Subtype-Specific Effects of Lithium on Glutamate Receptor Function

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Karkanias, Nikolas B. and Roger L. Papke. Subtype-specific effects of lithium on glutamate receptor function. *J. Neurophysiol.* 81: 1506–1512, 1999. We report that substitution of sodium with lithium (Li^+) in the extracellular solution causes subtype-specific changes in the inward and outward currents of glutamate receptors (GluRs), without a shift in reversal potential. Li^+ produces an increase of inward and outward currents of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors and decreases in the currents of kainate (KA) and *N*-methyl-D-aspartate receptors. The greatest effect of Li^+ was observed with GluR3. A concentration-response curve for GluR3 reveals that the potentiation caused by Li^+ is greatest at saturating agonist concentrations. GluR1, which shows no potentiation by Li^+ at 100 μM KA, shows a small but significant potentiation at saturating KA and glutamate concentrations. The effects of Li^+ on outward current, where Li^+ is not the primary charge carrier, and the lack of reversal potential shift argue for a mechanism of potentiation not associated with Li^+ permeation. This potentiation of current is specific for Li^+ because rubidium, although causing an increase of inward current, shifted the reversal potential and did not increase outward current. The effects of Li^+ are different for KA, a weak desensitizing agonist, and glutamate, a strong desensitizing agonist, suggesting that Li^+ might interact with a mechanism of desensitization. By using cyclothiazide (CTZ) to reduce desensitization of GluR3, we find that for low concentrations of KA and glutamate potentiation of the response by a combination of CTZ and Li^+ is no greater than by CTZ or Li^+ alone. However, at high concentrations of agonist, the potentiation of the response by a combination of CTZ and Li^+ is significantly greater than by CTZ or Li^+ alone. This potentiation was additive for glutamate but not for KA. At high agonist concentration in the presence of CTZ, the intrinsically lower desensitization produced with KA-evoked responses may preclude Li^+ from potentiating the current to the same degree as it can potentiate glutamate-evoked responses. The additive effects of CTZ and Li^+ were unique to the flop variant of GluR3.

INTRODUCTION

Ionotropic glutamate receptors (GluRs) are responsible for most of the fast excitatory neurotransmission in the mammalian brain, which includes activity-dependent synaptic modifications such as long-term potentiation and long-term depression (Bliss and Gardner-Medwin 1973; Bliss and Lynch 1988; Collingridge and Bliss 1987). A differential expression of GluR subunit genes gives rise to the functional diversity of GluRs among brain regions with unique permeability and kinetic properties for specific receptor subtypes. Characteristics of GluRs such as ionic selectivity and kinetics are vital to the understanding of fast excitatory synaptic transmission and how synaptic activity and neuronal plasticity may be coupled in various parts of the brain.

Several subtypes of GluRs contribute to fast excitatory transmission, and they can be pharmacologically distinguished into two major classes, non–*N*-methyl-D-aspartate (NMDA) and NMDA sensitive. The non-NMDA–sensitive channels contain the receptor subunits GluR1–GluR7. The channels composed of subunits GluR5–GluR7 can assemble with accessory subunits KA1 or KA2 to form receptors that are activated by kainate (KA). Channels composed of GluR1–GluR4 are activated by α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) as well as KA. The NMDA-sensitive channels are composed of NMDAR1 and NMDAR2a–NMDAR2d. Functional properties of these GluRs, such as their permeability to sodium, potassium, and calcium and their kinetics, are influenced by the specific subunit composition of the channel (Hollmann et al. 1991; Monyer et al. 1992) .

Receptor desensitization is another property of GluRs that may regulate synaptic function. When the glutamate transient time course is slow, because of the nature of the synaptic morphology, the duration of synaptic current may be determined primarily by desensitization kinetics (Barbour et al. 1994). Desensitization is promoted by agonist exposure, and experimental agonists can vary in their relative desensitizing effect. For example, KA produces less desensitization in AMPA-selective receptors than either AMPA or glutamate. The desensitization kinetics vary among the specific AMPA receptor subtypes as a result of RNA editing and alternative splicing. The flip/flop domain is a 38-amino acid cassette located extracellularly and N-terminal to the final transmembrane domain of AMPA receptors. Alternative splicing of this cassette yields mature flip or flop AMPA receptors that vary in their desensitization kinetics (Mosbacher et al. 1994; Sommer et al. 1990). Immediately before the flip/flop domain is the R/G site (Lomeli et al. 1994). RNA editing at the R/G site can also influence AMPA receptor desensitization kinetics with edited channels (G) recovering from desensitization faster.

Although pharmacological modulators such as cyclothiazide (CTZ) are thought to reduce desensitization of AMPA receptors thus providing pharmacological tools with which to study this property (Partin et al. 1993; Vyklicky et al. 1991; Wong and Mayer 1993), desensitization was largely assumed to be independent of the charge-carrying ion. In a previous study we reported the preliminary observation that Li^+ produced subtype-specific alterations of macroscopic current (Karkanias et al. 1998). Further investigation of Li^+ effects on GluR leads us to propose that modulation of receptor desensitization is a mechanism that causes the flop variant of GluR3 to display a modified conductance in the presence of Li^+.

