Epileptogenesis Up-Regulates Metabotropic Glutamate Receptor Activation of Sodium-Calcium Exchange Current in the Amygdala

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INTRODUCTION

Eight metabotropic glutamate (mGlu) receptors have been cloned and are subdivided into three groups, group I (mGlu1 and mGlu5), group II (mGlu2 and mGlu3), and group III (mGlu4, 6, 7, and 8; Conn and Pin 1997; Pin and Duvoisin 1996b; Keele et al. 1999). The purpose of this study was to test whether mGlu receptor-activation of Na\(^{+}\)–Ca\(^{2+}\) exchange current is enhanced by kindling-induced seizure activity and to analyze the possible mGlu subtype underlying activation of the exchange current. Here, we report that kindling-induced epilepsy up-regulates the Na\(^{+}\)–Ca\(^{2+}\) exchange current induced by activation of a group I, possibly mGlu5.

METHODS

Amygdala slices from control and kindled male Sprague-Dawley rats (90–200 g) were prepared as described (Keele et al. 1997). Rats were decapitated; the brains rapidly removed, placed in cold (4°C) artificial cerebrospinal fluid (aCSF) of the following composition (in mM): NaCl, 117; KCl, 4.7; CaCl\(_2\), 2.5; MgCl\(_2\), 1.2; NaH\(_2\)PO\(_4\), 1.2; Na\(_2\)HCO\(_3\), 25; and glucose, 11 (pH = 7.4), and aerated with a mixture of O\(_2\)/CO\(_2\) (95/5%). Coronal brain slices (500-μm thick) were prepared by using a vibrsilo and kept in aCSF at room temperature for ≥1 h. Slices were submerged in a recording chamber superfused with aCSF (2.5 ml/min, 31 ± 1°C). Blind whole-cell recordings were performed as described previously (Keele et al. 1997) by using patch electrodes (2–5 MΩ, pH = 7.2, 280 mosmol/kg) when filled with internal solution containing (in mM): Cs-gluconate, 122; NaCl, 5; CaCl\(_2\), 0.3; MgCl\(_2\), 2; EGTA, 1; HEPES, 10; Na\(_2\)ATP, 5; Na\(_3\)GTP, 0.4. Currents were acquired with an Axoclamp 2A amplifier with a switching frequency of 5–6 kHz (30% duty cycle; gain, 3–8 nA/mV; time constant, 20 ms). Signals were low-pass filtered at 1 kHz with a 4-pole Bessel filter and digitized at 5 kHz. Resting membrane potential was more negative than −60 mV and direct cathodal stimulation evoked action potentials overshooting 0 mV. Analog records were continuously acquired with a pen chart recorder.

Current-voltage (I-V) relationships of mGlu agonist-induced currents were obtained by either 1) applying voltage ramp commands from a holding potential (V\(_h\)) of −60 to −110 mV (80 mV/s) before and during the peak of the drug-induced current, or 2) applying voltage step commands (500 ms) from V\(_h\) = −60 to −110 mV in 10 mV intervals before and during the maximum evoked current. No difference between the two I-V protocols was noted.

Rats were anesthetized with Equithesin (35 mg/kg pentobarbital and 145 mg/kg chloral hydrate) and the right BLA implanted with tripolar electrodes, as previously described (Holmes et al. 1996b; Keele et al. 1999). Electrode tips were positioned at anteroposterior −2.0 mm and...
lateral −4.5 mm relative to Bregma at a depth of 7.3 mm from the dural surface (Paxinos and Watson 1986) and secured to the skull with stainless steel screws and dental cement. After 5 days, kindling stimulation (50–100 μA above the afterdischarge threshold) was initiated and consisted of a 2 s train (60 Hz) of monophasic square waves (2 ms), twice a day. Behavioral seizure severity was rated according to Racine (1972). Brain slices were prepared 3–7 days after the last of three consecutive stage five seizures. Control drug responses were obtained from naïve unoperated rats and confirmed in sham-operated animals (n = 9) which have been shown to exhibit mGlu responses similar to those in unoperated control animals (Holmes et al. 1996b).

