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AMPA and kainate-operated channels reconstituted in artificial bilayers

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Cationic channels which can be activated by α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and kainate play a key role in the generation of excitatory postsynaptic potentials in the brain of all vertebrates. On a protein carrying binding sites for both these ligands, affinity-purified from the *Xenopus* nervous system, electrical measurements have been performed to investigate its functional properties after the pure complex had been incorporated into planar lipid bilayers. Domoate, AMPA or kainate added to the reconstituted protein activated cationic channels, which were blocked by typical antagonists for this neurotransmitter system. These data suggest that the reconstituted protein is an ionotropic receptor of the unitary non-NMDA sybtype.

Unitary non-NMDA receptor; Reconstitution; Ion channel; *Xenopus*

1. INTRODUCTION

In the vertebrate nervous system, excitatory amino acid receptors containing an integral cation channel can be generally classified into those activatable by *N*-methyl-D-aspartate (NMDA receptors) and the non-NMDA receptors, of which the subtypes recognising kainate (KA) or quisqualate (QUIS) are the major forms [1]. For the QUIS subtype, AMPA is a more selective and potent ligand than QUIS itself [2] and the AMPA subtype is now a preferred nomenclature for the QUIS receptor. However, KA and AMPA sites can at some locations be closely associated, and the receptors there can be defined as unitary non-NMDA receptors [3,6]. For instance, in many cases, reveiwed by Barnard and Henley [4], QUIS or AMPA have been reported to block the conductance evoked by KA and to cross-densensitise with the latter. Recently we showed [3] in biochemical fractionation of extracts of *Xenopus* brain (an exceptionally rich source of KA and AMPA binding) that the great majority of the KA and AMPA sites could not be separated. A single protein was then purified from *Xenopus* brain, carrying both KA and AMPA sites, which show strong reciprocal interaction [5]. Functional reconstitution of this protein into planar lipid bilayers is here reported. Evidence is produced to demonstrate typical non-NMDA receptor properties for the reconstituted receptor channel.

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; NMDA, *N*-methyl-D-aspartate; KA, kainate; QUIS, quisqualate; OG, octylglucoside; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione

2. EXPERIMENTAL

2.1. *Purification procedure*

Total brain membranes prepared from adult *Xenopus* were solubilised in 1% octylglucoside (OG) as described elsewhere [3]. Affinity chromatography using domoate immobilised on AH-sepharose 4 B resin (Pharmacia) was based upon the method of Hampson and Wenthold [6]. All materials not specified were from Sigma.

2.2. *Planar lipid bilayers*

Bilayers were made from 9 parts asolectin (Sigma) and 1 part cholesterol (Sigma, Grade 1). The aqueous phase contained 0.2 M KCl, 0.2 mM $CaCl_2$, 10 mM Tris-acetate (pH 7.4), on both sides of the bilayer, unless stated otherwise. Lipid bilayers were formed over a 1 mm diameter aperture in a septum that separated two 4 ml Teflon chambers as described by Mueller and Rudin [7]. Proteoliposomes (50 μl of the suspension, see below), agonists and antagonists were added to the *cis* side, after formation of the bilayer. Voltage was applied to the *cis* side of the bilayer and the *trans* side was held at virtual ground. The currents were measured by a Ag/AgCl electrode system, using a voltage-clamp converter as input circuit and a current-voltage converter as output circuit with a 10^8 Ω feedback resistor. Positive current is the flow of positive ions into the *trans* compartment, and, in all records, channel opening is shown as an upward deflection if the applied potential is positive. The membrane currents were recorded on a modified digital audio processor (Sony PCM - 701 ES) connected to a video cassette recorder (Sony SL-HS 100 EC), filtered at 100 Hz and displayed on a Gould chart recorder. The liposomes containing the purified protein were prepared [8] by dialysis to give a final concentration of 0.025% lipids, with protein concentration in the suspension about 1.5 $\mu g \cdot ml^{-1}$. All experiments were at room temperature.