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METHODS

Oocyte preparation

Female *Xenopus laevis* frogs were purchased from Nasco (Fort Atkinson, WI) and kept in tanks at 17°C. Frogs were anesthetized for 30 min on ice in 2.2 g/1.5 l of 3-aminobenzoic acid ethyl ester purchased from Sigma (St. Louis, MO). Oocytes were obtained in lobes through a small abdominal incision made just above the leg and near the midline on the ventral surface of the frog. Two to three lobes were pulled from the frog, cut, and placed in collagenase from Worthington Biochemical (Freehold, NJ) (1 mg/ml in calcium-free Barth's solution containing 88 mM NaCl, 1 mM KCl, 15 mM HEPES, 0.33 mM $MgSO_4$, and 0.1 mg/ml gentamicin sulfate, pH 7.6) for 2 h to enzymatically remove the native follicular cell layer. After the follicular cell layer was removed, oocytes were washed several times with calcium-free Barth's and then washed several times with Barth's solution containing 88 mM NaCl, 1 mM KCl, 15 mM HEPES, 0.33 mM $CaNO_3$, 0.41 mM $CaCl_2$, 0.33 mM $MgSO_4$, and 0.1 mg/ml gentamicin sulfate (pH 7.6) and stored at 17°C. Mature oocytes were injected the same or following day with the appropriate RNA transcripts (20 ng/oocyte).

mRNA transcription and injections

cDNA clones containing the appropriate gene and T3/T7 bacterial promoters were isolated from bacteria and purified with a kit from Qiagen (Santa Clarita, CA). Purified cDNA clones were linearized with the necessary restriction enzyme and then purified to serve as template for in vitro transcription. Briefly, in vitro cRNA transcripts were prepared with the appropriate mMessage mMachine kit from Ambion (Austin, TX). Transcription reactions were performed with 1 μg cDNA as template, an RNA polymerase (T3 or T7, depending on clone), DTT, RNase inhibitor, dNTPs, and ^{32}P. Nucleotide incorporation was evaluated by DEAE81 filter binding assays and a liquid scintillation counter. RNA was stored in DEPC water stocks at −80°C, and aliquots were used for injection into the oocytes. The accession numbers for the clones used in this study were GluR1 (X17184), GluR2 (M85035), GluR3 (M85036), GluR6 (Z11548), NMDAR1–1a (L19708), NMDAR2a (AF001423), and NMDAR2b (U11419). Unless otherwise noted, we used the flop variants of AMPA receptors in our experiments.

Two-electrode voltage clamp

For the conventional two-electrode, voltage-clamp experiments, oocytes were placed in a Warner Instruments (Hamden, CT) recording chamber and perfused with frog Ringer solution [containing (in mM) 115 NaCl, 2.5 KCl, 1.8 $BaCl_2$, and 10 HEPES, pH 7.3]. Ringer solutions containing lithium (Li^+) or rubidium were made by substituting the ion for sodium. Osmolarity of different Ringer solutions was checked with a Precision Systems (Natick, MA) Osmette A osmometer. A Warner Instruments Oocyte Clamp OC-725B and Frequency Devices model 902 filter were interfaced with National Instruments (Austin, TX) LabVIEW software and a Macintosh computer for data acquisition. Electrodes were fabricated from glass capillary tubes (KG-33) from Garner Glass (Claremont, CA) with a DKI (Tujunga, CA) model 750 needle/pipette puller. Voltage electrodes were filled with 3 M KCl and had resistances on the order of 1–5 MΩ, whereas current electrodes were filled with 0.25 M CsCl, 0.25 M CsF, and 100 mM EGTA (pH 7.3) and had resistances of 0.5–3 MΩ. Experiments were performed at room temperature, and the oocyte membrane was clamped at −50 mV. Currents were measured to the nearest nanoampere. At least three and usually four or more oocytes were used for each measurement. Drugs were dissolved in Ringer and applied by filling a 2.0-ml length of tubing at the end of the perfusion line. A discrete volume of agonist was thereby administered over a 10-s period. Some drug stocks were dissolved in DMSO and then diluted in Ringer to <1% DMSO. No effect on control response was observed when the agonist was dissolved in DMSO. In most experiments, barium was used instead of calcium in the Ringer to minimize contributions of endogenous calcium-activated chloride current. However, similar results were obtained in the presence of calcium. For experiments with GluR6(Q/R), a 2.0-ml, 10-s pulse of concanavalin A (Sigma; St. Louis, MO, type IV; 1.2 mg/ml) was applied 5 min before applying agonist.

Current-voltage relationships were performed by delivering a voltage ramp, −50.0 mV to +50.0 mV, during the plateau phase of the response to agonist (pClamp 5.5, Axon Instruments; Foster City, CA). The passive current-voltage response of the cell membrane in the absence of agonist was subtracted from the current-voltage response in the presence of agonist. Permeability ratios for Li^+ and rubidium with respect to sodium were calculated with *Eq. 1*

$$\Delta E_{rev} = E_{rev,X} - E_{rev,Na} = (RT/zF) \cdot \ln P_X[X_0]/P_{Na}[Na_0] \quad (1)$$

E is the reversal potential in the presence of X (Li^+ or Rb^+) or Na^+, ΔE is the difference between the reversal potentials in X and Na, $[X_o]$ is the concentration of X outside of the cell (115 mM), $[Na_o]$ is the concentration of Na^+ outside of the cell (115 mM), and P is the permeability of the ion.

