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Ca^{2+} permeability of unedited and edited versions of the kainate selective glutamate receptor GluR6

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ABSTRACT **The Ca^{2+} permeability of the kainate selective glutamate receptor GluR6 depends on the editing of the RNA (or DNA). The unedited version of GluR6, GluR6Q, encodes a glutamine at position 621 (Q/R site) and exhibits a Ca^{2+}/monovalent ion permeability ratio of 1.2, while the edited version of GluR6, GluR6R, encodes an arginine at position 621 and exhibits a permeability ratio of 0.47. Kainate activation of the GluR6 receptor results in currents that are modulated by extracellular calcium ions. Permeability ratios of other divalent ions indicate that the Q/R site is not the only determinant for divalent ion permeability. The level of editing of the receptor will determine the Ca^{2+} influx through the GluR6 receptor channels and, consequently, may modulate the synaptic activity.**

Ca^{2+} flux through glutamate receptors is thought to have a key role in long-term potentiation, excitotoxic cell death, and epilepsy. In most neurons that have been studied the non-*N*-methyl-D-aspartate (NMDA) ionotropic glutamate receptors exhibit low permeability to Ca^{2+} (1), although in some neurons and glia cells the Ca^{2+}/monovalent ion permeability ratio (P_{Ca}/P_{mono}) is considerably higher (2–6). Two types of non-NMDA glutamate receptors have been observed in cultured rat hippocampal neurons. Type I receptors have a low P_{Ca}/P_{mono} and exhibit a linear current–voltage (*I–V*) relationship, while the type II receptors with a high P_{Ca}/P_{mono} exhibit a strongly inward rectifying *I–V* relationship (4). However, non-NMDA glutamate receptors present in salamander bipolar cells exhibit a high P_{Ca}/P_{Na} but a linear *I–V* relationship (5).

Recent cloning experiments have revealed the existence of at least three structurally distinct classes of non-NMDA receptors in mammals: GluR1–GluR4, which are activated by both kainate and DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (7–11); GluR5–GluR7, of which GluR5 and GluR6 generate homomeric channels activated by kainate but not by AMPA (12–15); and KA-1 and KA-2, which do not generate functional homomeric channels but bind kainate but not AMPA (16–18). Coexpression of KA-2 with GluR5 or GluR6 potentiates the response compared to the homomeric GluR5 or GluR6, respectively, and the heteromeric channels are activated by both AMPA and kainate (17, 18).

The Ca^{2+} permeability and rectification properties of the GluR1–GluR4 class of subunits depend strongly on the subunit composition of the receptor. GluR1, GluR3, and GluR4, in combination with each other or as homomeric channels, generate strongly inward rectifying receptors permeable to Ca^{2+}—i.e., high P_{Ca}/P_{mono}. However, when the GluR2 subunit is a constituent of the receptor complex, the *I–V* relationship becomes linear and Ca^{2+} permeability is greatly reduced (19). Mutagenesis studies have revealed that the presence of either a glutamine or an arginine in the putative transmembrane region II can account for the permeability and rectification properties (20, 21). GluR1, GluR3, and GluR4 subunits all have a glutamine, while GluR2 has an arginine. Thus, the presence of an arginine correlates with a linear *I–V* relationship and low P_{Ca}/P_{mono}.

Recently, it has been shown that this critical arginine is not encoded in the GluR2 gene but probably results from almost 100% editing of the mRNA. Similar editing takes place in the expression of GluR5 and GluR6 subunits but not to the same extent; ≈30% of the GluR5 and ≈70% of the GluR6 mRNAs are edited (22). In this report, we study the effect of this editing process on the divalent ion permeability of GluR6 receptors.

MATERIALS AND METHODS

The GluR6Q version was generated by mutagenesis of a cDNA coding for the GluR6R subunit (23).

