A Kainic Acid Receptor from Frog Brain Purified Using Domoic Acid Affinity Chromatography*

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A kainic acid receptor was purified from Triton X-100/digitonin-solubilized frog brain membranes. The purification was carried out in two steps: ion exchange chromatography using DEAE-Sepharose CL-6B and affinity chromatography with domoic acid immobilized on Sepharose 4B. The specific binding activity of the affinity-purified receptor is 481-fold higher than that of the crude solubilized preparation and 1617-fold higher than that of the whole membrane fraction. Scatchard analyses of the affinity-purified receptor showed a curvilinear plot which fit a two-site model with dissociation constants of 5.5 and 34 nm and B\text{max} values of 1700 pmol/mg protein and 4400 pmol/mg protein for the high and low affinity components, respectively. The dissociation constants of the purified receptor are similar to those of the crude soluble preparation (4.8 and 39 nm). Inhibition constants for several kainic acid analogs were also similar for the purified and crude preparations. The active purified receptor migrated with a \( M_\text{r} = 570,000 \) on gel filtration analysis using Sepharose 6B. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the affinity-purified receptor showed a single broad band with silver stain, migrating with a \( M_\text{r} = 48,000 \).

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There is now compelling evidence that glutamate (or related compounds, collectively known as excitatory amino acids) is a major neurotransmitter in the central nervous system. The development of selective pharmacological agents has allowed the electrophysiological characterization of neuronal receptors for excitatory amino acids. These studies have identified three major classes of excitatory amino acid receptors based on the agonist preferred by the receptor: kainate (KA),1 quisqualate, and N-methyl-D-aspartate receptors (Watkins and Evans, 1981). Discrete receptor classes similar to those determined electrophysiologically have also been demonstrated in radioligand binding studies using neuronal membranes and tissue sections (Monaghan et al., 1983; Foster and Fagg, 1984). While such studies give information on receptor distribution, amount, and pharmacological properties, a more thorough knowledge of the molecular properties of these receptors is required not only to understand the basis of excitatory amino acid neurotransmission but also to investigate the numerous reports of an involvement of these neurotransmiters in several human neurological disorders (Greenamyre, 1986; Meldrum et al., 1986).

We have recently reported the solubilization of an active KA binding protein from rat brain (Hampson et al., 1987b). While this represents the initial step toward the characterization of the KA receptor, the purification of this receptor is hampered by its low concentration in mammalian brain and the lack of an efficient method of purification. Such limitations are typical of receptor purifications but can often be circumvented by using an enriched source of receptor and purification using affinity chromatography. It has been reported that brains of several species have much higher levels of KA receptor than does rat brain, with frog brain being among the highest (London et al., 1980). In the present study we have used frog brain as a source for the purification of a KA receptor. This purification was facilitated by our development of a domoic acid affinity column.

**EXPERIMENTAL PROCEDURES**

**Materials**—[\( ^\text{3}^\text{H} \)]KA (60 Ci/mmol) and Aquasol were purchased from Du Pont-New England Nuclear. KA, dihydrokainic acid, glutamic acid, quisquicular acid, Triton X-100, digitonin, EGTA, polyethylene glycol, and all gel filtration and electrophoresis standards were from Sigma. Domoic acid was obtained from Cambridge Research Biochemicals. Tris and phenylmethylsulfonyl fluoride were from Bethesda Research Laboratories. EDAC and electrophoresis reagents were from Bio-Rad. DEAE-Sepharose CL-6B, AH-Sepharose 4B, and Sepharose 6B were purchased from Pharmacia LKB Biotechnology Inc.

Preparation of Domoic Acid Affinity Resin—One gram of AH-Sepharose 4B was swollen in 0.5 M NaCl for 30 min and washed in a sintered glass funnel with 200 ml of this solution followed by 200 ml of distilled water. All procedures were carried out at room temperature. Domoic acid (10 mg) was dissolved in 1 ml of water, and EDAC (4 mg) was dissolved in 1 ml of water. One ml of each of these solutions was added to 1 ml of the washed gel, and the pH was immediately adjusted to 6.1 using NaOH and HCl. The pH was monitored over the next 60 min and adjusted to pH 6.1 as necessary. The incubation was continued for 20 h on a slowly rotating shaker after which the gel was allowed to settle and the supernatant fraction aspirated. The gel was washed with three cycles of a solution of 0.1 M sodium acetate, pH 4.0, containing 0.5 M NaCl and a solution of 0.1 M sodium bicarbonate, pH 8.3, containing 0.8 M NaCl. The gel was then washed with a solution of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100, 10% glycerol, and 0.5 M NaCl. Prior to use, the affinity resin was equilibrated with 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100 and 10% glycerol.

