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Biochemistry

Coupling of a purified goldfish brain kainate receptor with a pertussis toxin-sensitive G protein

(kainic acid/glutamate receptors/guanine nucleotide-binding protein)

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ABSTRACT **Goldfish brain has a high density of [^{3}H]kainate-binding sites, a subpopulation of which appears to be coupled to a pertussis toxin-sensitive G protein. We show here that a purified kainate receptor preparation reconstituted into phospholipid vesicles exhibits guanine nucleotide-sensitive high-affinity [^{3}H]kainate binding. Pertussis toxin treatment abolishes the guanine nucleotide-sensitive portion of the [^{3}H]kainate binding, and kainate promotes [^{3}H]guanosine 5′-[β,γ-imido]triphosphate binding and [γ-^{32}P]GTP hydrolysis. Guanosine 5′-[γ-thio]triphosphate (GTP[γS]) decreases the apparent Stokes radius of the soluble purified receptor preparation, consistent with dissociation of the kainate receptor–G protein complexes. The affinity-purified preparations contain proteins of 45, 41, and 35 kDa. The 45- and 41-kDa proteins crossreact with antibodies against the kainate receptor cloned from frog brain. The 35-kDa protein is recognized by an antiserum (SW) directed against the β subunit of G proteins. When kainate receptors are purified in the presence of GTP[γS], the 35-kDa protein is no longer present. Also, [^{3}H]kainate affinity is decreased and is no longer guanine nucleotide sensitive. Upon reconstitution with purified G proteins, high-affinity guanine nucleotide-sensitive binding and kainate-stimulated GTPase activity can be restored. These observations indicate that a kainate receptor from goldfish brain functionally interacts with a pertussis toxin-sensitive G protein.**

Glutamate and related acidic amino acids are the primary excitatory neurotransmitters in the central nervous system of vertebrates (1). Glutamate receptors are involved not only in the mediation of synaptic transmission but also in a variety of other physiological effects (2). Traditionally, receptor classification has been defined by structurally related agonists [kainate, quisqualate, and *N*-methyl-D-aspartate (NMDA)] that selectively open cation channels, thereby mediating neuronal excitation. Recently, however, this scheme has been expanded to include glutamate receptors that are linked to the production of second messengers (2).

Kainate, a cyclic analog of glutamate, activates monovalent cationic conductances in vertebrate neurons (1), including goldfish retinal cells (3). More recently, other cellular responses to kainate, some of which may be mediated by heterotrimeric guanine nucleotide-binding proteins (G proteins), have been described, including the following: the stimulation of an influx of Ca^{2+} through voltage-sensitive Ca^{2+} channels in both central neurons (4) and astrocytes (5); the reduction of K^+ conductances in hippocampal neurons, in particular those involved in the slow inhibitory postsynaptic potential evoked by γ-aminobutyric acid type B receptors (6) and voltage-dependent K^+ channels (7); and the formation of the second messengers cGMP and nitric oxide (8) in the cerebellum.

The density of high-affinity kainate receptors in nonmammalian vertebrate brain is much greater than that of mammals. Kainate receptors from frog and chick brain have been purified (9, 10) with apparent molecular weights of approximately 50,000. Kainate-binding proteins from both *Rana pipiens* (11) and chick brain (12) have been cloned, and the amino acid sequences are very similar, suggesting that they may be versions of the same protein. Although there are sequence similarities with the ligand-gated channels, these proteins have not been shown to exhibit any ion channel activity. Recently, a high-affinity kainate receptor from rat brain has been cloned with a predicted molecular weight of 105,000 (13). The carboxyl-terminal half has approximately 37% identity with the lower molecular weight kainate-binding proteins. Again, no channel activity was observed in cells expressing this protein. A series of different glutamate receptor subunits have been cloned that show ion channel activity when mRNA is injected into oocytes (14–16). Although both kainate and α-amino-3-hydroxy-5-methylisoxazolepropionate (AMPA) activate the response, radioligand binding analysis shows that these channels are high-affinity AMPA-binding proteins (15). Recently, a related (40–80% identity) protein has been found that is activated by kainate and not AMPA (17). Thus, a portion of the ion channel activity elicited by kainate is through AMPA receptors, and, the function of high-affinity kainate-binding proteins has not been determined.