3. RESULTS AND DISCUSSION

Purification of a protein carrying binding sites for [^{3}H]KA and [^{3}H]AMPA was achieved by affinity

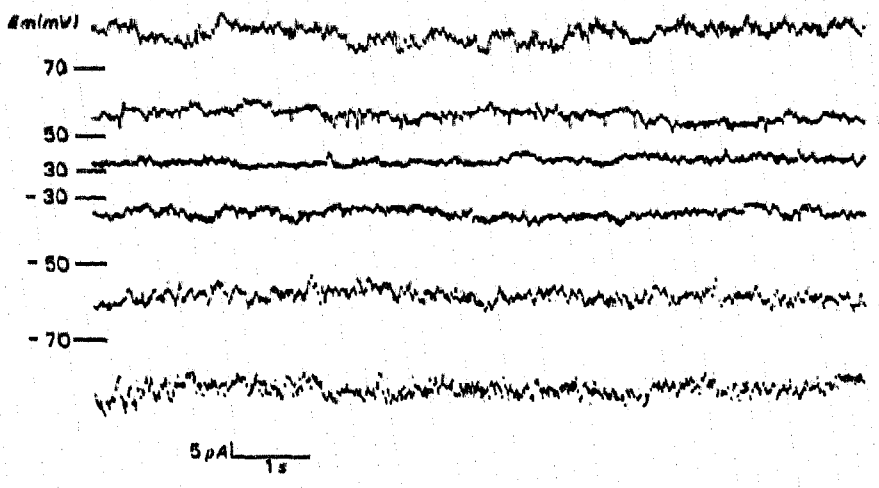

Fig. 1. Fluctuations of cationic channels in planar lipid bilayers containing the reconstituted AMPA/KA binding protein isolated from *Xenopus* brain. Representative channel records, at the indicted voltages (± 30, 50, 70 mV), for activation by 5 μM domoate applied to the *cis* side. (At least 4 independent experiments gave very similar results. The same is also true concerning records in Figs 2 and 3.) Evidence is seen of current jumps, indicating different values of channel conductance.

chromatography using immobilised domoate (a more potent analogue of KA) based upon a previously reported [6] method (details to be published elsewhere). Gel electrophoresis analysis revealed in silver staining a broad band around M_r 42 000 plus some weaker bands [5].

To determine whether the isolated protein is a functional receptor, it was reconstituted into artificial lipid bilayers [8,9]. The protein was incorporated into liposomes of asolectin: cholesterol (9:1). These were added to one side (*cis*) of a bilayer of similar composition, over a 1 mm diameter aperture. Current flow was observed, under voltage-clamp, only when the protein was present in the liposomes and a non-NMDA receptor agonist was added. Liposomes were very fusogenic and 17 membranes of 19 tested revealed incorporated channels in the presence of agonist. Results obtained from three different batches of protein were very similar. Fig. 1 shows current fluctuations of increasing amplitudes evoked by domoate (5 μM), applied at the *cis* side, at holding potentials in turn of +30, +50 and +70 mV, which reversed direction at negative potentials. Either KA or AMPA (tested in the 10-60 μM range) were able to induce fluctuations when added to the *cis* side (Fig. 2). The fluctuations were completely suppressed (Fig. 2) by 100 μM 6-cyano-7-nitroquinoxaline-2-,3-dione (CNQX), a selective non-NMDA receptor antagonist [10] or by 100 μM kynurenate (not shown). Differences in the agonist concentration required to evoke currents between domoate (Fig. 1), KA or AMPA (Fig. 2) are consistent with the rank order of potency for these ligands in displacing [^{3}H]KA and [^{3}H]AMPA binding, both in solubilised membranes [3] and in the affinity-purified product [5]. In asymmetric conditions, with 0.2 M KCl on the *cis* side and 0.6 M KCl on the *trans* side, and with the bilayer clamped at

