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Purified unitary kainate/α-amino-3-hydroxy-5-methylisooxazole-propionate (AMPA) and kainate/AMPA/*N*-methyl-D-aspartate receptors with interchangeable subunits

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ABSTRACT **We have purified and characterized two vertebrate excitatory amino acid ionotropic receptors from the *Xenopus* central nervous system. Each is a unitary receptor (i.e., having more than one class of excitatory amino acid agonist specificity within one protein oligomer). The first is a unitary non-*N*-methyl-D-aspartate (non-NMDA) receptor and the second is a unitary NMDA/non-NMDA receptor. The specific agonist-activated channel activity and pharmacology of each type were recognized by patch-clamping lipid bilayers in which the isolated protein was reconstituted. In the second case, the NMDA and the non-NMDA sites could not be physically separated and exhibited functional interaction. Parallel evidence for this was obtained when poly(A) RNA from *Xenopus* brain was translated in oocytes: a noncompetitive inhibition of the response to L-kainate is produced by NMDA to a maximum depression of 30% at 1 mM NMDA. Each isolated oligomer contains 42-kDa subunits of the non-NMDA ligand binding type, but the second type has an additional NMDA-receptor-specific 100-kDa subunit. Thus, a subunit-exchange hypothesis can account for the known multiplicity of excitatory amino acid receptor types.**

The glutamate receptors essential for fast excitation are classified into *N*-methyl-D-aspartate (NMDA)-sensitive and -insensitive receptors, and in the latter, separate types recognizing L-kainic acid (KA) and AMPA (1) have been generally accepted (2). However, electrophysiological evidence is accruing for unitary non-NMDA receptors of KA and AMPA selectivity *in situ* (for review, see ref. 3). Further, in a variety of fractionations after solubilization from *Xenopus* brain, the bulk of the KA and AMPA sites could not be separated (4), suggesting a unitary receptor type. This is supported by the ability of recombinant non-NMDA receptor subunits (5–7) to form channels opened by both KA and AMPA (6, 7). Since the nervous system of *Xenopus laevis* has (8), to our knowledge, the highest concentration of KA and AMPA binding sites known, we have used it to purify and reconstitute a native unitary non-NMDA receptor. Our findings show a receptor series comprising interchangeable subunits, namely, unitary KA/AMPA receptors, NMDA receptors, and hybrid unitary KA/AMPA/NMDA receptors, the latter having three sites on one protein.

EXPERIMENTAL PROCEDURES

Purification of Receptors. Membranes from the total brain plus spinal cord of adult *X. laevis* (20 animals per preparation, 140 mg of protein) were solubilized in 1% octyl glucoside (8), but using 1 mM *N*-ethylmaleimide, 1 mM phenylmethylsulfonyl fluoride, and aprotinin (0.4 international unit/ml) as additional protease inhibitors. Domoate-Sepharose was prepared and used according to Hampson and Wenthold (9), but with the following changes. The medium, except where stated otherwise, was Tris buffer (50 mM Tris citrate, pH 7.0/1 mM EGTA/1 mM EDTA) in 0.8% octyl glucoside. The extract was applied to the column (7 ml of resin) by recycling for at least 5 h (25 ml/h). All washes were in Tris buffer/0.8% octyl glucoside containing (first wash, 50 ml) 100 mM NaCl and (second wash, 100 ml) 250 mM NaCl and (third, 25 ml) in Tris buffer only. Elution was with 100 μM KA (or, where stated, 100 μM AMPA) in Tris buffer/0.8% octyl glucoside (30 ml). The eluate was concentrated to 2 ml with an Amicon PM10 filter, and free KA or AMPA was removed on a Sephadex G-25 column (1.2 × 30 cm). Ligands, binding assays, and methods not specified were as in refs. 4 and 8.