Membrane Preparation—Leopard frogs (Rana pipiens berlandieri) were obtained from Carolina Biological Supply Co. Frog brains (100) were dissected and homogenized in ice-cold water with a Brinkmann Polytron (6 brains/12 ml). The homogenate was centrifuged at 54,000 \( \times g \) for 15 min at 4 °C. All subsequent procedures, including all chromatographic steps, were conducted at 4 °C. The crude membranes were suspended in 50 mM Tris citrate, pH 7.0 (9 brains/12 ml) and centrifuged 54,000 \( \times g \) for 15 min. The washed crude membranes were stored at -70 °C.

Solubilization of Membranes and in Vitro Binding Assays—Solu-
bilization of frog brain membranes was carried out as described previously for rat brain membranes (Hampson et al., 1987b). Washed membranes from 100 frog brains were resuspended in 50 mM Tris citrate, pH 7.0, and incubated at 37 °C. This step has been shown to help remove endogenous inhibitors of KA binding (Sharif and Roberts, 1980). The suspension was centrifuged at 54,000 g for 30 min, centrifuged at 54,000 g for 15 min, and the supernatant was resuspended in 0.5 M potassium phosphate, pH 7.0, containing 10% glycerol to give a final protein concentration of 4-6 mg/ml. Phenylmethylsulfonyl fluoride and EGTA were added to final concentrations of 0.1 and 1.0 mM, respectively. Triton X-100 and digitonin were added to final concentrations of 1.0 and 0.2%, respectively. The mixture was incubated on ice with constant shaking for 30 min, centrifuged at 54,000 g for 15 min, and the supernatant was dialyzed against 1 liter (three changes) of 10 mM Tris citrate, pH 7.4, containing 0.1% Triton X-100, 10% glycerol, and 0.1 mM phenylmethylsulfonyl fluoride. After dialysis the preparation was centrifuged at 180,000 g for 1 h and immediately applied to the ion exchange column. The binding assays for membrane-bound and solubilized KA binding sites were carried out as described previously (Hampson et al., 1987b). Nonspecific binding was defined as the amount of [3H]KA binding remaining in the presence of 250 μM KA.

Ion Exchange Chromatography—After dialysis, the preparation was centrifuged at 180,000 g for 1 h. The supernatant fraction was applied to a column (2.7 x 10.5 cm) of DEAE-Sepharose CL-6B. After sample application, the column was washed with 10 mM Tris citrate, pH 7.4, containing 10% glycerol and 0.1% Triton X-100 for 10 h at a flow rate of 80 ml/h. After this time, protein was no longer being eluted from the column as determined by reading the absorbance at 280 nm. KA binding activity was eluted with a gradient consisting of 150 ml each of 10 mM Tris citrate, pH 7.4, containing 0.1% Triton X-100, 10% glycerol, and 0.1 mM Triton X-100, 10% glycerol, and 0.2 M NaCl. The flow rate was reduced to 40 ml/h, and 7-ml fractions were collected. Fractions were assayed for specific [3H]KA binding activity and protein.

Affinity Chromatography on Domoic Acid Sepharose—The pooled fractions from the DEAE-Sepharose CL-6B column were applied at a rate of 50 ml/h to a column containing 8 ml of the domoic acid affinity resin. The resin was washed sequentially with 50 ml of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100, 10% glycerol, and 100 mM NaCl; 250 ml of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100, 10% glycerol, and 250 mM NaCl; and 10 ml of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100 and 10% glycerol. KA receptors were specifically eluted with 24 ml of 62 μM KA in 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100 and 10% glycerol at 25 °C for 45 min. The eluate was collected on ice and dialyzed overnight against six changes (1.5 liters each) of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100 and 10% glycerol.