Concentration-response relationships

The responses of GluR3 expressing oocytes to various test concentrations of KA were normalized by the response to the EC_{50} KA concentration (100 μM) immediately preceding the test concentration. First, a response was recorded to 100 μM KA, and after a 5-min washout a response was recorded to a test dose of KA. After 5 min, 100 μM KA was applied again to determine any residual effects from the test dose of KA. If the response to 100 μM KA after the test dose was ≥75% of the response to 100 μM KA before the test dose, the oocyte was tested further at other concentrations of KA. The resulting concentration-response relationship was fitted with *Eq. 2* (Luetje and Patrick 1991)

$$\text{Response} = \frac{I_{max}[\text{agonist}]^n}{[\text{agonist}]^n + (EC_{50})^n} \quad (2)$$

RESULTS

Effect of Li^+ on neuronal GluR function

Specific GluR subtypes were evaluated for their potential modulation by Li^+. Effects on GluR function were noted in the range of 5–115 mM Li^+. For our standard agonist applications we used 100 μM KA for AMPA receptors, 100 μM glutamate for KA receptors, and 100 μM glutamate + 10 μM glycine for NMDA receptors. Under these conditions, KA/NMDA-receptor currents appeared to be reduced in Li^+ solutions, whereas AMPA receptor currents were potentiated in Li^+ solutions. Subtype-selective differences in potentiation were observed within the AMPA receptor class and were investigated further with ionic variation in extracellular solutions, different agonists at multiple concentrations, and chemical modulators.

Permeability and conductance of neuronal GluRs to Li^+

Compared with their respective sodium controls, the amplitude of the KA-evoked current in Li^+ varied across subtype of GluR. However, when extracellular sodium was replaced with Li^+, no shift in reversal potential was detected for any of the subtypes that were tested. For the AMPA-selective flop variants of GluRs, both inward and outward currents in Li^+ Ringer

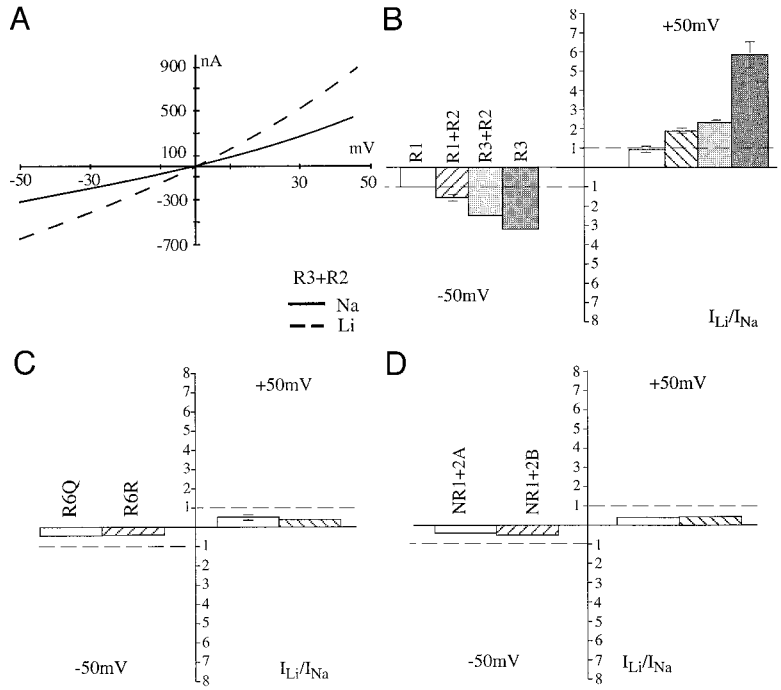

FIG. 1. Current-voltage relationships of various glutamate receptor (GluR) subtypes. *A*: current-voltage relationship a *Xenopus* oocyte expressing the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-selective ionotropic GluR3 + GluR2 in response to 100 μM kainate (KA). *B*: bar graph of the current recorded in lithium (Li^+) relative to Na^+ for GluR1, GluR1 + R2, GluR3 + GluR2, and GluR3 receptors with 100 μM KA at 2 different membrane voltages. Error bars represent SE. *C*: bar graph of the current recorded in Li^+ relative to Na^+ for GluR6Q and GluR6R receptors with 100 μM glutamate at 2 different membrane voltages. *D*: bar graph of the current recorded in Li^+ relative to Na^+ for NMDAR1a + NMDAR2a and NMDAR1a + NMDAR2b receptors with 100 μM glutamate + 10 μM glycine at 2 different membrane voltages.

were equal to or greater than the current in Na^+ Ringer. A representative *I-V* relationship for GluR3 + R2 is shown in Fig. 1*A*. The increase of both inward and outward currents without a shift in the reversal potential suggests an effect on the P_{open} rather than an increase in single channel conductance with Li^+. In general, the inward and outward currents of various AMPA receptors were potentiated by Li^+, ranging from 40 to 200% above control (Fig. 1*B*). However, the current evoked by 100 μM KA in Li^+ Ringer through GluR1 was not significantly increased. In contrast, under these conditions the current in Li^+ Ringer was decreased by 40–60% for KA and NMDA receptors (Fig. 1, *C* and *D*). Because GluR1 + R2 receptor currents were potentiated but GluR1 currents were not (Fig. 1*B*), we investigated the role of the GluR2 subunit in Li^+ potentiation. Wild-type GluR2 alone does not function well in oocytes. Therefore for these experiments we expressed wild-type GluR2 with a Q/R site mutant, GluR2(R586Q), as well as the mutant alone. The inward and outward currents of heteromeric GluR2 + GluR2(R586Q) receptors were increased in Li^+ by 74 ± 7.9% (n = 6) and 72 ± 10% (n = 6), respectively (Fig. 2*A*). The conductance of the mutant homomer was increased 134 ± 26% above control (n = 3) in the presence of Li^+ (Fig. 2*B*). These results indicate that GluR2 is intrinsically capable of Li^+ modulation. However, further experiments focused on the GluR3 subtype because it was most sensitive to Li^+ potentiation and easily formed homomeric receptors.

