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Lithium modulates desensitization of the glutamate receptor subtype GluR3 in *Xenopus* oocytes

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Abstract

Analysis of splice variants and site-directed mutants of the AMPA receptor GluR3 expressed in *Xenopus* oocytes has shown that lithium produces a large potentiation of the GluR3 flop splice variant and suggested that lithium might inhibit rapid desensitization, which is characteristic of this receptor (Karkanias, N. and Papke, R., Subtype-specific effects of lithium on glutamate receptor function. J. Neurophysiol., 81 (1999) 1506–1512). We now show that mutation of the 769R/G desensitization site (Lomeli, H.M.J., Melcher, T., Hoger, T., Geiger, J.R., Kuner, T., Monyer, H., Higuchi, M.B.A. and Seeburg, P.H, Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. Science, 9(266) (1994) 1709–1713) greatly attenuates the lithium-induced potentiation of GluR3. Additionally, experiments with the non-desensitizing site-directed mutant GluR3(L507Y) (Stern-Bach, Y., Russo, S., Neuman, M. and Rosenmund, C., A point mutation in the glutamate binding site blocks desensitization of AMPA receptors. Neuron, 21 (1998) 907–918) further confirms that lithium enhances GluR3 responses by reducing desensitization, since lithium's effects are reversed in this mutant. Lithium's effects on GluR3 desensitization are distinct from the effects of aniracetam on desensitization. Specifically, aniracetam, which potentiates wild-type AMPA receptors, is ineffective on the non-desensitizing GluR3(L507Y) mutant, but has synergistic effects with lithium on wild-type receptors.

Keywords: *Xenopus* oocyte; α-Amino-3-hydroxy-5-methyl-4-isoxazole propionate; Desensitization; Aniracetam; Glutamate; Kainate

Ionotropic glutamate receptors are responsible for virtually all of the fast excitatory neurotransmission in the mammalian brain. Such activity also underlies activity-dependent synaptic modifications such as long-term potentiation (LTP) and long-term depression (LTD) [2–4]. One important kinetic property of these receptors is desensitization, the induction of a conformational state of the channel that is functionally inactive in the prolonged presence of agonist. An appreciation for the significance of desensitization kinetics is vital to an understanding of fast excitatory synaptic transmission. For example, if the time course of glutamate removal from the synaptic cleft is slow then the duration of the synaptic current will be determined primarily by desensitization kinetics [1]. Thus, through the regulation of glutamate uptake processes, desensitization may be seen as a modulatory factor in synaptic function.

Recently [7], we reported evidence to suggest that Li^+ may also modulate desensitization of specific subtypes of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors. Lithium remains the major treatment for bipolar disorder and we have modeled potential mechanisms for its efficacy [6]. Based on our observation that lithium can modulate glutamate receptor desensitization, it is tempting to speculate that some therapeutic and/or toxic effects of lithium might be exerted through a direct modulation of AMPA receptor function. However, while some subtype-selective effects of lithium can be detected at concentrations that approach the range of physiological relevance, lithium's effects are primarily noted in a high concentration range most relevant to in vitro studies of receptor biophysics.

Several subtypes of glutamate receptors contribute to fast excitatory transmission and are pharmacologically distin-

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guished by their sensitivity to experimental agonists such as NMDA (*N*-methyl-D-aspartate), AMPA and kainic acid (KA). The non-NMDA sensitive channels contain the receptor subunits GluR1-GluR7 and KA1–2. Channels composed of GluR1-GluR4 are activated by AMPA as well as KA and represent an important class of postsynaptic receptors. These glutamate receptor subunits can assemble as homomeric or heteromeric complexes and the specific subunit composition of a receptor regulates its functional properties such as ionic permeability and the kinetics activation and desensitization [5,9]. While desensitization is promoted by agonist exposure, the relative desensitizing effects vary among different experimental agonists with KA producing less desensitization in AMPA receptors than either AMPA or glutamate.