We have observed that [^{3}H]kainate binding in goldfish brain membranes is shifted from high to low affinity in the presence of guanine nucleotides (18). Also, [^{3}H]kainate binds in a pertussis toxin-sensitive manner and a 40-kDa protein is [^{32}P]ADP-ribosylated in a kainate-sensitive manner. These data are consistent with the interaction of a population of kainate receptors with G proteins. In the present paper, detergent-solubilized kainate receptors were purified, and the results indicate that pertussis toxin-sensitive G proteins can be copurified and are functionally associated with the receptor. Furthermore, the kainate receptor, separated from its endogenous G protein, can be functionally reconstituted with purified bovine G protein.

EXPERIMENTAL PROCEDURES

Purification of Kainate Receptor. Synaptosomal membranes from whole goldfish brain (*Carassius auratus*; Grassyfork Fisheries, Martinsville, IN) were prepared as described previously (18). Kainate receptor was solubilized from syn-

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazolepropionate; ATP[γS], adenosine 5′-[γ-thio]triphosphate; CNQX, 6-cyano-7-nitroquinoxoline-2,6-dione; G protein, heterotrimeric guanine nucleotide-binding protein; GTP[γS], guanosine 5′-[γ-thio]triphosphate; NMDA, *N*-methyl-D-aspartate; p[NH]ppG, guanosine 5′-[β,γ-imido]triphosphate.

aptosomes (19) by using 2% *n*-octyl β-D-glucopyranoside [octyl glucoside; final detergent-to-protein ratio of 20:1 (wt/wt)] in HMEN buffer (20 mM Hepes–NaOH, pH 7.4/100 mM NaCl/5 mM $MgCl_2$/1 mM EDTA) with protease inhibitors (0.2 mM phenylmethylsulfonyl fluoride, aprotinin at 1 μg/ml, leupeptin at 1 μg/ml). The kainate receptor was purified by domoic acid affinity chromatography as described by Hampson and Wenthold (9) except that one column volume of 10 mM kainate was added to the column 10 min prior to elution and the DEAE separation was omitted. For some experiments, prior to elution with kainate, the column was first washed with buffer containing either 100 μM guanosine 5′-[γ-thio]triphosphate (GTP[γS]) or guanosine 5′-[β,γ-imido]triphosphate (p[NH]ppG) to elute the G proteins associated with kainate receptors. The eluate was collected on ice, concentrated 10- to 20-fold by ultrafiltration on an Amicon YM-30 membrane, and dialyzed against 500 ml HMEN containing 1.5% sodium cholate.

Incorporation of Purified Kainate Receptor–G Protein into Phospholipid Vesicles. One hundred microliters of asolectin vesicles (soybean phosphatide mixture from Associated Concentrates, Woodside, NY; 17 mg/ml) was added to 400 μl of soluble purified receptor [lipid-to-protein ratio (wt/wt) of 50–100:1] and incubated on ice for 30 min. One-milliliter Extractigel D columns (Pierce) were washed in 2.5 vol of HMEN containing bovine serum albumin at 2 mg/ml, equilibrated in HMEN, and centrifuged at 2000 rpm for 20 min. The protein/lipid mixture was applied to the column and the reconstituted material was collected during a repeat centrifugation.