Specificity of the effect of Li^+ on GluR currents

To test the specificity of Li^+ effect on GluR currents, we performed similar experiments in rubidium-based Ringer. Raw waveforms recorded from a GluR3-expressing oocyte in Na^+, Rb^+, and Li^+ Ringer are shown in Fig. 3*A*. Rubidium produced a mean increase in current of 56 ± 4.6% (n = 4) but correspondingly shifted the reversal potential 6.60 ± 1.03 mV (n = 7) for GluR3 (Fig. 3*A*, *inset*) and 4.05 ± 0.46 mV (n = 10) for GluR3 + R2 (Fig. 4, *A* and *B*). There is no potentiation of outward current by Rb^+ for GluR3 (Fig. 3*A*, *inset*) or for GluR3 + R2 (Fig. 4*B*). By using the shift in reversal potentials and solving for the ratio P_{Rb}/P_{Na} (see METHODS), we calculated a P_{Rb}/P_{Na} of 1.30 for GluR3 and 1.17 for GluR3 + R2.

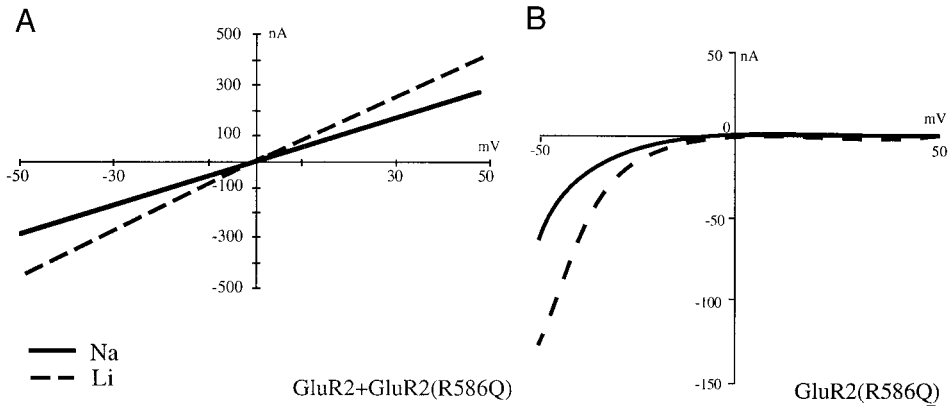

FIG. 2. Current-voltage relationships of Q/R site mutant heteromer and homomer. *A*: current-voltage relationship from a *Xenopus* oocyte expressing the AMPA-selective ionotropic GluR2 + GluR2(R586Q) in response to 100 μM KA. *B*: GluR2(R586Q).