Valuable insights into the process of desensitization have come from characterization of specific glutamate receptor mutants and splice variants, as well as from the identification of selective chemical modulators. For example, alternative splicing of a 'flip/flop' domain yields mature flip or flop AMPA receptors, and the flip variants appear to desensitize less than their flop counterparts [10,13]. Immediately prior to the flip/flop domain is the R/G site [8], where RNA editing can also influence AMPA receptor desensitization kinetics. Specifically, channels produced from the edited RNA (G-type) recover from desensitization faster than R-type channels. The chemical modulators of AMPA receptor desensitization cyclothiazide (CTZ) and aniracetam (AN) additionally provide pharmacological tools with which to study desensitization [11,15,16].

In our previous study [7] we noted that flop receptor currents were potentiated to a greater degree by Li^+ than were flip receptor currents. The potentiation induced by Li^+ was greatest for the GluR3 subtype of AMPA receptors and least for GluR1. Under conditions where desensitization was reduced with CTZ, Li^+ produced additional potentiation for flop variants yet produced an antagonism in flip variants, consistent with the idea that Li^+ produces potentiation of current in GluR3 by modulating the degree of receptor desensitization.

Recently the leucine (L) at site 507 in GluR3 was identified to be a critical residue for desensitization [14]. Mutation of L507 to an aromatic amino acid such as tyrosine (Y) seemed to abolish desensitization. Attempts to potentiate GluR3(L507Y) current by reducing desensitization with CTZ actually resulted in inhibition. In order to further investigate the effect of Li^+ on GluR3 function, we evaluate the significance of the R/G editing site and use the modulator aniracetam as well as the non-desensitizing mutant GluR3(L507Y).

Our experiments with GluR3 use the two-electrode voltage clamp technique in *Xenopus* oocytes. Oocytes were isolated from adult *Xenopus laevis* frogs as described previously [7] and injected with the appropriate RNA transcripts (20 ng/oocyte) of cDNA plasmids containing individual GluR genes. For two-electrode voltage clamp experiments, oocytes were placed in a RC-8 recording chamber from Warner Instruments Corporation (Hamden, CT) and perfused with frog Ringer solution (115 mM NaCl or LiCl, 2.5 mM KCl, 1.8 mM $BaCl_2$, 10 mM HEPES, pH 7.3). Experiments were performed at room temperature and the oocytes were clamped at −50 mV. At least three and usually four or more oocytes were used for each measurement. A discrete volume of agonist was administered over a 10-s period. Some drug stocks were dissolved in dimethyl sulfoxide (DMSO) and then diluted in Ringer to less than 1% DMSO. No effect on control response was observed when the agonist was dissolved in DMSO. 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. The GluR3o(G769R) mutant was constructed with a Quickchange site-directed mutagenesis kit from Stratagene (Cedar Tree, TX) and compared with the corresponding wild-type. The GluR3(L507Y) mutant was provided by Y. Stern-Bach.

We evaluated the effect of Li^+ on steady-state currents evoked with saturating agonist concentrations in Na^+ or Li^+ based Ringer solutions in GluR-expressing oocytes. Examples of wild-type and mutant GluR3 responses to 1 mM KA are shown in Fig,1A,C. The responses of GluR3 flop receptors (GluR3o) evoked by 1 mM KA in Li^+ Ringer were increased by 522 ± 40% ($n = 12$) compared with the Na^+ controls (Fig. 1A). We have previously reported that under similar conditions lithium causes only a 50% potentiation of GluR1 responses [7]. The GluR1 and GluR3 clones we tested vary in the R/G site described by Lomelli et al. [8]. This site influences recovery from desensitization, so that the sequence at this site regulates the magnitude of steady-state currents by altering the equilibrium between conducting and non-conducting states of the channel during the responses of these receptors. We hypothesized that the differences in Li^+ potentiation observed with GluR1 and GluR3 may also be associated with this R/G site. In order to test this hypothesis, the amino acid at the R/G site of GluR3(G) was mutated to the corresponding amino acid in GluR1(R). We observed that the responses of the R/G site mutant GluR3o(G769R) receptor were increased by only 31 ± 6% ($n = 7$). This level of Li^+ potentiation obtained with GluR3o(G769R) was essentially the same as with GluR1 [7], indicating that the R/G site is critical for the large Li^+ potentiation of wild-type GluR3o.