Binding and GTPase Assays. [^{3}H]Kainate (58 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq) binding was measured as described previously (18). Nonspecific binding was defined in the presence of 100 μM unlabeled kainate, and saturation analyses were performed with a nonlinear least-squares fit to a one-site model or to the Hill equation. Total [^{3}H]p[NH]ppG (18 Ci/mmol, Amersham) binding sites were determined by a preincubation with 50 nM GTP in HMEN buffer for 1 hr followed by incubation for 1.5 hr at room temperature with 1 μM [^{3}H]p[NH]ppG in a final volume of 100 μl containing 20 mM Tris·HCl at pH 8, 1 mM EDTA, 1 mM dithiothreitol, 100 mM NaCl, 10 mM $MgCl_2$, and 0.05% Tween-20. The binding reaction was terminated by dilution in 4 ml of wash buffer containing 20 mM Tris·HCl at pH 8, 100 mM NaCl, and 25 mM $MgCl_2$ followed by rapid filtration through Millipore HAWP filters. The filters were washed with an additional 4 ml of wash buffer and dried under suction, and bound radioactivity was measured. Nonspecific binding was measured in the presence of 100 μM nonradioactive p[NH]ppG. For measurements of GTPase activity, 25-μl (6–8 μg of protein) aliquots of reconstituted vesicles were incubated at 30°C for 20 min in a final volume of 50 μl consisting of 20 mM Hepes–NaOH at pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM p[NH]ppA, 1 mM dithiothreitol, and 0.5 μM [γ-^{32}P]GTP (100,000 cpm; New England Nuclear). The release of inorganic phosphate was measured as described by Brandt and Ross (20). Nonspecific GTP hydrolysis was determined by the addition of 250 μM nonradioactive GTP.

Pertussis Toxin Treatment. Aliquots of purified reconstituted preparations (6–8 μg of protein) were incubated for 3 hr at room temperature in the presence or absence of 1 μg of the A protomer of pertussis toxin in a final volume of 50 μl consisting of 10 mM Hepes–NaOH at pH 7.4, 50 mM NaCl, 2.5 mM $MgCl_2$, 1 mM ATP, 1.5 mM dithiothreitol, 0.5 mM GTP, and 10 μM NAD. These mixtures were then diluted to a total of 350 μl with HMEN buffer for [^{3}H]kainate binding measurements.

Gel Electrophoresis and Immunoblotting. Protein samples (3–5 μg) were mixed with SDS sample buffer and heated to 60°C for 5 min prior to separation on SDS/10% or 12% polyacrylamide minigels. These were fixed in 5% glutaraldehyde and stained with silver. Immunoblotting with KAR-B1 (1:25 dilution) was done as described by Hampson *et al.* (21), except that proteins were transferred to Immobilon membranes (Millipore). Blocking and incubation buffers contained 0.1% Tween-20. Protein concentrations were determined by using the bicinchoninic acid (BCA) protein assay kit from Pierce.

RESULTS

Affinity-Purified Kainate Receptor Copurifies and Interacts with an Endogenous G Protein. Binding isotherms with Scatchard transformations for [^{3}H]kainate binding to various receptor preparations are shown in Fig. 1. As described previously (18), synaptosomal membranes (Fig. 1*A*) exhibited high-affinity [^{3}H]kainate binding (K_d = 34 ± 3.5 nM), which was decreased 6-fold in the presence of GTP[γS] (K_d 212 ± 34 nM). Solubilized membranes (Fig. 1*B*; K_d = 101 ± 27 nM) and affinity-purified preparations incorporated into phospholipid vesicles (Fig. 1*D*; K_d = 76 ± 4.5 nM) bound [^{3}H]kainate with a somewhat lower affinity; however, the affinities were also decreased (2- to 3-fold; K_d of 202 ± 89 nM, soluble; 167 ± 18 nM, reconstituted) in the presence of GTP[γS] (but not in the presence of ATP[γS]; Fig. 1*D*). GTP[γS] treatment had no effect on B_{max}. To determine the guanine nucleotide specificity, equilibrium binding of 25 nM [^{3}H]kainate to purified-reconstituted vesicles was performed in the presence of increasing concentrations of various nucleotides (Fig. 2*A*). The rank order of potency was: GTP[γS] ≈ p[NH]ppG > GDP ≈ GTP. cGMP had very little effect and ATP[γS] had none at concentrations of 1 mM (data not shown). These data suggest that a G protein remained coupled to the receptor when membranes were solubilized with octyl glucoside and during the purification procedure. [^{3}H]Kainate binding was positively cooperative in membranes in both the absence and the presence of GTP[γS] (Hill