The purified receptor was concentrated 10-fold in an Amicon PM10 cell at 0°C and 0.2 ml was centrifuged on a 7–15% sucrose density gradient (8) in 0.8% octyl glucoside/0.15 M NaCl. From the 0.25-ml fractions, 10 μl was assayed with [^{3}H]KA or [^{3}H]AMPA, and 170 μl was rotated overnight at 4°C with 100 μl of wheatgerm lectin-Sepharose 6MB (Pharmacia) beads. These beads were then washed with 50 mM Tris citrate (pH 7.2), shaken with [^{3}H]glycine (100 nM, 45 Ci/mmol; 1 Ci = 37 GBq) at 23°C for 90 min, filtered (Whatman GF/C), and washed rapidly twice with 2.5 ml of ice-cold buffer, and radioactivity was measured. Nonspecific binding (with 0.1 mM glycine) was low and has been subtracted. Specific [^{3}H]glycine binding was not decreased by 10 μM strychnine. After pooling, fraction I or II as defined was depleted of sucrose by water washing and concentrated for subunit analysis with a Centracon filter.

Reconstitution and Patch-Clamping. Bilayers (≈30 GΩ resistance) were formed from 9 parts asolectin (Sigma) and 1 part cholesterol (Sigma grade 1), at the tips (3 μm) of hard borosilicate glass pipettes (10). The purified receptor was incorporated (11) into liposomes of the same composition; these were dialyzed against 100 mM NaCl/10 mM Tris citrate, pH 7.4, to 1.5 μg/ml and taken into the pipette. Bath solution was 100 mM NaCl/5 mM KCl/2 mM $CaCl_2$/10 mM Tris·HCl, pH 7.4. Insertion into the bilayer was enhanced by applying pipette potentials of +180 mV for 10–60 min, after which at least 50% of patches responded to AMPA. All patches that

Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisooxazolepropionate; NMDA, *N*-methyl-D-aspartate; EAA, excitatory amino acid; KA, kainate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione.

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responded to AMPA responded equally well to KA. Each observation reported was made on at least six patches (with exceptions, due to scarcity of material, only in the two cases noted). Bath solutions were changed as described (10). Amplitudes of channel currents filtered at 1 kHz on playback (sampling at 5 kHz) were fitted to histograms by sums of Gaussian distributions; the peak amplitudes were fitted by linear regression vs. pipette potential to give the open channel conductance levels. When the liposomes containing fraction I or fraction II protein were separated from any residual free receptor on a 5–20% sucrose density gradient, the channel activity for each protein sample was not changed.

Subunit Analyses. The protein was SDS-denatured at 90°C and subjected to SDS/PAGE (12). For immunoblots, the gel was blotted on to Problot membrane (Applied Biosystems) at 70 V for 30 min at 23°C and incubated with the monoclonal antibody (13). Detection (13) employed biotin/peroxidase labeling and rabbit anti-mouse IgG antibody.

RESULTS

High-Affinity KA and AMPA Binding Sites Are Present on the Same Purified Protein. In the affinity chromatography in octyl glucoside, >97% of the binding sites for both KA and AMPA were retained by the domoate resin. After specific elution, B_{max} values (Table 1) showed that ≈1000-fold purification had occurred. Due to the abundance of the binding sites for both AMPA and KA in the extract, the final B_{max} values are the same as or greater than those found for other completely purified ligand-gated ion channels of similar size [e.g., the nicotinic or γ-aminobutyrate (14) receptors]. A single population of high-affinity sites for both [^{3}H]KA and [^{3}H]AMPA (Table 1) was observed, and the agonists KA, AMPA, and domoate showed similar affinities in competition at the [^{3}H]KA and [^{3}H]AMPA sites, whereas the non-NMDA competitive antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (2) showed a significant difference (Table 1). As expected, NMDA competed very poorly with KA or AMPA binding; e.g., only 12% of the total [^{3}H]KA binding (at 20 nM) was displaced by 1 mM NMDA. The total number of binding sites present on the affinity-purified protein is (within the experimental error) the same for KA and AMPA.