While the R/G site appears to be critical for determining the relative potentiation of GluR1 and GluR3 by lithium, the desensitization process itself seems to be the key basis for the effects observed. Specifically, when the non-desensitizing mutant GluR3o(L507Y) was tested, it was observed that Li^+ actually inhibited the responses of these receptors to 1 mM KA by 34 ± 2% ($n = 5$).

Lithium's potentiation of wild-type GluR3 and inhibition of GluR3(L507Y) responses was consistent between splice variants and was seen with other agonists such as glutamate.

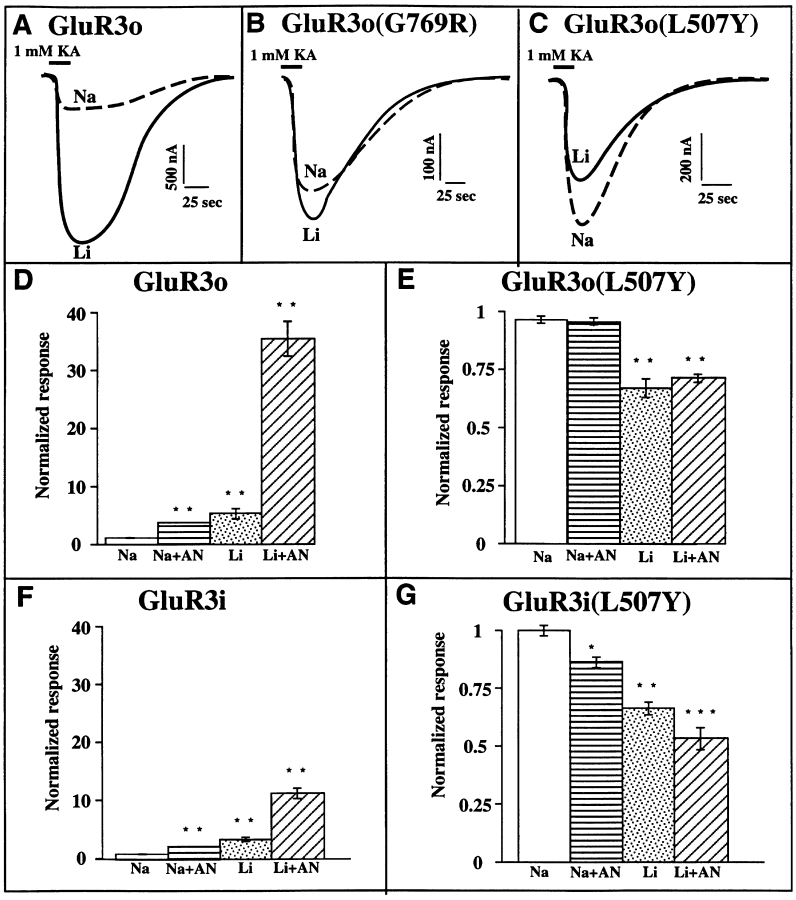

Fig. 1. Mutational analysis of GluR3 Sensitivity to lithium and aniracetam (AN). (A) Raw currents evoked with 1 mM KA from wild-type GluR3o expressing oocytes showing the potentiation by Li^+. (B) The R/G site mutant GluR3o(G769R) displays little potentiation by Li^+. (C) The non-desensitizing mutant GluR3o(L507Y) is inhibited by Li^+. (D–G) Responses of wild-type and mutant GluR3 to 1 mM glutamate in the presence of Na^+, Na^+ + AN, Li^+, and Li^+ + AN. (D) Li^+ potentiates GluR3o and is synergistic with AN. (E) Li^+ inhibits GluR3o(L507Y) and AN has no effect. (F) Li^+ potentiates GluR3i and is synergistic with AN. (G) Li^+ and AN inhibit GluR3i(L507Y). Responses are normalized to 1 mM glutamate in Na^+ for each receptor. Each bar represents the mean ± SEM of three to ten oocytes. Asterisks indicate significant differences (*$P < 0.05$, **$P < 0.005$ compared with Na^+, ***compared with Li^+).