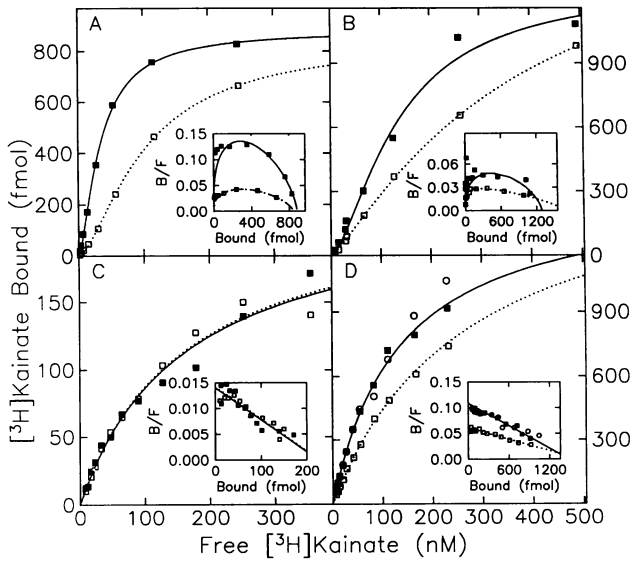

FIG. 1. Saturation binding of [^{3}H]kainate to kainate receptor preparations. *Insets* show Scatchard transformations of the data; *B/F*, bound/free. Binding assays were performed both in the absence (■) and in the presence of 100 μM GTP[γS] (□) or adenosine 5′-[γ-thio]triphosphate (ATP[γS]) (○), using synaptic plasma membranes (12 μg of protein per assay) (*A*), solubilized membranes (20 μg of protein per assay) (*B*), receptors purified with GTP[γS] wash (0.1 μg of protein per assay) (*C*), or receptors purified routinely (1.6 μg of protein per assay) (*D*). The effect of ATP[γS] (○) is shown in *D*. Purified preparations were reinserted into phospholipid vesicles before assaying.

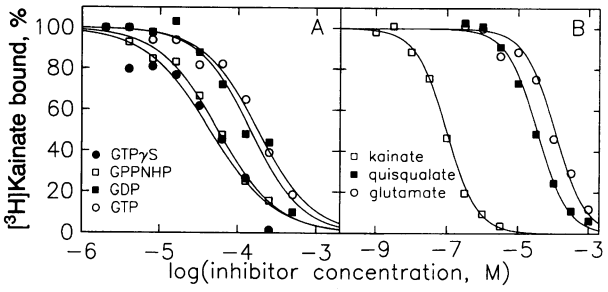

FIG. 2. Effects of nucleotides (*A*) and glutamatergic ligands (*B*) on equilibrium binding of [^{3}H]kainate in purified-reconstituted preparations (25 nM [^{3}H]kainate in *A*, 50 nM in *B*). Control binding was determined in the absence of inhibitor and nonspecific binding in the presence of 100 μM kainate. The data are normalized to control binding. IC_{50} values of 44, 55, 144, and 178 μM were obtained for GTP[γS], p[NH]ppG, GDP, and GTP, respectively.

coefficients of 1.6 and 1.4, respectively; Fig. 1 *A* and *B*). Upon purification, linear Scatchard plots were observed. The loss of positive cooperativity in the purified preparations could reflect either a purification of one subtype of receptor or the loss of interaction between two binding sites.

The effect of glutamatergic ligands on [^{3}H]kainate binding to reconstituted preparations is shown in Fig. 2*B*. The K_i values for unlabeled kainate, quisqualate, and glutamate were 0.04 ± 0.01, 18 ± 7, and 54 ± 22 μM, respectively. These results are similar to those for membrane-bound and soluble kainate receptor in goldfish brain (18, 19).