Table 1. Affinity purification of a KA/AMPA receptor

Parameter	Prior to chromatography		After chromatography	
	[^{3}H]KA	[^{3}H]AMPA	[^{3}H]KA	[^{3}H]AMPA
K_d, nM	20	150	15	34
B_{max}	14	17	7000–13,000	8000–12,000
n_H	0.96	1.00	1.06	0.97
r	0.98	0.99	0.99	0.93
Overall yield, %	—	—	12	10
K_i, nM				
Domoate			0.38	0.75
KA			16.2	9.2
AMPA (+0.1 M KSCN)			28	26
AMPA (alone)			85	—
L-Glutamate			850	395
CNQX			1490	5800
NMDA			>1,000,000	>1,000,000

B_{max} (pmol/mg of protein) and K_d values (both from Scatchard transformations, linear in the 1–200 nM ^{3}H-labeled ligand range), the Hill number (n_H), its correlation coefficient (r), and the K_i values (for competitive inhibition, determined over at least a 1000-fold concentration range) were all obtained by best-fit (one-site) analysis with the LIGAND program. The mean from at least three preparations (SEM ≤10%) is shown in each case, except that the range (to the nearest 1000) for four preparations is illustrated for B_{max} (purified). Two other preparations made with AMPA replacing KA for elution gave similar results but the yield was 70% of that with KA (attributed to the initial lower affinity of AMPA).

The affinity for AMPA (but not for KA) was increased considerably during purification (Table 1), suggesting that a selective inhibitor of AMPA binding (15) was removed. Moreover, the purified AMPA/KA-binding peak had an $s_{20,w}$ of 7 S (Fig. 1*A*), whereas from the crude extract (in the same medium) it had an $s_{20,w}$ of 8.6 S (8), suggesting this inhibitor is a large receptor-associated regulatory polypeptide.

Functional Non-NMDA and NMDA Receptors Are Purified. Elution with either KA or AMPA also yielded, surprisingly, binding sites typical (2) of NMDA receptors (Table 2). A mean of 24% (in four preparations) of the total binding sites for [^{3}H]glutamate (100 nM) present in the purified material was displaceable by 100 μM NMDA (while the rest were resistant to 1 mM, but displaceable by <1 μM KA or AMPA). In sucrose density gradient centrifugation, the high-affinity binding sites (Table 2) for [^{3}H]glycine [resistant to 10 μM strychnine, a characteristic of the NMDA receptor glycine site (18)] and the NMDA-sensitive [^{3}H]glutamate binding sites were concentrated together in a second partly resolved peak in the gradient (Fig. 1, fraction II), centered at an $s_{20,w}$ of 10.1 S. Dizocilpine [MK-801 (19)] binding sites were also present in fraction II and showed agonist and spermidine enhancement (Table 2). Fraction I (≈7 S) had none of those characteristic NMDA receptor binding properties. Signifi-

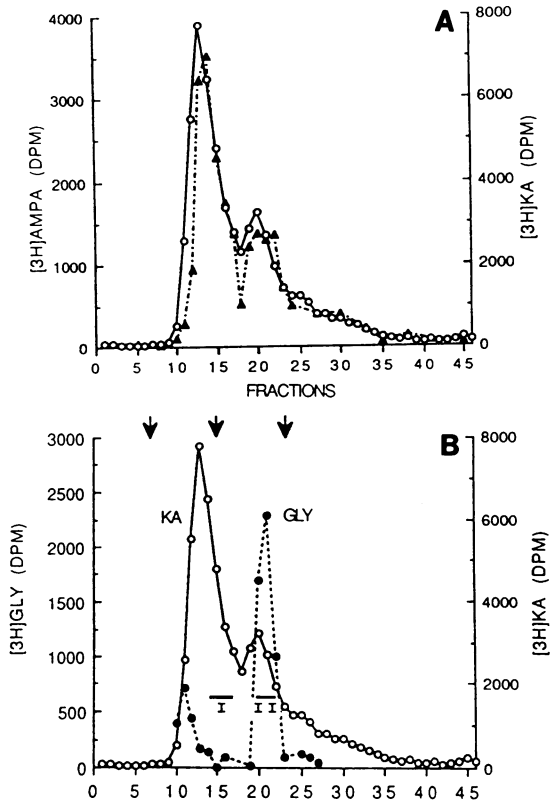

FIG. 1. (*A*) Gradient centrifugation of purified receptors, with specific binding of [^{3}H]KA (○) and [^{3}H]AMPA (▲). (*B*) High-affinity [^{3}H]glycine (●) and [^{3}H]KA (○) binding (after detergent removal) of samples from the gradient in *A*. Arrows show the location of markers, from left to right, hemoglobin (4.5 S), yeast alcohol dehydrogenase (7.4 S), and catalase (11.4 S). Pooled fractions I and II are shown by bars. The small initial fraction of [^{3}H]glycine binding seen was always present but varied in size between preparations; it was excluded from the analyses. NMDA-sensitive [^{3}H]glutamate binding (Table 2) paralleled the [^{3}H]glycine profile. Pretreatment with and inclusion in the gradient of dithiothreitol (10 mM), prior oxidation to disulfides by diamide, or peak recentrifugation did not interconvert the peaks; i.e., they behaved as independent species. Similar profiles were obtained in four such experiments, each from an independent purification.