Affinity Chromatography on Domoic Acid Sepharose—The pooled fractions from the DEAE-Sepharose CL-6B column were applied at a rate of 50 ml/h to a column containing 8 ml of the domoic acid affinity resin. The resin was washed sequentially with 50 ml of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100, 10% glycerol, and 100 mM NaCl; 250 ml of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100, 10% glycerol, and 250 mM NaCl; and 10 ml of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100 and 10% glycerol. KA receptors were specifically eluted with 24 ml of 62 μM KA in 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100 and 10% glycerol at 25 °C for 45 min. The eluate was collected on ice and dialyzed overnight against six changes (1.5 liters each) of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100 and 10% glycerol.

Electrophoresis and Staining—Samples from various stages of the purification were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing or reducing conditions. The gels were silver-stained according to the procedure of Giulian et al. (1983).

Affinity Chromatography on Domoic Acid Sepharose—The pooled fractions from the DEAE-Sepharose CL-6B column were applied at a rate of 50 ml/h to a column containing 8 ml of the domoic acid affinity resin. The resin was washed sequentially with 50 ml of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100, 10% glycerol, and 100 mM NaCl; 250 ml of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100, 10% glycerol, and 250 mM NaCl; and 10 ml of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100 and 10% glycerol. KA receptors were specifically eluted with 24 ml of 62 μM KA in 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100 and 10% glycerol at 25 °C for 45 min. The eluate was collected on ice and dialyzed overnight against six changes (1.5 liters each) of 50 mM Tris citrate, pH 7.0, containing 0.1% Triton X-100 and 10% glycerol.

Preparation of Domoic Acid Affinity Resin—Preliminary experiments conducted to investigate the feasibility of using KA and KA analogs as immobilized ligands for affinity purification of KA receptors indicated that KA and domoic acid, immobilized via carbodiimide linkage, resulted in retention of solubilized binding activity. Attempts to immobilize KA and KA analogs through the amino group resulted in loss of binding activity in all cases. Based on this finding, EDAC/AH-Sepharose-linked KA and domoic acid were compared for their ability to bind solubilized KA receptors. Since domoic acid is not available in radiolabeled form, [3H]KA was used as a tracer to monitor the approximate efficacy of the coupling reaction. [3H]KA was found to couple to AH-Sepharose 4B in high yield using EDAC as a linker. Both immobilized compounds bound solubilized KA receptors (data not shown); domoic acid appeared to retain a higher percentage of solubilized binding sites. Based on these results, experiments were conducted to further characterize immobilized domoic acid.

The structures of domoic acid and KA are shown in Fig. 1. Although the compounds are structurally similar, domoic acid has an extended side chain substitution on carbon atom 3 of the heterocyclic ring which terminates in a carboxylic acid group (labeled 1 in Fig. 1). This additional functional group, not present in KA, may participate in the EDAC/AH-Sepharose 4B reaction but apparently is not critically responsible for the observed binding activity since immobilized KA also displayed activity, albeit lower than that observed for domoic acid. However, although all 3 carboxylic acid groups in the molecule (or any combination of these 3 groups) may be involved in the coupling reaction, the higher activity observed for domoic acid may be attributed in part, to immobilization via this functional group.

Purification of Kainic Acid Binding Sites—Membranes from frog brain were solubilized using a protocol developed for solubilization of KA receptors from rat brain (Hampson et al., 1987b). The suspension was centrifuged at 54,000 g for 1 h and immediately applied to the ion exchange column. The binding assays for membrane-bound and solubilized KA binding sites were carried out as described previously (Hampson et al., 1987b). Nonspecific binding was defined as the amount of [3H]KA binding remaining in the presence of 250 μM KA.
al., 1987b). Twenty-one percent of the total membrane bound sites were not solubilized by this procedure, a value similar to that observed in rat brain. However, unlike rat brain, the quantity of receptors solubilized in frog brain was equivalent to almost 1.8 times the amount of receptors measured in the untreated membranes. For data presented in Table I, a total amount of binding of 471 pmol (specific activity = 1.07 pmol/mg protein) was found in the membrane fraction, while 864 pmol was found in the crude solubilized fraction. Such an apparent increase in the numbers of receptors after detergent treatment has been reported previously for both excitatory amino acid receptors (Yoneda and Ogita, 1987) and other receptor systems (e.g. benzodiazapinely-aminobutyric acid system, see Yoneda et al., 1985; Kuriyama and Taguchi, 1987). It has been proposed (Kuriyama and Taguchi, 1987) that this phenomenon may be attributed to the removal of phospholipids by detergent since similar observations have been made on brain membranes treated with phospholipases (Havoundjian et al., 1986). It has also been suggested that the binding of glutamate and its analogs to membrane preparations is inhibited by endogenous compounds such as glutamate (Sharif and Roberts, 1980). Such compounds would likely be removed from the soluble preparation by the extensive dialysis used in our solubilization procedure.