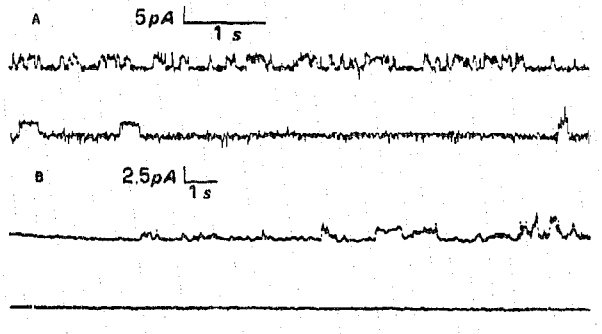

Fig. 2. KA and AMPA activation of single channel conductances in planar lipid bilayers. The figure displays sample records at different time scales and current amplitudes. Channel openings induced by: (A) 50 μM KA (upper trace), holding potential = +90 mV; the fluctuations are greatly reduced by the addition of 100 μM CNQX (lower trace); (B) 25 μM AMPA (upper trace), holding potential = +50 mV; 100 μM CNQX (lower trace) completely inhibits the agonist-induced currents. (Due to the noise level and the high cutoff filtering required, the amplitude of the single channel conductances in part B are not well resolved.)

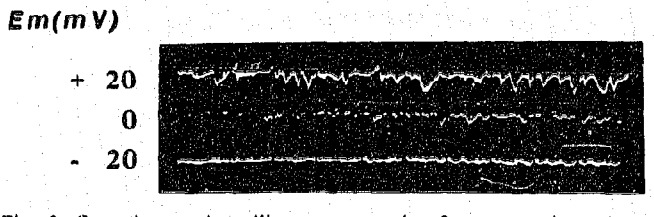

Fig. 3. Superimposed oscilloscope records of currents throughout reconstituted channels activity by KA (50 μM). Recording was under asymmetric conditions, i.e. 0.2 M KCl on the *cis* side and 0.6 M KCl on the *trans* side (each in 0.2 M $CaCl_2$, 10 mM Tris-acetate, pH 7.4). At this KCl ratio, the Nernst equilibrium potential for monovalent cation transport is −24 mV. In the record, the applied potential of −20 mV shows very small positive current fluctuations, which increase at 0 and +20 mV.

several voltages between −30 mV and +30 mV, the agonist-evoked current reversed near −20 mV, in close agreement with that predicted by the Nernst equation for a cationic selective channel (in these conditions −24 mV). In the records shown in Fig. 3 the applied potential of −20 mV elicited only very small positive current fluctuations, which increased at 0 mV and +20 mV. These results are expected for a cation-selective channel.

Our study shows that the purified AMPA/KA binding protein isolated from *Xenopus* brain and reconstituted into lipid bilayers displays properties of a chemically-gated ion channel, as expected for a functional receptor. Indeed, the agonist activation, the antagonist competition and the selectivity for cations over anions are in important respects the same as the unitary non-NMDA ionotropic receptor studied in neuronal cultures [11,12] and in the *Xenopus* oocyte translation system [13,14].

Recently, molecular cloning and functional expression of rat cDNAs encoding for subunits of non-NMDA receptors have been reported [15,16], showing that QUIS/AMPA and KA can bind to and activate a common protein of relative molecular mass 100 000. The purified protein used here is partly homologous to those (to be published elsewhere) and is related to a family of KA-binding proteins identified from non-mammalian species, cloned members of which have M_r values of 48 000 and 49 000 [17,18]. However, an important difference from the latter is that the protein we isolated is an ionotropic receptor, activatable both by AMPA and KA, whereas no channel activity was observed when the cDNAs of known frog and chick KA-binding proteins were expressed in translational systems [17,18]. The structural and functional relationship between the 100 000 M_r polypeptide family, identified from rat, and these KA-binding proteins, identified from non-mammalian nervous systems, still remains to be clarified.

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