Table 2. Binding of NMDA receptor ligands and KA

Binding	Ligand bound, pmol/ml	
	In solution	In liposomes
[^{3}H]Glu (NMDA displaceable)	2.5	1.05
[^{3}H]CGP-39653* (50 nM)	1.7	1.1
[^{3}H]MK-801 (20 nM)	<0.06	<0.06
[^{3}H]MK-801 + Glu/Gly	0.63	—
[^{3}H]MK-801 + Glu/Gly + Sper	0.95	1.5
[^{3}H]Gly (100 nM)	—	1.2
[^{3}H]Gly + (+)-HA-966* (10 μM)	—	0.17
[^{3}H]KA (20 nM)	4.42	2.2

Purified receptor was assayed in solution (with dilution of the octyl glucoside to 0.04% in the assay mixture) or in liposomes (washing with detergent-free assay medium and filter assay) (the mean of triplicates from two preparations is shown; SEM <10%). Volumes for liposomes and receptor are unrelated. Assays (on 20 μl) for [^{3}H]MK-801 (22 Ci/mmol; 2 h), [^{3}H]glutamate (100 nM, 49 Ci/mmol; ± 1 mM NMDA), and [^{3}H]glycine were at 25°C and assays for [^{3}H]CGP-39653 (34 Ci/mmol; 1 h; 0.5% lubrol) were at 0°C. Enhancers were glutamate and glycine at 10 μM and spermidine (Sper) at 0.1 mM. The entire protein was used, so the NMDA receptor sites will be only a fraction (see Fig. 1) of the total KA/AMPA sites present. [^{3}H]Glycine binding cannot be measured in the detergent.

*Specific competitive antagonist (2, 17) for the NMDA receptor of rat brain, at its agonist site (for CGP-39653) or its glycine site [for (+)-HA-966].

cantly, binding sites for [^{3}H]KA and for [^{3}H]AMPA (equimolar) were present throughout both peaks (Fig. 1).

The second distinct peak with [^{3}H]glycine, [^{3}H]KA, and [^{3}H]AMPA binding was consistently observed. Elution of the affinity column with 1 mM NMDA, instead of with 100 μM KA or AMPA, failed to release any binding sites of [^{3}H]KA or [^{3}H]AMPA. All of these findings strongly suggest that a proportion of the NMDA receptors is physically linked to a set of non-NMDA receptors.

The purified protein (either entire or from fraction I or II; Fig. 1) was incorporated into liposomes, which were fused with bilayers in patch-clamp pipettes. When AMPA, KA, or domoate was applied on the outside, abundant channel opening activity was evoked with the intact protein and both fractions I and II (Fig. 2 *A–C*). With fraction II (or the intact protein), the patches also responded to 1–10 μM NMDA, but only in the presence of glycine (16) (Fig. 2 *F* and *J*). Glycine (up to 0.1 mM) had no effect on KA or AMPA responses. The NMDA response was never observed in patches with fraction I.

The frequency of openings increased from 1 μM to 100 μM NMDA, AMPA, or KA. No channel currents were observed when the protein was omitted or with 0.1 mM nicotine, acetylcholine, or γ-aminobutyrate. With symmetrical saline solutions, the current–voltage relationship with all three ligands was linear throughout (Fig. 2*E*), to give single-channel conductances of ≈6 pS (AMPA), 9 pS (KA or domoate), and 50 pS (NMDA). Less-frequent higher conductance levels were observed with AMPA or KA but these were not distinguished from simultaneous 6- to 9-pS openings. With NMDA (plus glycine) alone, only 50-pS openings were seen (Fig. 2 *E*, *F*, and *J*), although openings of mainly 30–40 pS, possibly subconductances, were also present when Mg^{2+} was added (Fig. 2 *G* and *I*).