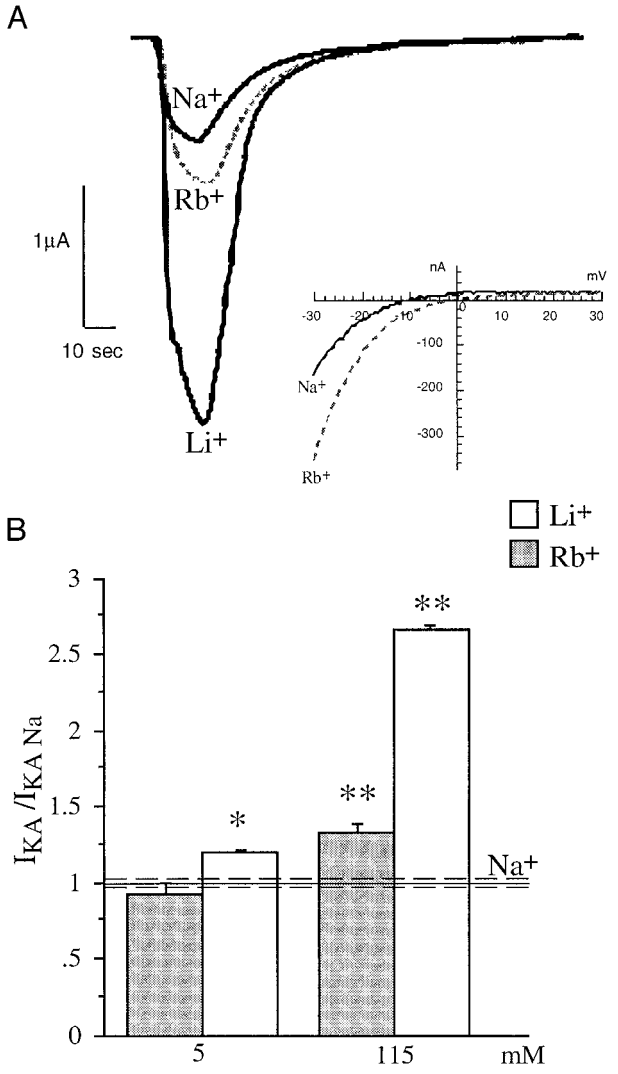

FIG. 3. Comparison of the effects of Rb^+ and Li^+. *A*: response waveforms evoked with 100 μM KA from a GluR3 expressing oocyte in Na^+, Rb^+, and Li^+ Ringer solutions. The response of GluR3 in Na^+ Ringer is the smallest of the 3 solutions tested. The response of GluR3 in Rb^+ Ringer is ~56% larger than in Na^+ and is approximately 170% larger in Li^+ Ringer than in Na^+. The inset shows a representative *I-V* for GluR3 in Na^+ and Rb^+. The reversal potential is shifted 6.60 ± 1.03 mV in the positive direction. *B*: comparison of the potentiation caused by 2 different concentrations of Li^+ (open bar) and Rb^+ (shaded bar), relative to Na^+ (solid line; SE, dashed line). Asterisks indicate significant differences compared with Na^+. * $P < 0.001$, ** $P < 0.0001$.

Concentration dependence of extracellular Li^+

We wished to determine if the magnitude of Li^+ potentiation of GluR3 response varies linearly with external Li^+ concentration. We noted that when 100 μM KA is used as the agonist an increase of the extracellular Li^+ concentration causes a current potentiation that could be fit by *Eq. 2* with an EC_{50} of 14.85 ± 1.71 mM ($n \geq 4$) (Fig. 5). However, when saturating concentrations (1 mM) of KA were used we saw a further anomalous increase in potentiation at the highest extracellular Li^+ concentration. To test if this peculiar increase with 1 mM KA in 100% Li^+ (115 mM Li^+, 0 mM Na^+) could be associated with the total absence of sodium we performed experiments at the same Li^+ concentration but with added Na^+ (115 mM Li^+, 10 mM Na^+) as well as an osmotic control (115 mM Li^+, 20 mM sucrose). The presence of 10 mM Na^+ in 115 mM Li^+ Ringer did not significantly reduce the response to 1 mM KA (data not shown). These results indicate that the extreme potentiation reported for 1 mM KA responses obtained in the presence of 115 mM Li^+ was not due to a specific effect associated with the removal of sodium. Note that when 1 mM Glu was used as the agonist the GluR3 current displayed an increased threshold for potentiation by Li^+ that did not appear to saturate and reached a maximum potentiation of 357% with 100% mole fraction of Li^+ (Fig. 7*B*).

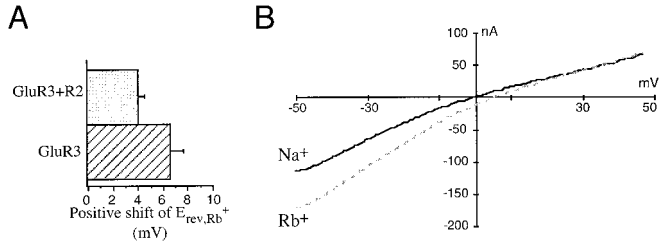

FIG. 4. Effects of Rb^+ on GluR3 + GluR2. *A*: mean data for the reversal potential shift of GluR3 and GluR3 + R2 caused by Rb^+. *B*: *I-V* relationship for GluR3 + GluR2 in Na^+ and Rb^+. Note the rightward shift in reversal potential and that the outward current is not affected as in Fig. 1*A*. The agonist used was 100 μM KA.

It is interesting to note the receptor sensitivity to potentiation by concentrations of Li^+ that approach levels used for treatment of bipolar disorder. We observed an increase of 21 ± 3% ($n = 10$, $P < 0.001$) for GluR3 and 26 ± 3% ($n = 6$, $P < 0.05$) for GluR3 + R2 in response to 100 μM KA at 5 mM extracellular Li^+. If the potentiation was linearly dependent on extracellular Li^+ concentration, at 5 mM extracellular Li^+ (1/23 of 115 mM) one would expect that the potentiation would be ~1/23 of the maximum potentiation observed with 100 μM KA and 115 mM Li^+ (or 7–8%). This observation further supports the hypothesis that the effects of Li^+ are most likely due to effects on P_{open} rather than channel conductance. The response waveforms recorded in 115 mM Na^+, 115 mM Li^+, and 5 mM Li^+ from GluR3-expressing oocytes are shown in Fig. 6, *A–C*. Rubidium has no effect on GluR3 current at 5 mM extracellular concentration in contrast to Li^+ (Figs. 3*B* and 6). Potentiation by Li^+ is readily reversible at both high and low concentrations on washout of the Li^+ Ringer. In Fig. 6*D*, an

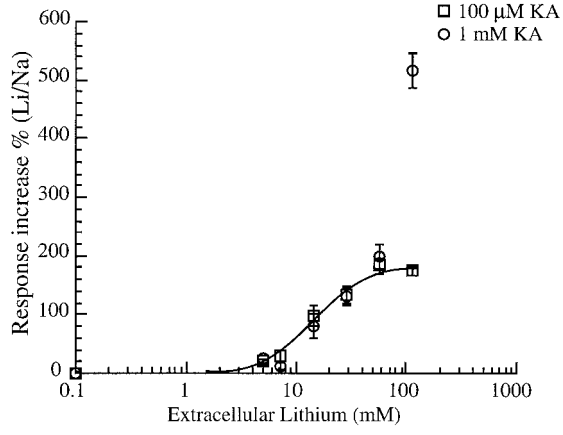

FIG. 5. Effect of extracellular [Li^+] titration on GluR3 response potentiation. The percent increase in response caused by Li^+ relative to Na^+ Ringer is plotted vs. extracellular [Li^+] for GluR3 expressing oocytes. Two different agonist concentrations were used; 100 μM KA (□) and 1 mM KA (○). Each point represents the means ± SE of 4–11 oocytes.