SDS/polyacrylamide gels, run under reducing conditions, consistently revealed three polypeptides (Fig. 3, lane A). The major band migrated with a molecular mass of ≈41 kDa, with less intensely staining bands of ≈45 and 35 kDa. The 41- and 45-kDa bands were labeled on Western blots by a monoclonal antibody (KAR-B1) raised against the frog kainate binding protein (Fig. 3, lane C). Therefore, these protein bands appear to share an epitope with the frog receptor, identifying these bands as possible kainate receptor components. We attempted to identify the copurified G protein by immunoblotting with several antisera directed against synthetic peptides that correspond to amino acid sequences from several G protein subunits. The antisera RM, AS, and GO recognize the carboxyl-terminal decapeptide of subunits α_s, α_t, and α_o, respectively (22). The antiserum GA was raised against a peptide common to many of the cloned α subunits (amino acids 40–54 of α_{i1}) and SW, against a β-subunit peptide. The 35-kDa band was strongly recognized by SW, identifying this

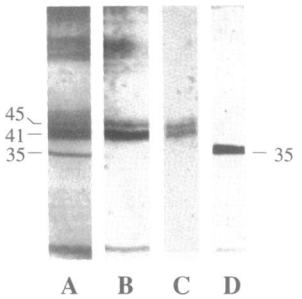

FIG. 3. SDS/polyacrylamide gel electrophoresis of affinity-purified receptors. Lane A is a silver-stained gel of a routinely purified receptor preparation. Lane B is a silver-stained gel of purified eluates washed with 100 μM GTP[γS] while immobilized on the affinity column prior to elution. Lane C is an immunoblot of a gel of routinely purified receptor preparations allowed to react with KAR-B1, a monoclonal antibody directed against purified frog kainate binding protein. Lane D is an immunoblot of a gel of routinely purified receptor preparations allowed to react with SW, an antiserum to G protein β subunit. Molecular masses are given in kDa.

polypeptide as a β subunit (Fig. 3, lane D). All anti-α-subunit antisera recognized proteins in crude goldfish membranes; however, only GO showed immunoreactivity with purified preparations (data not shown). The signal was weak but consistently present at ≈40 kDa in all (n = 3) preparations tested. Specific elution of the β subunit with the kainate receptor was further confirmed by the fact that no proteins were detected in samples taken directly before elution with kainate, and after elution, the 35-kDa band coeluted with the 45- and 41-kDa bands.

Fig. 4*A* shows specific [^{3}H]kainate binding, in both the absence and the presence of guanine nucleotides, to a purified-reconstituted preparation, part of which had been pre-