The reversal potential (≈0 mV) for the AMPA, KA, or NMDA currents was unchanged when SO_4^{2-} was unilaterally substituted for 90% of the Cl^-, confirming that the single-channel currents are cationic. This is supported by a recent initial study of the KA-induced current across a planar bilayer containing our unfractionated purified protein (16), in which a unilateral increase in K^+ gave a Nernstian shift of the reversal potential.

The AMPA/KA (but not the NMDA) channel openings were prevented by 30 μM CNQX (Fig. 2 *C* and *D*); the NMDA (but not the AMPA/KA) channel openings were suppressed likewise by the selective NMDA receptor competitive antagonist (2) D-aminophosphonovalerate at 10 μM. In each case the effect could be progressively overcome by increasing the agonist concentration (data not shown). With Mg^{2+} (0.1–15 mM; in 20 of 20 tested channel-containing patches), NMDA receptor blockade (at −100 mV) always occurred and was complete at 10 mM, whereas 0.1–1 mM Mg^{2+} caused a partial blockade of the NMDA activity with pronounced "channel flickering" (Fig. 2 *G–J*). Zn^{2+} (0.1–4 mM; 15 of 15 patches) also blocked (fully at 4 mM) the response to NMDA/glycine (only), but in a voltage-

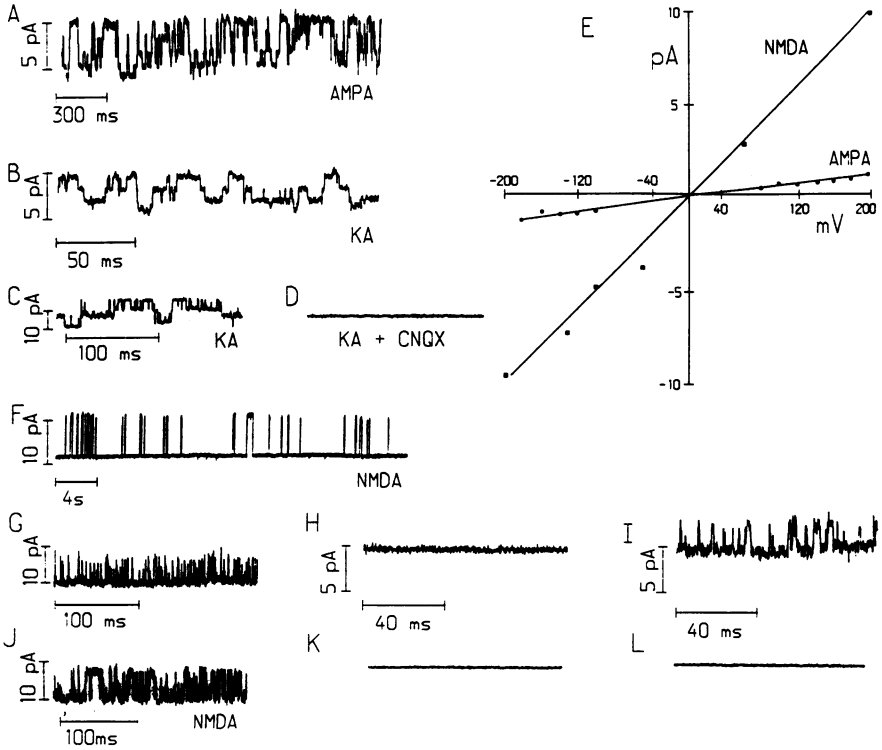

FIG. 2. Recordings from representative bilayer patches containing the purified receptors as follows: fraction I (*A* and *B*) or fraction II (*C* and *D*) or both (*F–L*). Openings are downward except in *F* and *J*. (All recordings were low-pass-filtered at 2 or 3 kHz; also note different scales). (*A*) Response to 10 μM AMPA at −160 mV. (*B*) Response of same patch to 10 μM KA at −180 mV. (*C* and *D*) A fraction II patch responds likewise to KA at −160 mV (*C*) and is fully and reversibly blocked by 50 μM CNQX in a voltage-independent manner (*D*). The response of this patch to AMPA was likewise blocked by CNQX (data not shown). (*E*) Current–voltage plots for the lowest of the conductance levels (6 pS) with AMPA and for NMDA (slope, 50 pS). (*F* and *J*) Responses to NMDA showing 50-pS openings at 180 mV (*F*) and −180 mV (*J*). (*G*) Flickering block of NMDA channel caused by 1 mM Mg^{2+} at −120 mV. (*H* and *I*) Another patch, showing a complete block of NMDA response by 20 mM Mg^{2+} at −150 mV (*H*) but an unblock at +100 mV (*I*). The channel then had a conductance of 30 pS. (*J–L*) Antagonism by 4 mM Zn^{2+} of NMDA responses (−180 mV) (*J*) at negative (−180 mV) (*K*) and positive (180 mV) (*L*) potentials. (*F–L*) NMDA is at 10 μM with 2 μM glycine.

independent manner (Fig. 2 *J–L*). MK-801 (19) at 1 μM fully blocked NMDA activity, but only at negative potentials (data not shown). All of this behavior and the conductance values are characteristic of the respective neuronal excitatory amino acid (EAA) receptors (2, 18–20).