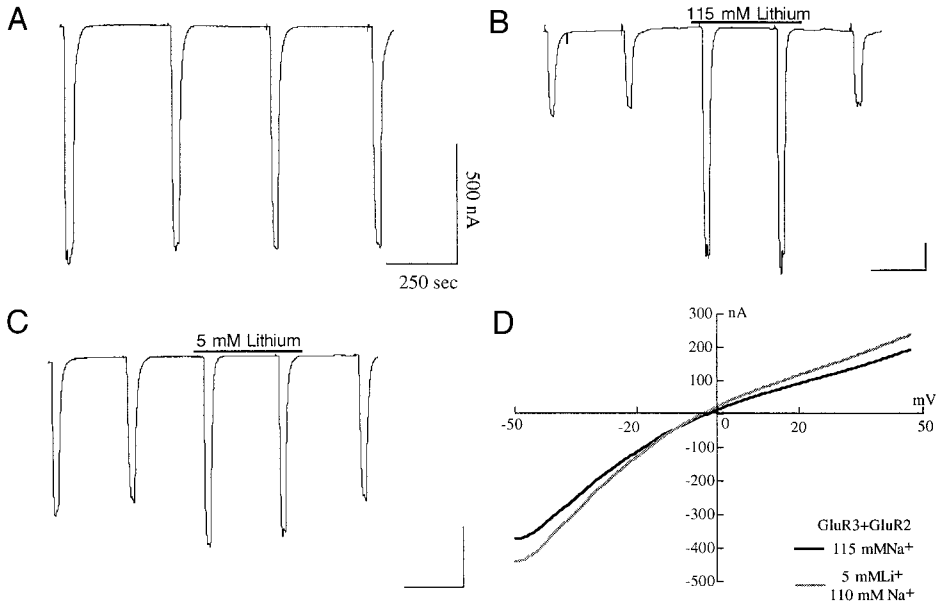

FIG. 6. Effect of Li^+ concentrations in the therapeutic window. *A*: depicted here are responses to 100 μM KA in Na^+ Ringer from oocytes expressing GluR3 channels. *B*: first 2 responses are recorded in Na^+ Ringer, the next 2 in Ringer with 115 mM Li^+, and the final response is a washout back to Na^+ Ringer. *C*: paradigm is the same as in *B* except the third and fourth responses are recorded in 110 mM Na^+/5 mM Li^+. *D*: *I-V* relationship for a *Xenopus* oocyte expressing GluR3 + GluR2 recorded in 110 mM Na^+/5 mM Li^+ showing potentiation of both inward and outward current.

I-V relationship of a GluR3 + R2-expressing oocyte recorded in 5 mM Li^+ showed an increase of both inward and outward currents analogous to that shown in Fig. 1*A*.

Li^+ effects on GluR agonist potency

We examined the potency and efficacy of KA in the presence and absence of Li^+. The concentration-response relationship determined for GluR3 in Na^+ Ringer yielded a Hill slope of 1.14 ± 0.07 and an EC_{50} of 125 ± 8 μM (Fig. 7*A*, ●). In Li^+ Ringer, the concentration-response relationship yielded a Hill slope of 2.19 ± 0.56 and an EC_{50} of 211 ± 28 μM (Fig. 7*A*, ▲). Li^+ increased the maximal attainable response by >500% and increased the EC_{50} by 60% compared with sodium. The total percent increase caused by Li^+ for glutamate and KA at low and high concentrations relative to the same agonist and concentration in Na^+ is presented in Fig. 7*B*. GluR1 does not show the same magnitude of increase compared with GluR3 with either KA or glutamate at saturating concentrations.

Li^+ effects and desensitization

We examined the effect of Li^+ when desensitization of GluR3 channels was reduced with the compound CTZ (Fig. 8). With 100 μM KA or glutamate as the agonist, both Li^+ and 100 μM CTZ potentiate GluR3 responses to the same extent. There was no further increase when the treatments were combined (Fig. 8, *A* and *B*). With 1 mM KA as the agonist, the combination of 100 μM CTZ and Li^+ substitution produced a current increase that was 30% larger than the increase CTZ produced in Na^+ Ringer (Fig. 8*C*, $P < 0.05$, unpaired *t*-test). At a high glutamate concentration (1 mM), the combination of 100 μM CTZ and Li^+ substitution produced a current increase that was 353 ± 54% larger than the increase CTZ produced in Na^+ Ringer (Fig. 8*D*, $P < 0.001$, unpaired *t*-test). Although CTZ potentiated GluR1 currents in sodium, there was no apparent interaction between the combination of CTZ with Li^+ at either agonist concentration (data not shown).