The solubilized and dialyzed receptor preparation from frog brain was applied to a column of DEAE-Sepharose CL-6B (Fig. 2). Solubilized KA receptors were retained on the column during sample application and during the subsequent wash phase while most of the protein was eluted. The binding activity was eluted in a broad peak early in the gradient. This step resulted in a 21-fold purification over the crude solubilized preparation (Table I). Fractions from the DEAE-Sepharose CL-6B column were pooled as indicated in Fig. 2 and applied to the domoic acid affinity column. Most of the protein was recovered in the unretained fraction while less than 1% of the [3H]KA binding activity was recovered in this fraction (Fig. 3). Approximately 98% of the total protein recovered from the column was collected in the unretained and NaCl wash fractions. During the extended wash steps, particularly the buffer wash containing 250 mM NaCl, a portion (13%) of the binding activity was eluted. However, most of the binding activity was specifically eluted using 62 μM KA; the protein eluted in this fraction represented 1% of the total protein applied. This two-step purification procedure resulted in a 481-fold purification over the solubilized preparation. Thus, the final purified preparation with an average specific activity of 1730 pmol/mg protein represented a 1617-fold purification over that of the membrane-bound receptor concentration.

**Pharmacological Analyses**—Saturation analyses were conducted on the crude solubilized and purified preparations (Fig. 4). In both receptor preparations, the Scatchard plots were curvilinear in all cases suggesting the presence of more than one binding site. The Hill coefficient for both the crude solubilized and the purified preparation was 0.8. The computerized curve fitting program (LIGAND) used to analyze the data revealed that a two-site model provided a better fit to the data than did a one-site model as determined by a lesser sum of squares of the two-site model. The data did not fit a three-site model. The difference between the one- and the two-site model was not significant at the p = 0.05 level as determined by the F test. However, since the sum of the squares was less for a two-site model and previous pharmacological studies have consistently reported the presence of two binding sites for KA (London and Coyle, 1979; London et al., 1980; Foster et al., 1981; Honore et al., 1986), we have assumed a two-site model in the present study. The pharmacological properties of the purified binding sites were further...
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FIG. 4. A, saturation and computer-generated Scatchard plots for specific \[^{3}H\]KA binding to purified KA receptors. Each point represents the average of three experiments done in duplicate. The $K_d$ and $B_{max}$ values (mean ± S.E.) determined using a computerized nonlinear curve-fitting program (LIGAND; Munson and Rodbard, 1980) were $5.5 ± 1.1 \text{nM}$ and $1700 ± 420 \text{pmol/mg protein}$ and $34 ± 9 \text{nM}$ and $4400 ± 1200 \text{pmol/mg protein}$ for the high and low affinity components, respectively. B, representative saturation and Scatchard plots of the crude solubilized \[^{3}H\]KA binding sites. Values are the average of five experiments done in duplicate. The $K_d$ and $B_{max}$ values were $4.8 ± 1.5 \text{nM}$ and $3.8 ± 1.4 \text{pmol/mg protein}$ for the high affinity site and $39 ± 6.7 \text{nM}$ and $12.8 ± 1.3 \text{pmol/mg protein}$ for the low affinity site.

FIG. 5. Competition profiles for the crude solubilized and purified KA receptor preparations. Binding assays were conducted using 5 nM \[^{3}H\]KA. Aliquots of the crude solubilized or purified preparation were preincubated for 10 min with 6–10 concentrations of inhibitor prior to performing the soluble binding assay. •, domoic acid; ○, quisqualic acid; ▲, glutamic acid; ●, dihydrokainic acid.