Oocytes were isolated from *Xenopus* frogs and injected with 5–15 ng of RNA *in vitro* transcribed as described (7). Recordings were performed 4–14 days after injection under a two-electrode voltage clamp with an Axoclamp 2A amplifier. Both recording and current electrodes were filled with 3 M KCl. *I–V* relationships were obtained by applying 2-sec voltage ramps in the presence of agonist and subtracting the average resting *I–V* curve obtained before and after agonist application. When changing solutions, the oocyte would in general be equilibrated with the new solution for 2 min before applying the agonist. *I–V* curves obtained from successive applications of agonist in the new solution established that the 2-min perfusion was sufficient to exchange the solutions and the internal ion concentrations were constant for the duration of the experiments. The following solutions were used: low-Ca^{2+}/Ringer's, 15 mM Hepes·NaOH, pH 7.4/90 mM NaCl/1 mM KCl/0.1 mM $CaCl_2$/1 mM $MgCl_2$. The solution used for the divalent permeability studies was 15 mM Hepes/80 mM *N*-methylglucamine (pH adjusted to 7.4 by HCl)/10 mM $MgCl_2$, $CaCl_2$, $SrCl_2$, or $BaCl_2$. The Ca^{2+} solutions contain 15 mM Hepes (pH 7.4) and either 2, 5, 10, or 20 mM $CaCl_2$ and *N*-methylglucamine for osmotic balance. A correction for solution-dependent junction potentials (3–6 mV) is included in the reversal potentials.

Oocytes were injected with 50–100 nl of 20 mM 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid (pH 8.0) (BAPTA) 5–180 min prior to recording. Each oocyte was exposed to 10 μM concanavalin A (Con A; Sigma type IV) for 5 min in the recording chamber if not otherwise indicated. Activities were calculated by Guggenheim's modification of the Debye–Hückel expression for activity coefficients (24).

RESULTS

The permeability properties for divalent cations of homomeric channels generated from GluR6 subunits were studied

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Abbreviations: NMDA, *N*-methyl-D-aspartate; P_{Ca}/P_{mono}, Ca^{2+}/monovalent ion permeability ratio; AMPA, DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid.

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in *Xenopus* oocytes injected with *in vitro* transcribed RNA. The receptors were activated by either kainate or domoate and current responses were recorded with a two-electrode voltage clamp. Most experiments were performed after Con A treatment of the oocytes, which generally potentiated the responses at least 100-fold (13). This treatment was particularly important for analysis of the GluR6R variant, which only gave 5- to 50-nA responses to kainate before Con A treatment. Control experiments using the more efficacious agonist domoate established that Con A treatment did not influence the permeability properties (data not shown).

The Q/R site has been shown to control the rectifying properties of the GluR1–GluR4 class of subunits when cells are studied in high sodium solutions (20, 25). We and others have reported a similar role for the Q/R site in controlling rectification in GluR6 (17, 23). GluR6Q receptors exhibited a strongly inward rectifying *I–V* relationship in contrast to GluR6R receptors, which exhibited a slight outward rectification (Fig. 1*B*). The experiments were performed in low Ca^{2+} (0.1 mM)/Ringer's solution to avoid interference from endogenous Ca^{2+}-activated Cl^- channels. Substitution of the external chloride by the impermeable methanesulfonate did not change the reversal potential of GluR6R (13) or GluR6Q (data not shown), indicating that there is no significant Cl^- contribution to the current.

To investigate the Ca^{2+} permeability of the two variants of the receptor, we performed experiments in solutions containing different concentrations of Ca^{2+} as the only permeable external ion. The external Na^+ and K^+ ions were substituted by isoosmolar concentrations of the impermeable cation *N*-methylglucamine. To avoid activation of endogenous Ca^{2+}-activated Cl^- channels (26), which are activated in the rising phase of the GluR6 response, the fast Ca^{2+} ion