Because flip/flop variants were reported to vary in their intrinsic desensitization, we set out to determine the interaction of the flip/flop domain with Li^+ potentiation. We investigated GluR3 flip currents for their capacity to be modulated by Li^+ at saturating agonist concentrations in the presence and absence of CTZ and Li^+ (Fig. 8, *E* and *F*). We found that Li^+ potentiated the current evoked from GluR3 flip channels less

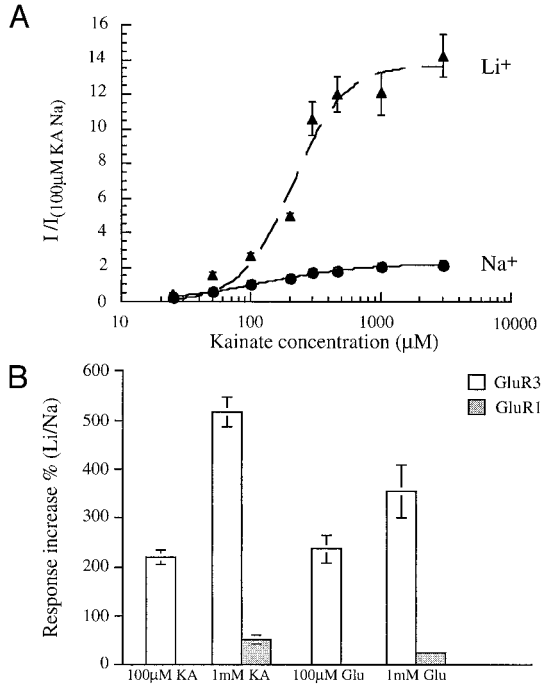

FIG. 7. Concentration-response relationship. *A*: concentration-response relationship of KA for GluR3 in Na^+ and Li^+ Ringer solution. All responses were normalized to the response of 100 μM KA in Na^+ Ringer. *B*: percent increase in response caused by Li^+ with different agonists and agonist concentrations. For 100 μM KA the increase was 220 ± 14% ($n = 19$), for 1 mM KA the increase was 518 ± 30% ($n = 19$), for 100 μM glutamate the increase was 237 ± 29% ($n = 5$), and for 1 mM glutamate the increase was 357 ± 54% ($n = 11$). The increase is presented normalized to the response to the respective agonist concentration in Na^+.

than from GluR3 flop. Potentiation of responses to 1 mM KA and 1 mM Glu was only 19 and 67% of the potentiation obtained with GluR3 flop. We also examined the effect of Li^+ when desensitization of GluR3 flip channels was reduced with the compound CTZ (Fig. 8, *E* and *F*). In contrast to the results obtained with GluR3 flop, Li^+ actually decreased 1 mM KA + 100 μM CTZ-evoked GluR3 flip currents by 19% ($n = 6$) compared with the 1 mM KA + 100 μM CTZ-evoked currents in Na^+ Ringer ($n = 6$) (Fig. 8*E*, $P < 0.05$, unpaired *t*-test). Similarly, Li^+ decreased 1 mM Glu + 100 μM CTZ-evoked GluR3 flip currents by 35% ($n = 11$) compared with the 1 mM Glu + 100 μM CTZ-evoked currents in Na^+ Ringer ($n = 11$) (Fig. 8*F*, $P < 0.05$, unpaired *t*-test). This reduction in GluR3 flip current when CTZ and Li^+ are combined was also seen when lower agonist concentrations were used (100 μM Glu), indicating that this effect is not specific to high agonist concentrations ($n = 3$, $P < 0.05$, data not shown).