Whereas 10 mM Mg^{2+} had no effect on the channels gated by AMPA or KA alone, when fraction II patches (2 of 2) were tested with 10 mM Mg^{2+} present, using 0.1 mM NMDA (+2 μM glycine) plus 10 μM AMPA (or KA), the AMPA- (or KA)-evoked channels also became blocked at negative potentials (recordings appeared as for Fig. 2*H*). Therefore, when Mg^{2+} gains access to the NMDA-gated channel, it also blocks subsequent responses to AMPA (or KA). All of the observations here strongly suggest that both NMDA and non-NMDA ligands can operate a common ion channel in a unitary form of the EAA receptor. It is important to note, however, that the latter cross-reaction was not seen with fraction I, which on reconstitution exhibited only the characteristics of pure AMPA and KA receptors.

Independent confirmation of this unusual cross-reaction was sought in the total *Xenopus* brain EAA receptor population as expressed in oocytes (21) injected with *Xenopus* brain poly(A) RNA. The NMDA-evoked currents and their blockade by MK-801 and Mg^{2+} were as reported for oocytes injected with rat brain RNA (22, 23). When KA (0.1 mM) was applied as a pulse (10 s) during perfusion of an oocyte with AMPA (0.1 mM), the response to the KA was reduced by ≈90%. Such cross-blockade has been reported with rat RNA (for review, see ref. 3) and was used to conclude that KA and AMPA share the same receptor. However, NMDA (0.1 mM) also antagonized the responses to KA (Fig. 3 *A–C*) and to domoate. NMDA (1 mM) caused a reduction of ≈30% in the maximum response but no change in their EC_{50} values (Fig. 3*D*). This effect of NMDA was identical in the presence and absence of glycine (1 or 10 μM) or of Mg^{2+} (1 mM) and showed no voltage dependence.

It could be envisaged that NMDA is a competitive desensitizing agonist [as is AMPA (22, 23)] or is a weak antagonist at the KA receptor. This was excluded because the observed effect is distinctly noncompetitive (Fig. 3*D*), and when expressed in the oocyte, the recombinant pure non-NMDA receptor Glu-R1 (5) responds strongly to KA and to AMPA (6, 7), and NMDA has no effect on the non-NMDA response (ref. 5 and unpublished data). Thus, NMDA at 0.1 mM (used as in Fig. 3*D*) gave no shift of the KA dose–response curve; even with 1 mM NMDA, the EC_{50} for KA increased from 36.4 μM to 44 μM, but the maximum response to KA was unchanged. Hence, on a purely non-NMDA receptor, NMDA acts only extremely weakly and competitively.

Subunits of the Two Receptor Types. Coomassie blue-stained SDS/PAGE gels of the total purified protein revealed consistently a slightly broad band at 42 kDa and a sharp band at 100 kDa. However, only the 42-kDa component was present in fraction I, whereas the 42-kDa and the 100-kDa bands were present in fraction II (Fig. 4). Likewise, when liposomes with the fraction I protein, showing the KA/AMPA-dependent channels (Fig. 2), were themselves analyzed (data not shown), only the 42-kDa band was seen (as in Fig. 4*A*).