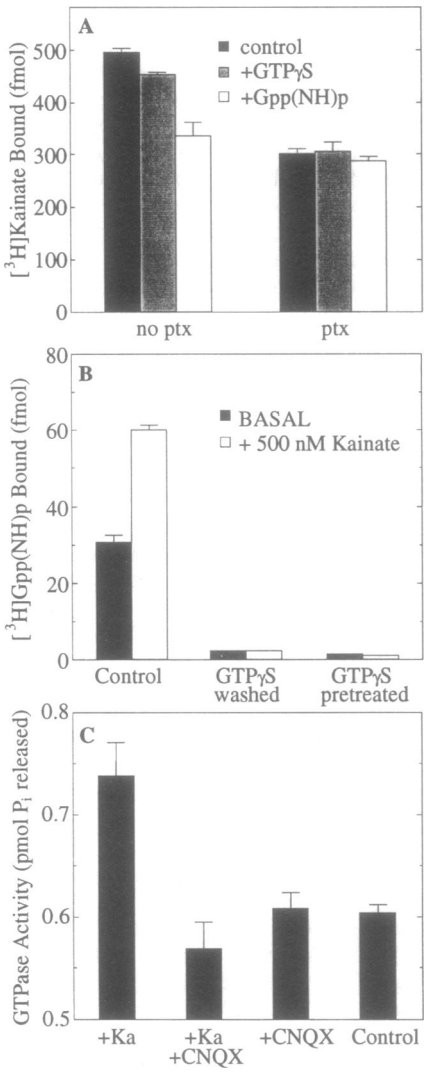

FIG. 4. (*A*) Pertussis toxin effect on [^{3}H]kainate binding. Purified-reconstituted receptor preparations were treated with the A protomer of pertussis toxin at 20 μg/ml. Specific [^{3}H]kainate binding (50 nM) is shown in the absence of nucleotides or in the presence of either GTP[γS] or p[NH]ppG at 100 μM (1.4 μg of protein per assay). (*B*) Effect of kainate on endogenous binding of [^{3}H]p[NH]ppG. Affinity-purified and reconstituted preparations were incubated with [^{3}H]p[NH]ppG (250 nM) in the absence and presence of 500 nM kainate for 30 min at room temperature (0.6 μg of protein per assay). Control is routinely purified receptors, "GTPγS washed" are receptor eluates obtained after exposure to 100 μM GTP[γS] while adsorbed to the affinity column, and "GTPγS pretreated" are receptors purified from membranes solubilized in the presence of 10 μM GTP[γS] and 10 μM p[NH]ppG. (*C*) Effect of kainate on GTPase activity. As indicated, aliquots were incubated with 500 nM kainate, 250 μM 6-cyano-7-nitroquinoxaline-2,6-dione (CNQX), or both. Control represents the basal level of GTP hydrolysis.

incubated with pertussis toxin at 20 μg/ml. Pertussis toxin decreased the guanine nucleotide-sensitive portion of the [^{3}H]kainate binding. This suggests that treatment with pertussis toxin can uncouple the kainate receptor from copurified G protein.

[^{3}H]p[NH]ppG and [^{3}H]kainate binding activities were coeluted from the agonist affinity column with a molar ratio of 0.1–0.4 (n = 5). Evidence for a functional interaction between the receptor and G protein is shown in Fig. 4 *B* and *C*. Kainate stimulated both [^{3}H]p[NH]ppG binding (Fig. 4*B*) and [γ-^{32}P]GTP hydrolysis (Fig. 4*C*) in purified-reconstituted vesicles. CNQX, a kainate antagonist, blocked the kainate-stimulated GTPase activity. The increase in [^{3}H]p[NH]ppG binding and GTPase activity in the presence of agonist is characteristic of G-protein-linked receptors and results from the increased rate of exchange of GTP (or analogs) for GDP.

The copurification of a G protein with the kainate receptor and the decrease, in the presence of guanine nucleotides, of [^{3}H]kainate affinity in solubilized preparations suggest that these proteins form tight complexes in detergent solution. To test this, we solubilized reconstituted preparations with 1% cholate and applied these to a Superose 12B FPLC column (Pharmacia; separation on the basis of Stokes radii). Preincubation of samples with GTP[γS] converts the [^{3}H]kainate binding component to a smaller species (180 kDa to 115 kDa; Fig. 5*A*). These data are consistent with the GTP[γS]-induced separation of a heterotrimeric G protein from receptor–detergent complex.

Separation of the Kainate Receptor from Endogenous G Protein During Purification and Coupling of the Receptor with Exogenous G Proteins. To dissociate the kainate receptor completely from its endogenously coupled G proteins, the soluble extracts, adsorbed to the agonist affinity column, were washed with either 100 μM GTP[γS] or 100 μM p[NH]ppG prior to eluting with kainate. In these purified-reconstituted preparations, the affinity for [^{3}H]kainate was low both in the presence (K_d = 183 ± 12 nM) and in the absence (K_d = 166 ± 4.5 nM) of guanine nucleotides (Fig. 1*C*). In these same vesicles, virtually no [^{3}H]p[NH]ppG binding was observed (Fig. 4*B*). While the 45- and 41-kDa polypeptides were still present, the 35-kDa polypeptide,

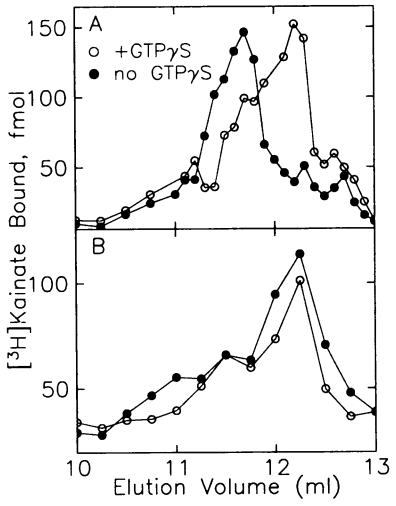

FIG. 5. Elution profile of specific [^{3}H]kainate binding activity with Superose 12B FPLC chromatography. (*A*) Aliquots of purified-reconstituted preparations were incubated for 30 min on ice with 0.5 μM kainate or 0.5 μM kainate and 200 μM GTP[γS], as indicated. The vesicles were solubilized in 1% cholate and applied to the column. The column was developed at 0.5 ml/min with HMEN containing 1% cholate. (*B*) Representative chromatograph of a purified preparation that had been exposed to GTP[γS] while bound to the affinity column.