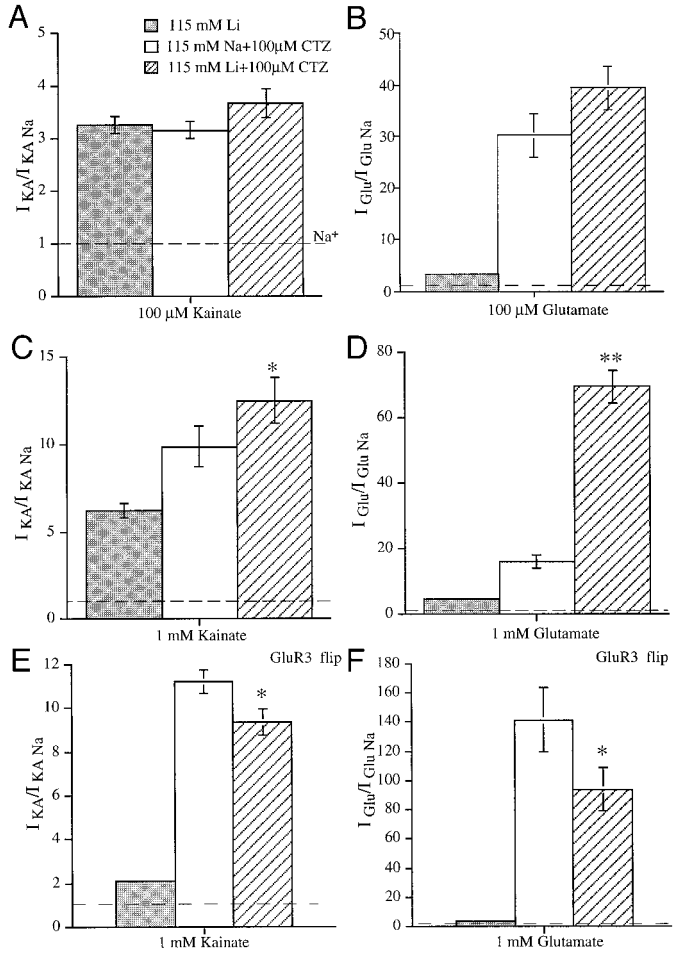

FIG. 8. Interactions among cyclothiazide (CTZ), agonist, and Li^+. Shaded bars represent mean current potentiation in Li^+ relative to Na^+ (dashed line). Open bars represent mean current potentiation by 100 μM CTZ relative to Na^+ (dashed line). Striped bars represent mean current potentiation in Li^+ +100 μM CTZ relative to Na^+ (dashed line). Error bars represent SE. *A*: GluR3 flop responses to 100 μM KA in 115 mM Li^+ ($n = 13$), 115 mM Na^+ + 100 μM CTZ ($n = 13$), and 115 mM Li^+ + 100 μM CTZ ($n = 14$). *B*: GluR3 flop responses to 100 μM glutamate in 115 mM Li^+ ($n = 7$), 115 mM Na^+ + 100 μM CTZ ($n = 7$), and 115 mM Li^+ + 100 μM CTZ ($n = 8$). *C*: GluR3 flop responses to 1 mM KA in 115 mM Li^+ ($n = 9$), 115 mM Na^+ + 100 μM CTZ ($n = 9$), and 115 mM Li^+ + 100 μM CTZ ($n = 7$). *D*: GluR3 flop responses to 1 mM glutamate in 115 mM Li^+ ($n = 4$), 115 mM Na^+ + 100 μM CTZ ($n = 4$), and 115 mM Li^+ + 100 μM CTZ ($n = 4$). *E*: GluR3 flip responses to 1 mM KA in 115 mM Li^+ ($n = 6$), 115 mM Na^+ + 100 μM CTZ ($n = 6$), and 115 mM Li^+ + 100 μM CTZ ($n = 6$). *F*: GluR3 flip responses to 1 mM glutamate in 115 mM Li^+ ($n = 11$), 115 mM Na^+ + 100 μM CTZ ($n = 11$), and 115 mM Li^+ + 100 μM CTZ ($n = 11$). Asterisks indicate significant differences compared with Na^+ + CTZ. * $P < 0.05$, ** $P < 0.001$.

DISCUSSION

We characterized the subtype-selective potentiation of GluR3 by Li^+ through an evaluation of Li^+ effects with agonist concentration, mole fraction of extracellular Li^+, and compounds affecting receptor desensitization. Our results permit us to propose that the effect of Li^+ is to modify desensitization in a manner that depends on the flop domain.

Li^+, rubidium, and sodium are all monovalent cations in group I of the periodic table, but only Li^+ is used for the treatment of the mental illness bipolar disorder. Studies involving Li^+ inhibition of second-messenger systems often use rubidium to confirm the specificity of the inhibition by Li^+ (Ebstein et al. 1980). We therefore compared GluR3 currents in Li^+-, Na^+-, and Rb^+-based Ringer. Although we observed a potentiation of the responses in both Rb^+ and Li^+ Ringer compared with Na^+, the potentiation in Rb^+ was qualitatively different than in Li^+ because it coincided with a shift of the reversal potential in the positive direction and was not observed on outward currents. These data suggest that the effects of Rb^+ were largely due to an increased permeability of Rb^+ through the channel.

The magnitude of Li^+ potentiation increased with the concentration of extracellular Li^+ independently of agonist concentration except at saturating agonist concentration in the presence of 115 mM Li^+. One potential explanation for this observation is that there are two processes that contribute to Li^+ potentiation of GluR current. One process, which is independent of agonist concentration, may predominate at lower Li^+ concentrations. At very high Li^+ concentrations, a second form of potentiation may manifest that selectively enhances responses to high, potentially desensitizing concentrations of agonists.

The results of our concentration-response experiments further suggest that Li^+ caused a dramatic change in the apparent efficacy of the agonist. A raised maximal response suggests an increase in the probability of a channel being open (P_o) or an increase in the single-channel conductance (γ). However, the effects of Li^+ on outward current, where it is not the primary charge carrier, favors the interpretation that there is an alteration in the percentage of time that channels are open.

CTZ was reported to potentiate AMPA receptor currents by reducing receptor desensitization (Partin et al. 1996). Because Li^+ effects were greatest under desensitizing conditions, we hypothesized that the effect of Li^+ might also be to reduce desensitization, and we sought to determine if CTZ and Li^+ acted through similar or different mechanisms. If Li^+ and CTZ work through distinct mechanisms, the potentiation that each causes individually might be additive when they are applied in combination. Our results indeed suggest that, at high concentrations of KA, Li^+ and CTZ do not act entirely through the same mechanism. Moreover, when a high concentration of glutamate (1 mM) was used, the effects of Li^+ and CTZ appeared to be completely additive for GluR3. These observations are consistent with the idea that Li^+ produces potentia-

tion of current in GluR3 by modulating the amount of receptor desensitization and that desensitization of the channel can be influenced by specific agonists. Desensitization is also controlled by the presence of specific protein domains, as in the alternative splice variants, flip and flop, in AMPA receptors. Flop receptors are thought to desensitize more than flip variants. Indeed, it appears that flop receptor currents are also potentiated to a greater degree by Li^+ than are flip receptor currents. The specificity of the Li^+ effect for the flop domain is further supported by the observation that, under conditions of the additive CTZ effect for flop variants, there was an antagonism of the CTZ effect in flip variants.

The inconsistent interaction of Li^+ with CTZ suggests a novel mechanism for this effect, although with a similar requirement for the flop domain. The modulation of desensitization could involve alterations of rate constants into or out of the desensitized state as well as alterations of rate constants leading toward the desensitized state. Our data suggest that further detailed studies of desensitization may exploit the use of Li^+ as a tool to dissect the mechanisms of desensitization.

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