Further, a monoclonal antibody to an avian cerebellar 50-kDa KA-binding protein (13) strongly recognized the *Xenopus* 42-kDa protein (Fig. 4*A*). This antibody also coprecipitated the KA and the AMPA binding activities of fraction I (data not shown). The 42-kDa component, on gel elution and peptide microsequencing (J. Rossier, A.A., and E.A.B., unpublished work), was shown to be homologous to the recombinant GluR series (5–7) of non-NMDA receptors. All of these data clearly assign the 42-kDa polypeptide(s) to KA binding and to the channels gated by KA and AMPA.

The Unitary Non-NMDA Receptor. We conclude, therefore, that in fraction I a functional unitary KA/AMPA receptor has been isolated. It is striking that both the KA and the AMPA activities are bound to a KA-type column, that both are coeluted with either ligand in a 1:1 ratio (Table 1) and could not be separated, likewise, that KA and domoate are more potent than AMPA in inhibiting [^{3}H]AMPA binding, and that AMPA inhibits [^{3}H]KA binding with the AMPA binding affinity and KA inhibits [^{3}H]AMPA binding with the KA binding affinity (Table 1). We deduce that, in this receptor type, KA selects a KA conformation and AMPA selects an alternative AMPA conformation of a single binding site. A channel is opened by either, but the channel characteristics (e.g., desensitization) can differ in the alternative conformations. The concept of two alternative conformations of the AMPA site on one receptor has been used to explain the binding behavior of [^{3}H]AMPA to mammalian brain membranes under various conditions (15, 24).

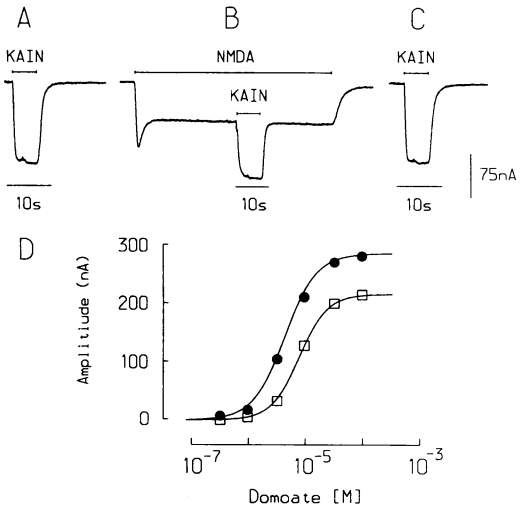

FIG. 3. Noncompetitive antagonism of KA-evoked responses (KAIN) by NMDA in an oocyte injected with *Xenopus* brain mRNA. (*A*) Response to 33 μM KA. (*B*) In a subsequent application of 33 μM NMDA (plus 2 μM glycine), the response to KA is antagonized (by 28%). (*C*) Full recovery. Forty-four KA-responsive oocytes were used with quantitatively similar results throughout. (*D*) Dose–response plots for domoate in the absence (●) and presence (□) of 1 mM NMDA (plus 2 μM glycine). The antagonized response was measured from the NMDA plateau (three oocytes; SD <5%).

The KA/AMPA/NMDA Receptor. In immunoblots of fraction II, using the anti-KA binding protein monoclonal antibody (13), the 42-kDa and the 100-kDa protein bands present were clearly reactive (Fig. 4*B*). This demonstrates that they are related proteins and, hence, neither is a nonreceptor contaminant of the other.