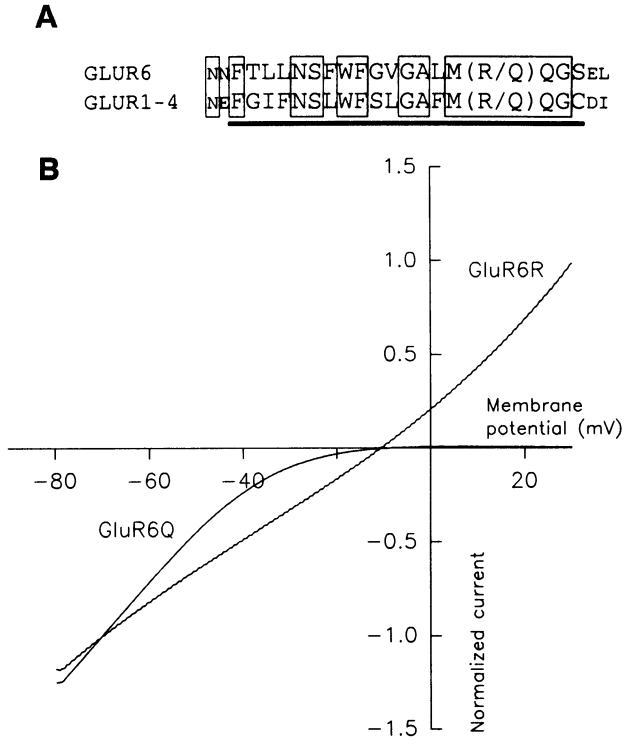

FIG. 1. (*A*) Comparison between regions of GluR6 and GluR1–GluR4. The putative transmembrane region is underlined. (*B*) *I–V* relationships for either of the homomeric GluR6 variants obtained from oocytes injected with RNA encoding either GluR6Q or GluR6R. *I–V* was recorded during a 2-sec voltage ramp from −80 to 30 mV and then subtracting the average resting *I–V* curve obtained before and after agonist application. *I–V* curves were normalized to the current observed at −70 mV.

chelator BAPTA was injected prior to the recordings. A number of tests were performed to ensure an efficient block of the Ca^{2+}-induced Cl^- currents after BAPTA treatment. After recordings from each oocyte (normally one to four agonist applications of 5 sec), the oocyte was exposed to agonist in high Ca^{2+} (20 mM). Only oocytes that did not show any change in the reversal potential after 30 sec were used for the analysis (70% and 90% of the GluR6Q and GluR6R injected oocytes, respectively). The strong rectification of the GluR6Q receptor current makes determination of the reversal potential of the GluR6Q receptor very sensitive to an additional Cl^- current. The rectification permitted an additional test of whether BAPTA could control calcium and prevent secondary activation of the Cl^- current. If this current had been activated, then a large outward current would be expected at positive potentials. Indeed, this was the case in oocytes not injected with BAPTA, but only a very small outward current was seen in BAPTA-injected oocytes (Fig. 2*B*). Since the *I–V* curve of the Cl^- current is known (ref. 27; unpublished data), a worst-case estimate of the contribution of the Cl^- current at the reversal potential can be made by assuming that all the current at 50 mV is Cl^- current (e.g., 10 nA in Fig. 2*B*), the Cl^- contribution to the total current at −40 mV is, because of the outward rectification of the Cl^- current, estimated to be 0.2 nA, and this would change the reversal potential by <1 mV in the negative direction. Only the GluR6Q-injected oocytes that did not develop any additional outward current after 30 sec of agonist application in 20 mM Ca^{2+} solution were used for the analysis. In summary, injection of BAPTA to a final internal concentration of ≈2 mM in the oocyte is sufficient to inhibit Ca^{2+}-induced activation of the Cl^- channels, and under these conditions Ca^{2+} permeation can be assessed.

The reversal potentials were used to assess the permeation of Ca^{2+} for the homomeric GluR6 channels. Fig. 2 shows that the reversal potentials for both the GluR6Q and GluR6R channels become more depolarized as a function of Ca^{2+} activity in the extracellular solution, indicating that both channels are permeable to Ca^{2+} ions. The reversal potentials for GluR6R channels are obviously more negative than for GluR6Q at a given Ca^{2+} concentration, suggesting a higher P_{Ca}/P_{mono} for GluR6Q than for GluR6R (Fig. 2).

To examine the Ca^{2+} permeability more quantitatively, the reversal potentials were recorded in low Ca^{2+}/Ringer's solution (containing Na^+ and K^+ as charge carriers) and afterwards in Na^+,K^+-free 10 mM Ca^{2+} solution. The reversal potential in the low Ca^{2+}/Ringer's solution was used to estimate the intracellular monovalent ion activity, which showed some variability between oocytes from different batches and between oocytes from the same batch at different times after injection. Assuming that the intracellular monovalent ion concentration varies only marginally during the shift of bathing solution, the permeability ratios were calculated from the Goldman–Hodgkin–Katz equation modified to include divalent cations (1), assuming no anion permeability and equal permeability for sodium and potassium. The latter was confirmed by substituting extracellular sodium with potassium, which did not change the reversal potential significantly (0.4 ± 0.5 mV; n = 6). Fig. 2 *B* and *C* shows two typical *I–V* curves for the GluR6Q and -R variants. The permeability ratio was calculated for each oocyte and averaged. GluR6Q showed ΔV_{rev} = 29 ± 2 mV (n = 10) negative shift of the reversal potential in the Ca^{2+} solution compared to normal Ringer's solution, implying P_{Ca}/P_{mono} = 1.2 ± 0.1. The numbers for the GluR6R variant were ΔV_{rev} = 46 ± 2 mV (n = 10) and P_{Ca}/P_{mono} = 0.47 ± 0.03. A similar experiment was performed with 60 mM Ba^{2+} as the external charge carrier, showing a P_{Ba}/P_{mono} of 0.8 (n = 6) and 0.6 (n = 6) for GluR6Q and GluR6R, respectively. The reversal potentials were less than or equal to −110 mV in the absence of external