FIG. 6. Gel filtration chromatography on Sepharose 6B of purified KA receptors (A) and the crude solubilized preparation (B). Five ml of either preparation were applied to the column, the flow rate was adjusted to 9 ml/h, and 2-ml fractions were collected. Standards indicated at the top are: BD, blue dextran; THY, thyroglobulin; FER, ferritin; CAT, catalase; ALD, aldolase; and CYT, cytochrome c. The mobile phase consisted of 50 mM Tris citrate, 7.0, containing 100 mM NaCl, 0.1% Triton X-100, and 5% glycerol.

compared to the crude solubilized sites in competition experiments (Fig. 5, Table II). The IC$_{50}$ values obtained for the purified binding sites were very similar to those of the crude solubilized sites with domoic acid being the most potent inhibitor in both preparations.

**Biochemical Characterization**—Gel filtration chromatography of the purified binding sites was carried out on columns of Sepharose 6B and in the presence of Triton X-100 (Fig. 6). The purified KA receptors eluted with $M_r = 570,000$; the crude solubilized binding sites eluted with a similar relative molecular weight ($M_r = 550,000$) and slightly ahead of the main protein peak.

Samples from various stages of the purification were dialyzed, lyophilized, and subjected to electrophoresis on 10% SDS-polyacrylamide gels. The gels were stained using the highly sensitive glutaraldehyde/ammonia silver staining method of Giulian et al. (1983). A large diffuse band migrating at $M_r = 48,000$ was observed (Fig. 7). In some preparations, multiple bands were seen within the diffuse band centered at $M_r = 48,000$. The presence of other faintly staining bands was observed in some preparations in which the final specific activity was lower than that reported in Table I. However,
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FIG. 7. SDS-polyacrylamide gel electrophoresis of (A) crude solubilized preparation from frog brain (10 µg of protein), (B) pooled fractions from the DEAE-Sepharose CL-6B column (2.5 µg of protein), (C) unretained fractions from the domoic acid affinity column (2.5 µg of protein), and (D) purified KA receptor fraction (1 µg of protein). The gel was silver-stained as described by Giulian et al. (1983). Markers indicate standards (myosin, M₂₀ = 205,000; phosphorylase b, M₀ = 97,400; bovine serum albumin, M₂₀ = 67,000; ovalbumin, M₀ = 44,300; and carbonic anhydrase, M₂₀ = 29,000).

the band at M₀ = 48,000 was observed in all purified preparations.

DISCUSSION

This paper describes the purification of an active KA receptor from Triton X-100/digitonin-solubilized frog brain. Purification is facilitated by the use of frog brain which is an enriched source of KA receptor containing at least 40-fold more total KA binding sites than rat brain (London et al., 1980; Hampson et al., 1987a). A key step in this purification is the use of a domoic acid-Sepharose affinity column from which the bound receptor is eluted in the presence of KA. Domoic acid, an analog of KA, has the highest affinity for the KA receptor of all known compounds (Slevin et al., 1983). The immobilization of domoic acid on amino-alkyl Sepharose through a carbodiimide linkage yields an active receptor ligand which efficiently bound solubilized KA receptors. Scatchard analysis shows that the purified receptor preparation binds [³H]KA with dissociation constants similar to those determined for the crude soluble preparation. The specific binding of [³H]KA in these two preparations is also similarly inhibited by pharmacologically related compounds.

Attempts to purify excitatory amino acid receptors have previously met with little success because of the difficulty in obtaining active soluble receptors and the lack of specific affinity resins. A glutamate binding protein has been purified from rat and bovine brain (Michaelis et al., 1983; Kuni and Roberts, 1987), but the rather large amounts of this protein present in brain as well as its atypical pharmacological properties suggest that it may not be related to a glutamate synaptic receptor. This protein has M₀ = 14,000 determined with SDS-polyacrylamide gel electrophoresis. While this low molecular weight may be due to proteolysis, its pharmacological properties show it to be dissimilar to the receptor we have purified. A glutamate binding protein was solubilized from guinea pig brain, but this binding site displayed the pharmacological properties of a quisqualate-like receptor with only a weak affinity for KA (Koshiya, 1985). KA binding sites have been solubilized from pigeon cerebellum (Dilber et al., 1983). While these binding sites may share some properties with the receptor we have characterized in this study, they differ considerably in their affinities with dissociation constants of 30 and 330 nM compared to 4.8 and 39 nM we obtain for the solubilized frog brain receptor.