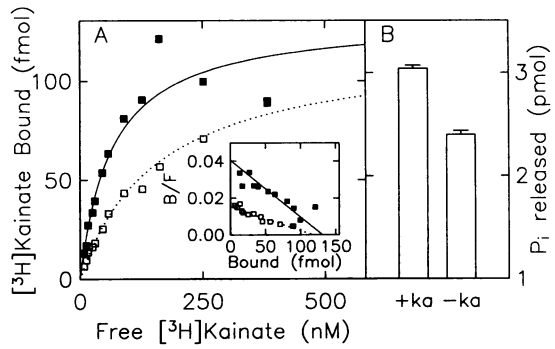

FIG. 6. Reconstitution of exogenous G protein with purified kainate receptor separated from endogenous G protein. (*A*) Saturation binding of [^{3}H]kainate in purified receptor preparations (0.5 μg of kainate receptor protein per assay), reconstituted with a mixture of partially purified G proteins. (*Inset*) Scatchard transformations of the data. Binding assays were performed in the absence (■, K_d = 64 ± 5.6 nM) and presence (□, K_d = 158 ± 6.2 nM) of 100 μM GTP[γS]. (*B*) GTPase activity was assayed in the presence (+ka) and absence (−ka) of 0.5 μM kainate with purified receptor preparations, reconstituted with purified α_o and $\beta\gamma$ subunits. Similar results were obtained with a mixture of G_i proteins and G_o.

identified as a β subunit of a G protein, no longer appeared on silver-stained SDS/polyacrylamide gels (Fig. 3, lane B). Fig. 5*B* shows that the [^{3}H]kainate-binding component of these purified preparations was eluted from the Superose 12B FPLC column at a position corresponding to the GTP[γS]-treated G-protein-linked receptor (Fig. 5*A*). Additionally, preincubation with GTP[γS] did not affect the migration in these preparations. These data demonstrate that treatment with GTP analogs can separate the kainate receptor from its coupled G protein.

Preparations of bovine brain G proteins were reconstituted with kainate receptor from which the endogenous G protein had been removed during purification. High-affinity, guanine nucleotide-sensitive [^{3}H]kainate binding was restored by the addition of a mixture of partially purified G proteins at a 20:1 molar ratio of G protein to receptor (Fig. 6*A*). Additionally, when purified α_o and $\beta\gamma$ subunits were reconstituted with receptor at a molar ratio of 2:5:1 ($\alpha/\beta\gamma$/receptor), hydrolysis of [γ-^{32}P]GTP by G_o was increased in the presence of kainate (Fig. 6*B*). This apparent functional interaction between kainate receptor and purified bovine G_o suggests that the G protein purifying with the kainate receptor may be related to G_o.

DISCUSSION

Previously, we have shown that goldfish synaptosomes contain a population of kainate receptors that couple to a pertussis toxin-sensitive GTP-binding protein (18). This study provides direct evidence for this interaction by examining purified kainate receptor and G protein in a reconstituted system. Several lines of evidence indicate that the kainate receptor and G protein were purified as a functional complex: (*i*) Guanine nucleotides decreased the affinity of [^{3}H]kainate binding in reconstituted affinity-purified preparations. One explanation for this effect is the guanine nucleotide-mediated dissociation of receptor–G protein complexes. This interpretation is supported by the decrease in apparent molecular weight of the receptor complex in the presence of GTP[γS], consistent with dissociation of a heterotrimeric G protein. (*ii*) In the presence of kainate, purified-reconstituted preparations showed an increase in [^{3}H]p[NH]ppG binding and [γ-^{32}P]GTP hydrolysis. The stimulation of G protein activity produced by kainate is somewhat lower than has been observed in other receptor–G protein systems. The reason for this is unknown but could be intrinsic properties of the goldfish kainate receptor–G protein com-