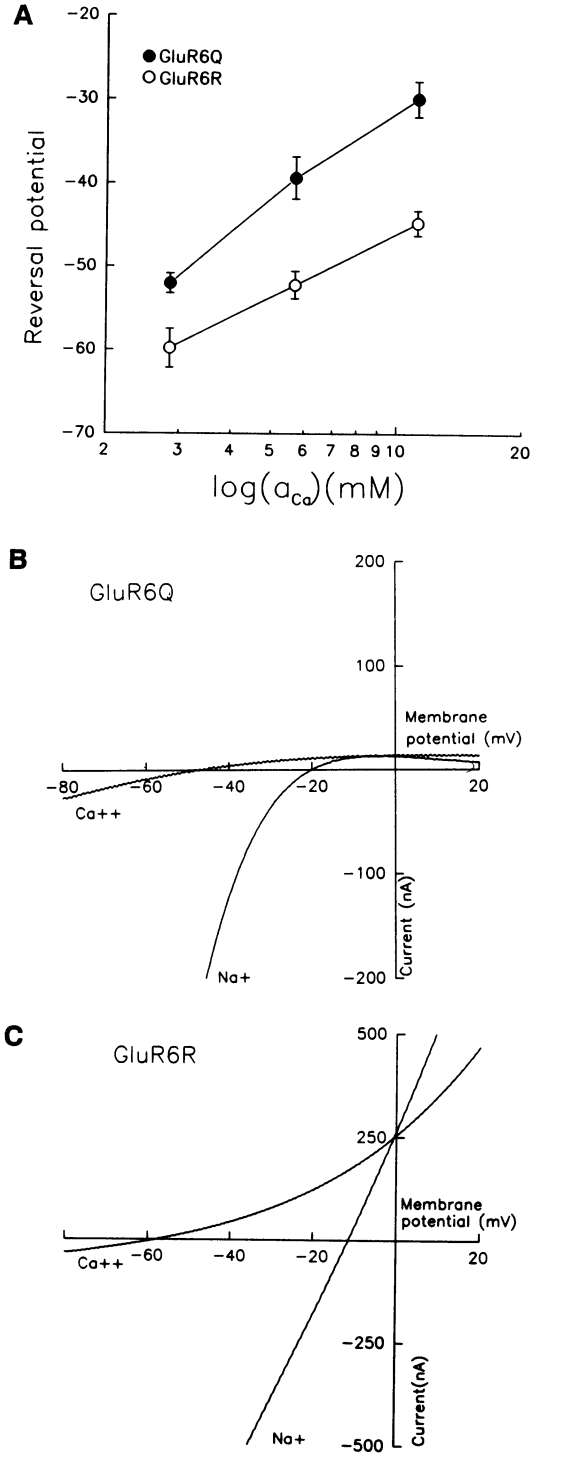

FIG. 2. (*A*) Reversal potentials obtained in Ca^{2+}/Ringer's solution containing 2–20 mM Ca^{2+} for GluR6Q or GluR6R. (*B*) *I–V* curves for homomeric GluR6Q receptors in either low Ca^{2+}/Ringer's (Na^+) or 10 mM Ca^{2+}/Ringer's (Ca^{2+}) solution. (*C*) As in *B* for GluR6R. Oocytes were injected with 100 nl of a 20 mM BAPTA solution prior to recording in order to avoid activation of the endogenous Ca^{2+}-activated Cl^- channel (see text).

Na^+, K^+, and divalent ions, indicating a negligible contribution of *N*-methylglucamine to the inward current.