Gel filtration chromatography of the soluble and purified preparations show that the KA receptor migrates with a similar relative molecular weight (M₀ = 550,000 for the soluble and M₀ = 570,000 for the purified). These values likely represent protein-detergent complexes, and the actual molecular weight of the receptor may be much smaller. A similar molecular weight (M₀ = 650,000) was also obtained for a KA receptor solubilized in rat brain (Hampson et al., 1987b).

SDS gel analysis of the purified receptor from frog brain shows a single broad band migrating at M₀ = 48,000. The broadness of the band may reflect the presence of several similar forms of the receptor due, for example, to partial proteolysis or microheterogeneity in carbohydrate residues. The KA receptor we have isolated appears to be a glycoprotein based on binding to several agarose-conjugated lectins (Hampson et al., 1987a). A similar behavior of the purified β-adrenergic and muscarinic receptors on SDS-polyacrylamide gel electrophoresis was also attributed to carbohydrate microheterogeneity (Shorr et al., 1981; Haga and Haga, 1985). The total KA binding sites obtained for high and low affinity components for the purified receptor is 6100 pmol/mg protein. For one binding site/molecule, the theoretical binding capacity for a pure receptor with a molecular mass of 48 kDa is 20,800 pmol/mg protein. Based on such a model, the purified receptor is about 29% pure. With the absence of additional bands on the silver-stained gel and the apparent high degree of purity of the receptor, it is reasonable to conclude that the binding site is present in the band migrating at M₀ = 48,000. At present this cannot be verified since neither covalent ligands for the KA receptor nor specific monoclonal antibodies have been developed. The comparison of the binding capacity that we obtain for the purified preparation with the theoretical binding capacity suggests that the receptor represents only part of the protein migrating at M₀ = 48,000. However, the determination of the purity of the receptor may be an underestimate due to limitations of the protein assay or the binding assay. While the soluble binding assay is reproducible, it is uncertain if all the binding activity is being measured. For example, some dissociation may occur during centrifugation and wash, and not all the receptor may precipitate. It is also possible that some of the protein in the purified preparation is denatured receptor. In support of this possibility is the fact that we have not recovered more than 40% of the binding activity from the affinity column, although conditions of elution, wash, and dialysis have been varied. Denaturation may also take place during dialysis, but this does not appear to be extensive since similar recoveries of binding activity are obtained if free KA is removed more quickly by gel filtration. A curvilinear Scatchard plot is obtained from the purified receptor preparation as well as from the crude solubilized preparation. Binding analyses have generated curvilinear Scatchard plots for KA receptors from most sources studied, and these have been interpreted as having two binding sites of high and low affinities, with dissociation constants similar to those we have obtained for the frog brain (Biziere and Coyle, 1979; London and Coyle, 1979; London et al., 1980; Foster et al., 1981; Honore et al., 1986). While ligand binding to these two sites has been studied by several laboratories, only the radiation inactivation analysis by Honore et al. (1986)
sought to determine if different molecules are responsible for the two binding sites in rat brain. These studies give molecular sizes of 76 and 52.4 kDa for the high and low affinity components, respectively, suggesting that the two sites are associated with different molecules. We see no evidence for a 76-kDa component in the purified frog brain receptor. The value we obtain by SDS-polyacrylamide gel electrophoresis for the purified frog brain receptor (M_r = 48,000) is similar to that determined by radiation inactivation for the low affinity component from rat brain. The curvilinear Scatchard plot for the purified receptor migrates as a single broad band in SDS-polyacrylamide gel electrophoresis at Mr = 48,000. These findings show that the frog and rat brain receptors are biochemically similar to those of mammals (Watkins, 1978). Our results also show that the frog and rat brain receptors are biochemically alike (Hampson et al., 1987a, 1987b). They migrate with similar molecular weights on Sepharose, bind to lectins, and have similar inhibition constants and dissociation constants.

In summary, these studies present the first purification of a KA receptor which has properties characteristic of a physiologically active excitatory amino acid receptor. The purified receptor migrates as a single broad band in SDS-polyacrylamide gel electrophoresis at Mr = 48,000. These findings should facilitate the molecular characterization of this receptor and the production of specific anti-receptor antibodies.

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