plex or a partial loss of activity during the lengthy purification and reconstitution procedure. (*iii*) Kainate receptors, separated from the endogenous G protein by exposure to guanine nucleotides prior to elution, displayed a decrease in affinity for [^{3}H]kainate, and the binding affinity was no longer affected by guanine nucleotides. These changes reflect absence of endogenous G proteins and not alterations in receptor protein, as restoration of guanine nucleotide-sensitive, high-affinity binding occurred by reconstitution with bovine G proteins. Furthermore, kainate receptors (in the presence of kainate) activated GTPase activity when reconstituted with purified G_o.

The characteristics that classically define G-protein-linked receptors are displayed by the kainate receptor described in this paper (23). This suggests that the physiological response of this receptor may be mediated by second messengers. As observed with native membranes (18), treatment with pertussis toxin eliminated the interaction of the kainate receptor–G protein complex. Attempts to label the G protein by pertussis toxin-catalyzed ADP ribosylation using [^{32}P]NAD showed only a very weak signal at approximately 40 kDa. The anti-β-subunit antibody SW strongly labeled a 35-kDa polypeptide on Western blots, while an antibody raised against the carboxyl terminus of the α subunit of mammalian G_o, exhibited weak but consistent crossreactivity with a 40-kDa polypeptide. This suggests the G protein copurifying with the kainate receptor may be related to mammalian G_o.

Previously, kainate receptors have been purified from frog (9) and chick (10) brains with molecular masses of 48 and 49 kDa, respectively. In this paper, protein bands present in the purified preparations migrated with apparent molecular masses of 41 kDa (most prominent) and 45 kDa under reducing conditions. Using a monoclonal antibody against the frog kainate-binding protein (21), we identified these proteins as kainate receptor components on immunoblots. The receptors from frog (11) and chick (12) have been cloned and the amino acid sequences determined. These proteins are closely related (55% identity). Taken together, these data indicate that the goldfish kainate-binding protein may be related to the frog and chick receptor. Although to date no function of these kainate-binding proteins has been described, the amino acid sequences suggest that these proteins are not homologous to known G-protein-coupled receptors.

We have defined the initial steps in the signal transduction pathway of a kainate receptor subtype; however, the subsequent events are presently unknown. Classically, kainate receptors are considered integral parts of ion channels (1). However, recent evidence indicates a portion of the kainate response actually may be mediated through AMPA receptors (14–16). Our results suggest that a high-affinity kainate receptor is coupled to and probably mediates its effect through a G protein. A quisqualate-ibotenate type of glutamate receptor has been described that activates phospholipase C through a pertussis toxin-sensitive G protein (24). Recently, cDNAs coding for this receptor have been cloned from the rat, and the molecular mass is predicted to be approximately 130 kDa (25, 26). Kainate does not activate this protein when expressed in oocytes. Furthermore, kainate can inhibit the inositol phosphate response stimulated by certain other neurotransmitters such as carbachol, histamine, and norepinephrine (27). The binding data in the present report indicate that this purified receptor is classically of the kainate subtype of glutamate receptor, with quisqualate only poorly displacing [^{3}H]kainate. Therefore, it is highly unlikely that the kainate receptor described in the present paper is this metabotropic quisqualate receptor.

Although the effector system remains unknown, we have identified an additional G-protein-linked glutamate receptor. We have demonstrated that a kainate receptor copurifies with an endogenous G protein with which it can reciprocally interact. This is an intriguing finding, because until now there has been no direct evidence linking a kainate receptor to a G-protein-mediated signal transduction mechanism. Since kainate receptors are the most abundant class of neurotransmitter receptors in nonmammalian brains and because of the vital importance of non-NMDA receptors in a wide variety of cellular processes, understanding the signal transduction mechanism of this receptor is of considerable interest.

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