We also studied the permeability properties of other alkaline earth metal ions. As shown in Fig. 3, all ions tested were able to permeate the channels. Determination of the reversal potentials (Table 1) indicated a different order of permeability for the two GluR6Q and -R variants with the order $Ca^{2+} \geq$

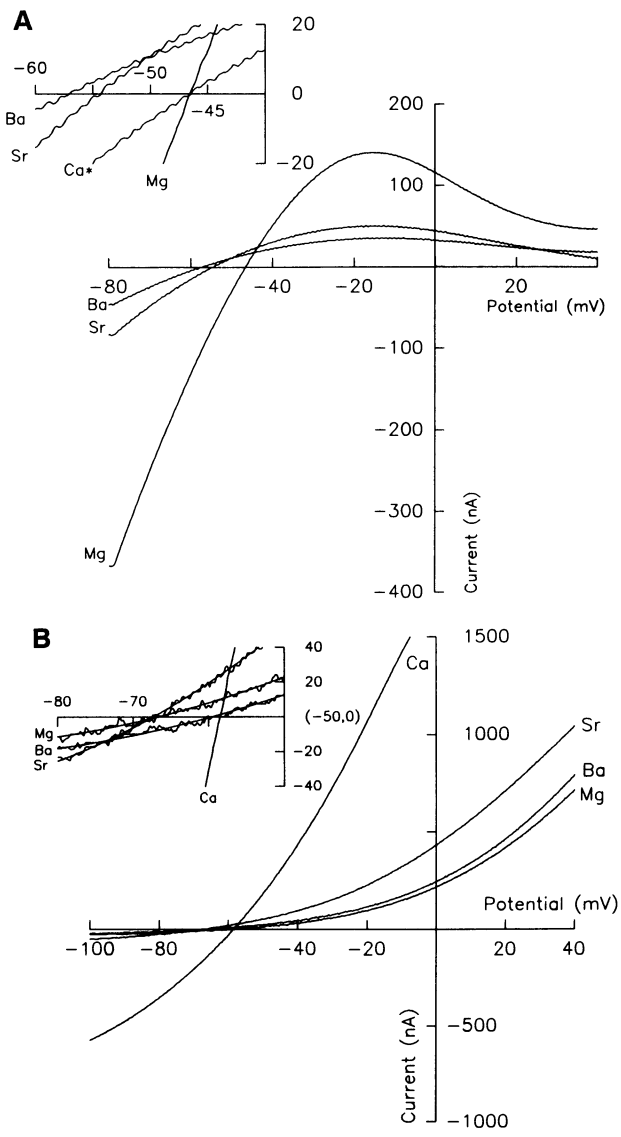

FIG. 3. *I–V* relationship for GluR6Q (*A*) and GluR6R (*B*) in solutions containing the indicated ion at 10 mM as the only extracellular charge carrier. *I–V* curves were obtained as 2-sec voltage ramps from −80 or −100 to 40 mV. *, Recording in Ca^{2+} solution for GluR6Q was performed after 30-sec Con A treatment, since a longer treatment would potentiate the response to a level where the Ca^{2+} influx exceeds the Ca^{2+} buffering capacity of the injected BAPTA, consequently making inhibition of the Ca^{2+}-activated Cl^- current impossible. An additional 5-min exposure to Con A was performed before agonist applications in the other solutions. The GluR6R-injected oocyte was treated for 3 min with Con A before kainate was applied in the Ca^{2+} solution and for an additional 3 min before kainate application in the other solutions. (*Inset*) To assess the reversal potential more precisely, the *I–V* curves for GluR6R obtained in the Mg^{2+}, Sr^{2+}, and Ba^{2+} solutions were fitted to a third-order polynomial function.

$Mg^{2+} > Sr^{2+} \geq Ba^{2+}$ for GluR6Q and $Ba^{2+} \geq Ca^{2+} > Sr^{2+} \approx Mg^{2+}$ for GluR6R.

An interesting finding is that the magnitude of the current was strongly dependent on the type of divalent ion (Fig. 3; Table 1). At a holding potential of −80 mV, the inward current in the Ca^{2+} solution was much larger than currents measured in either Mg^{2+}, Sr^{2+}, or Ba^{2+} solutions (Table 1). The currents in the Ca^{2+} solution were, for both variants, ≈5% of the currents measured in the low Ca^{2+}/Ringer's solution. This low current indicated that divalent ions might inhibit the current carried by monovalent ions. To analyze

Table 1. Permeability ratios determined in solutions containing the indicated ions at 10 mM as the only external charge carrier (n = 5–9) and relative current carried by the indicated ions compared to the current recorded in 10 mM Ca^{2+} solution at a holding potential of −80 mV (n = 4–6)

