect [23]. Nevertheless, the observation of positive cooperativity suggests that more than one binding site may be present on a single putative kainate receptor.

Some evidence regarding the physiological function of kainate receptor has been obtained using evoked field potentials in goldfish optic tectum slices in vitro [16,17]. These workers used a variety of selective L-glutamate receptor subtype antagonists and recorded their effects on optic tract evoked field potentials in the isolated optic tectum. Three antagonists produced almost complete inhibition of the postsynaptic field potentials. The most potent inhibitor was kynurenic acid followed by γ-D-glutamylglycine (γ-D-GG) and *cis*-2,3-piperidine dicarboxylic acid (PDA). An antagonist selective for the NMDA receptor, 2-amino-5-phosphonovalerate (APV), had negligible effects on postsynaptic field potentials and some evidence has suggested that γ-D-GG has little effect on quisqualate receptor at concentrations that block kainate receptors [3,33–35]. These data may suggest that the retinotectal transmission in goldfish could be mediated by kainate receptors.

We have recently studied the effects of eye removal on the number of glutamatergic and nicotinic binding sites in the contralateral tectum

of goldfish [39]. As has been previously demonstrated, enucleation results in a 40–50% decrease in the number of [^{125}I]α-Bgt and (−)-[^{3}H]nicotine binding sites in the contralateral tectum. There was no alteration, however, in the number of L-[^{3}H]glutamate, NMDA displaceable L-[^{3}H]glutamate, [^{3}H]AMPA or [^{3}H]kainate binding sites following eye removal. These results do not rule out a role for kainate receptors in this system but suggest that their number is not modulated by the removal of visual stimulation over the time period investigated (up to 18 days).

A brief account of the solubilization of kainate binding sites from pigeon cerebellum has been reported previously [36]. These workers used Triton X-100 to solubilize both a high- and low-affinity kainate binding site with K_d values of 20 nM and 440 nM, respectively, in the detergent extracts. The low-affinity site was reported to display positive cooperativity following solubilization and had an apparent molecular weight of 440 000 on gel filtration and a sedimentation coefficient of 12 S on sucrose density gradients.

In comparison to nicotinic acetylcholine receptors the level of knowledge about the biochemistry glutamate receptors is rudimentary. The rapid dissociation rate of [^{3}H]kainate from its binding sites reported in this study suggests that purification of this protein by affinity chromatography may be difficult in the absence of specific ligands with higher affinity (e.g., monoclonal antibodies). We have, however, obtained preliminary data to suggest that solubilized [^{3}H]kainate binding sites can be reconstituted into crude soya bean phospholipid vesicles (Henley, Horne and Oswald, unpublished data). Experiments are currently in progress to characterize the reconstituted binding sites and to attempt to obtain kainate activated radioisotope fluxes in vesicle preparations.

An interesting and potentially important aspect of the study of kainate receptors lies in the fact that the selective neurotoxic actions of kainate in the vertebrate central nervous system are reminiscent of damage observed in the brains of patients suffering from Huntington's disease and epilepsy [9]. Kainate binding sites have been demonstrated in human brain and appear to be present in the caudate nucleus which is known to degenerate in Huntington's disease. An understanding of the biochemistry of kainate receptors, therefore, may provide some clues to the etiology of these neurodegenerative diseases [23,37].

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