We cannot, as yet, prove that the 42-kDa band from fraction II contains exactly the same subunit(s) as the 42-kDa band from fraction I. We assume so, since both are recognized by

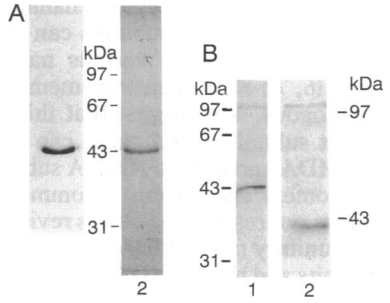

FIG. 4. Subunits (in 10% gels). Staining with Coomassie blue (lanes 1) or antibody (lanes 2). (*A*) The fraction I protein in a 10% gel. (*B*) The fraction II protein. The two bands in each gel in *B* (electrophoresed for different times) correspond.

a monoclonal antibody to a KA-binding protein from a distant species and since the KA and AMPA high-affinity binding sites and receptor activities are present in both fractions. In fraction II, the NMDA-evoked channels were often very abundant and the NMDA receptor binding activities present (Table 2) were too great (per μg of protein) to be due to some other subunit that is so rare that it is not detected on the gels or immunoblots. Because of this and because the 100-kDa protein is antigenically an EAA receptor type, we deduce that it is the 100-kDa protein that confers the NMDA receptor properties. Since there is evidence for functional interaction between the NMDA and the KA sites in fraction II (see above) and in expressed *Xenopus* brain RNA, for ≈30% of the total KA receptors (Fig. 3), the simplest interpretation of the data is that subunits of 42 and 100 kDa in *Xenopus* constitute a KA/AMPA/NMDA receptor oligomer.

DISCUSSION

We have provided evidence that in *Xenopus* brain there is a unitary non-NMDA receptor and also a unitary KA/AMPA/NMDA receptor. In addition, since many NMDA receptor sites do not coincide in their autoradiographic distribution with KA or AMPA sites in mammalian (17) or *Xenopus* (25) brain, a solely NMDA receptor class must also be postulated. However, this class of receptor would not be retained by the domoate affinity column (since we confirmed that domoate does not interact appreciably with NMDA binding sites). Such a population of separate NMDA receptor sites is indeed present since, after recycling the initial extract (freed from endogenous low molecular weight components) through the domoate column for 7 h at 4°C, <2% of the [^{3}H]KA-binding sites, but at least 30% of the NMDA-displaceable [^{3}H]glutamate sites initially present (mean of five experiments) were left unbound.

The channel opened by NMDA (50 pS) in the unitary KA/AMPA/NMDA receptor does not have the same properties as that opened by KA or AMPA (6–9 pS). We account for this with the proposal that the 100-kDa subunit binds NMDA and mediates a different conformational change to that evoked in the 42-kDa subunits alone. At saturating NMDA concentrations, then, it should not be possible to increase the current by subsequent addition of KA or AMPA. Likewise, when the 50-pS conductance evoked with NMDA is blocked by Mg^{2+}, subsequent addition of KA or AMPA should not result in a channel current. Both of these effects have been observed for one fraction of the NMDA receptors. Desensitization (not evident in the liposomes) can give rise to an additional form of NMDA–KA interaction within a common receptor, when it occurs in the metabolizing oocyte (Fig. 3). Particularly relevant here is the only report (apparently largely overlooked) analyzing in detail the effect of NMDA on non-NMDA receptor currents in neurons (26), which shows a failure of KA activation to summate with NMDA activation and concludes that some of the NMDA and KA channels reside in a common receptor–channel complex.

The recombinant mammalian subunits can readily assemble in heterooligomers to form various native-type non-NMDA receptors (6, 7), all of their transmembrane domains being very homologous. We suggest that this situation extends further, that subunit interchanges can also occur between the non-NMDA and certain NMDA subunits, and that the resultant oligomers again form a common channel. A model that explains our results and others reviewed above (3, 22, 23, 26) is that unitary non-NMDA receptors contain only AMPA/KA subunits and pure NMDA receptors contain only NMDA-type subunits, whereas the unitary AMPA/KA/NMDA receptors can arise from the partial replacement (during receptor gene expression) of one type by the other. The subunit size difference in *Xenopus* renders this situation more readily detected; however, we have seen similar effects in rat brain poly(A) RNA oocyte expression. Such an exchange, if it produced a stable oligomer with two 42-kDa and three 100-kDa subunits, would account for the size difference seen (Fig. 1) between the KA/AMPA and the KA/AMPA/NMDA receptors. This subunit-exchange hypothesis adds an explanation for the great multiplicity of EAA receptor types and subtypes observed electrophysiologically. Also, if neuronal activity controls the expression of particular subunits, it provides another mechanism for the major role of EAA receptors in synaptic plasticity.

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