	Permeability ratio (P_x/P_{mono})		Relative current (I_x/I_{Ca}, %)	
	GluR6Q	GluR6R	GluR6Q	GluR6R
Mg^{2+}	1.0 ± 0.1	0.41 ± 0.05	5.0 ± 0.7	2.1 ± 0.1
Ca^{2+}	1.2 ± 0.1	0.47 ± 0.03	100	100
Sr^{2+}	0.77 ± 0.05	0.41 ± 0.04	1.5 ± 0.3	3.8 ± 0.4
Ba^{2+}	0.71 ± 0.04	0.60 ± 0.06	0.6 ± 0.1	3.2 ± 0.0

Current ratio between Ca^{2+} and Mg^{2+} was measured after a short Con A application, while current ratios between Mg^{2+}, Sr^{2+}, and Ba^{2+} were measured after extended Con A treatment (see Fig. 3).

this effect, the currents were measured in Ringer's solution containing different concentrations of Ca^{2+} or Ba^{2+}. The EC_{50} for kainate did not change over the Ca^{2+} concentration range used when corrected for Ca^{2+}–kainate complexes (28). To ensure maximal responses, 30 μM kainate was used (i.e., 30 times the EC_{50}). The shapes and minima of the curves were independent of the magnitude of current responses, indicating that the BAPTA injection sufficiently inhibited activation of the Cl^- channels. Fig. 4*A* shows that Ca^{2+} at ≈2 mM gives the maximal inhibition with an increased current observed for higher Ca^{2+} concentrations, presumably reflecting the increase of the extracellular concentration of charge carrier and perhaps a Ca^{2+}-dependent change in the unitary conductance or the open probability of the receptor. The inhibitory effect of Ba^{2+} is not overcome at higher Ba^{2+} concentrations, suggesting a difference in the mechanism of Ba^{2+} permeation (see *Discussion*).

DISCUSSION

The present study shows that homomeric GluR6Q receptors exhibit a high P_{Ca}/P_{mono}, while the GluR6R receptor is less Ca^{2+} permeable. Permeability studies performed on the GluR1–GluR4 receptor subunits revealed a similar pattern. When the two variants of GluR2 were studied in a mammalian expression system P_{Ca}/P_{Cs} was found to be 1.2 for the GluR2Q variant and 0.05 for the GluR2R variant (21), compared to 1.2 and 0.47 for the GluR6Q and GluR6R variants, respectively. P_{Ba}/P_{mono} was estimated to be 2–3 for homomeric GluR1 and GluR3 but it was <0.02 for heteromeric receptors containing GluR2 (20). However, the GluR6 variants exhibit a similar P_{Ba}/P_{mono} of 0.8 and 0.6 for GluR6Q and GluR6R, respectively. These differences in divalent ion selectivity between different GluR subunits suggest that divalent ion permeability is not solely dependent on the amino acid at the Q/R site.

The Ca^{2+}-dependent reduction of the current mediated by the GluR6 variants makes the activity of the receptors sensitive to extracellular variations in Ca^{2+} concentrations. That dependency is not observed for the GluR1–GluR4 class of glutamate subunits (19). If receptors generated from this class of subunits contain GluR2, they exhibit a weak reduction in total current at high extracellular Ca^{2+} concentrations, while receptors without GluR2 carry more current at higher Ca^{2+} concentrations in accordance with the increased concentration of charge carrier (19). The mechanism underlying the Ca^{2+}-induced modulation of the response could involve a high-affinity divalent ion binding site in the channel or a Ca^{2+} (or divalent ion)-dependent change in open probabilities or unitary conductance as observed for the neuronal acetylcholine receptors (29, 30). The dependence of the current on Ca^{2+} concentration suggests a blocking mechanism analogous with the block of the voltage-gated Ca^{2+} channel (31, 32). The increased current at high Ca^{2+} concentrations might then reflect an increased exit rate due to electrostatic repulsion between Ca^{2+} ions in the channel. A blocking model could explain the apparent paradox for GluR6R, where Ba^{2+}, despite a permeability ratio to monovalent ions higher than that of Ca^{2+}, shows a decrease in total current at higher Ba^{2+} concentrations and not an increase as observed for Ca^{2+} (Fig. 4). If indeed Ba^{2+} binds in the channel with a higher affinity than Ca^{2+} it may inhibit the monovalent current more efficiently than Ca^{2+}. The consequently slower passage of Ba^{2+} ions will then reduce the total current mostly carried by Na^+ more efficiently.