As shown in (Fig. 1D–G), Li^+ potentiated responses to 1 mM glutamate for both wild type GluR3 flip and flop variants and inhibited both GluR3(L507Y) flip and flop variants (Fig 1E,G, $P < 0.001$).

We evaluated the responses of wild-type GluR3 in Na^+ and Li^+ based Ringer when desensitization was reduced with the modulator, aniracetam. Using 1 mM glutamate as the agonist, we observed that both the flip and flop variants of wild-type GluR3 were potentiated by aniracetam in the absence of Li^+ (Fig. 1D,F). However, the combination of aniracetam and Li^+ caused a significant additional potentiation ($P < 0.005$) of the wild-type receptors, which suggested that Li^+ and aniracetam worked independently to potentiate the current (Fig. 1D).

If Li^+ and aniracetam are working through modulation of receptor desensitization, then the elimination of desensitization in the GluR3(L507Y) mutants should prevent potentiation of their response by Li^+ as well as the synergy between aniracetam and Li^+. As noted above, Li^+ inhibited the glutamate-evoked responses of both the flip and flop variants of the GluR3(L507Y). While aniracetam potentiated GluR3 wild-type current (Fig. 1D,F), we observed no potentiation of the GluR3o(L507Y) responses (Fig. 1E) and a small inhibition of GluR3i(L507Y) responses (Fig. 1G; $P < 0.05$). The combination of aniracetam and Li^+ caused significant additional inhibition specifically for GluR3i(L507Y) responses with saturating glutamate as the agonist (Fig. 1G; $P < 0.05$).

In summary, we have investigated the nature of the GluR3 current potentiation by Li^+ through the evaluation of lithium's effects with non-desensitizing mutants of GluR3 and with compounds affecting receptor desensitization. Our results confirm that the potentiating effect of Li^+ on GluR3 is achieved through a modification of desensitization.

Specifically, we observed that the capacity of aniracetam to potentiate AMPA receptor currents was eliminated in the mutant GluR3(L507Y). Since Li^+ no longer potentiates the

current from GluR3(L507Y), but instead causes a slight inhibition, the results indicate that the absence of receptor desensitization removes the capability for lithium or aniracetam to further potentate current and support the hypothesis that Li^+ potentiates current in wild-type GluR3 by partially eliminating desensitization.

CTZ and aniracetam are each thought to potentiate AMPA receptor current by reducing desensitization. Aniracetam and Li^+ both potentiated the responses of the wild-type flip and flop variants of GluR3. The combination of aniracetam and Li^+ produced greater potentiation than either one alone, making their effects appear additive. In contrast, our previous work with CTZ showed that the combination of CTZ and Li^+ further potentiated only GluR3o responses while it antagonized GluR3i [7]. Interestingly, CTZ appeared to antagonize the glutamate-evoked currents of both flip and flop GluR3(L507Y) [14].

The mutant GluR3o(G769R), obtained when the R/G site of GluR3 was engineered to match GluR1, demonstrated the critical importance of the R/G site for the effects of lithium. This mutant was only slightly potentiated by lithium (Fig. 1B) and still maintained sensitivity to modulators such as CTZ and aniracetam (data not shown), similar to GluR1. The mutation of the R/G site therefore appears to dissociate the sensitivity of GluR3(G769R) to lithium from its sensitivity to other chemical modulators. This dissociation of function could explain the apparent additive effect observed when Li^+ is combined with CTZ or aniracetam and suggests that lithium may act through distinct kinetic states to alter receptor function.

In conclusion, our experiments demonstrate that lithium can be added to the list of chemical agents and/or genetic modifications that can modulate desensitization. However, it remains unclear whether chemical and genetic modulators exert identical effects on shifting the kinetic balance between activatable and desensitized states of AMPA-selective glutamate receptors. While mutations, or changes in the energy associated with small molecule interactions (e.g. CTZ, aniracetam and lithium), might affect desensitization by altering the effectiveness of agonists to induce the receptor conformations associated with desensitized states, mutations may also sterically eliminate specific receptor conformations or permit normally desensitized states to conduct current [12]. Our data suggest that further detailed studies of desensitization may exploit the use of lithium as a tool to dissect these potential mechanisms for the modulation of desensitization.

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