The observation that subtypes of the non-NMDA receptors are permeable to Ca^{2+} (4, 6, 19, 20) suggests an additional mechanism for glutamate-induced Ca^{2+} influx. This mechanism is most efficient at polarized or hyperpolarized potentials in contrast to the Ca^{2+} flux through the NMDA subtype of the glutamate receptors, which requires a depolarization of the membrane to relieve the Mg^{2+} block of the NMDA

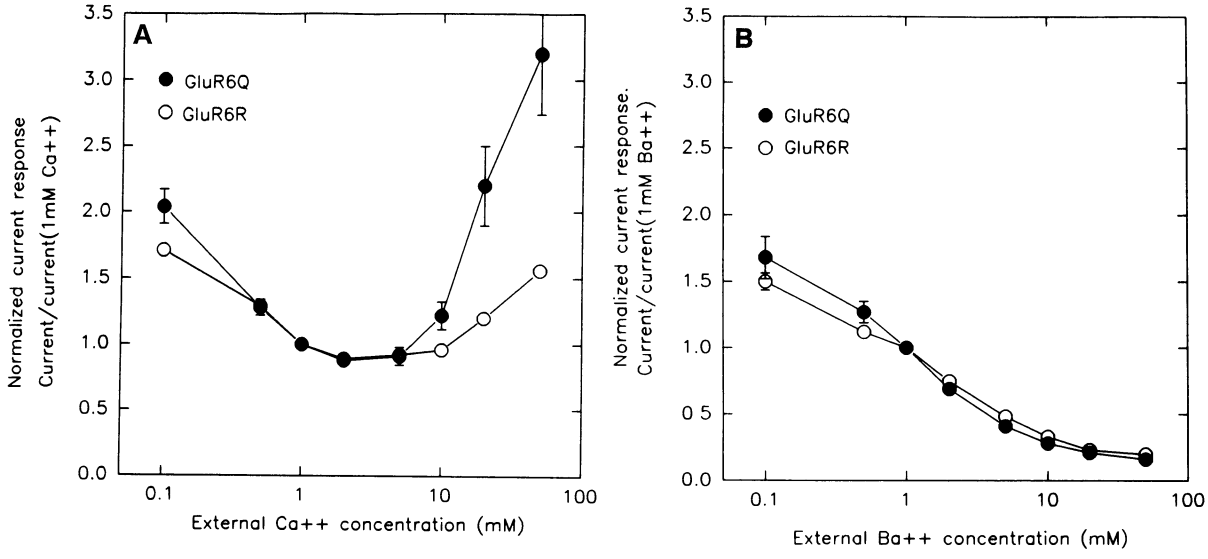

FIG. 4. Inward current measured at a holding potential of −70 mV in solutions containing different Ca^{2+} (*A*) or Ba^{2+} (*B*) concentrations. The Ca^{2+} or Ba^{2+} concentration was varied from 0.1 to 50 mM in Ringer's solution containing 90 mM NaCl, 1 mM KCl, and 15 mM Hepes·NaOH (pH 7.5). Oocytes were injected with BAPTA and treated with Con A prior to the recordings.

receptor. The glutamate-induced Ca^{2+} flux through the non-NMDA receptors may therefore be most significant for stimuli that do not depolarize the membrane sufficiently to activate the NMDA receptor or voltage-activated Na^+ or Ca^{2+} channels. The membrane potential may remain polarized due to simultaneous inhibitory stimuli, or the Ca^{2+}-permeable receptors may generate a positive feedback system through Ca^{2+}-activated Cl^- or K^+ channels. The increased internal Ca^{2+} concentration may induce additional long-term effects by affecting kinases and, consequently, the phosphorylation level of other receptor systems.

Note Added in Proof. Control experiments using a whole-cell patch-clamp technique on transfected mammalian 293 kidney cells confirmed that both versions of the GluR6 (R/Q) have significant permeability ratios. P_{Ca}/P_{mono} ratios were the same in oocytes and 293 kidney cells for GluR6Q. However, the GluR6R showed a higher P_{Ca}/P_{mono} (3.3 ± 0.5) in 293 cells. This unexpected difference remains to be investigated.

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