(S)-3,5-dihydroxyphenylglycine [(S)-DHPG], (R,S)-2-chloro-5-hydroxyphenylglycine [(R,S)-CHPG], glutamate, and quisqualate (QUIS) were applied to the inlet of the recording chamber in a 10-μL drop from an Oxford pipetter as described previously (Keele et al. 1997, 1999). Drop entry and emergence were monitored with food dye. The final concentration in the bath (1 ml) was estimated as 1% of that of the drop. Rapid onset/offset of drug responses minimized effects of desensitization. (S)-DHPG, (R,S)-CHPG, (R,S)-1-aminoindan-1,5-dicarboxylic acid (AIDA), α-aminophosphonovaleric acid (α-APV), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were purchased from Tocris Cookson (St. Louis). Tetrodotoxin (TTX), QUIS, and glutamate were obtained from Sigma.

Data were compared by using a paired Student’s t-test, a one-way analysis of variance (ANOVA), or unpaired analysis (two-way ANOVA). Concentration-response relationships were plotted and fit.

**FIG. 1.** Inward currents evoked by application (arrows) of mGlu receptor agonists to basolateral amygdala (BLA) neurons in the presence of t-2-amino-5-phosphovaleric acid [t-2-APV (50 μM)], 6-cyano-7-nitroquinoxaline-2,3-dione [CNQX (30 μM)], and tetrodotoxin [TTX (1 μM)]. A: quisqualate [QUIS (30 μM)] inward current is associated with inward shift (I-V) curve but no change in slope conductance (B). Group 1 agonist, (S)-3,5-dihydroxyphenylglycine [(S)-DHPG], (C), glutamate (300 μM, E), and the mGlu 5 agonist, (R,S)-2-chloro-5-hydroxyphenylglycine [(R,S)-CHPG] (2 mM, G) induce inward currents without a change in slope conductance (D, F, H). B, D, and F: current-voltage (I-V) relationships obtained with voltage ramp commands; H: the I-V curve obtained by applying voltage step commands. Long downward deflections numbered in A, C, and E correspond to the I-V relationships shown in B, D, and F, respectively. Repetitive downward deflections result from 5 mV (600-ms duration) hyperpolarizing voltage steps as a monitor of input conductance. V_H = −60 mV. Calibration (A, C, and E) = 50 pA, 20 s, and 60 pA, and 26 s (G). Agonists were applied to different neurons.
Agonist pharmacology of mGlu receptor-activated inward current in control animals

In basolateral amygdala neurons, QUIS evokes an inward current mediated by Na\(^+\)-Ca\(^{2+}\) exchange (Keele et al. 1997). Figure 1 compares typical exchange currents activated by mGlu agonists in BLA neurons in the presence of \(\alpha\)-APV (50 \(\mu\)M), CNQX (30 \(\mu\)M), and TTX (1 \(\mu\)M). Drop-application of QUIS (30 \(\mu\)M; Fig. 1A) induces a peak inward current amplitude of 81 ± 13 pA \((n = 14)\), whereas the group I selective agonist (S)-DHPG (300 \(\mu\)M) elicits an inward current of 45 ± 6 pA \((n = 17)\). The endogenous ligand, glutamate (300 \(\mu\)M), at a 10-fold higher concentration than QUIS, evokes an equivalent current of 88 ± 18 pA \((n = 8\); Fig. 1E). The selective mGlu5 agonist, (R,S)-CHPG (4 mM), induces a peak inward current of 103 ± 15 pA (Fig. 1G), suggesting mediation of the exchange current by the mGlu5 subtype.

The QUIS inward current was accompanied by a parallel inward shift in the I-V relationship indicating that membrane slope conductance was not increased (Fig. 1B). Membrane conductance during the peak of the current was 96 ± 2\% \((n = 11)\) that of control. Membrane slope conductance was 7.2 ± 0.7 nS before and 6.9 ± 0.7 nS \((P > 0.05)\); paired t-test; \(n = 11\) during the QUIS (30 \(\mu\)M) inward current. Similarly, membrane conductance during the (S)-DHPG current (Fig. 1D) was 98 ± 1\% \((n = 18)\) of control conductance [control: 8.6 ± 0.6 nS; (S)-DHPG (300 \(\mu\)M): 8.4 ± 0.6 nS; \(P > 0.05\); \(n = 18\)], whereas that of the glutamate- (300–100 \(\mu\)M) induced current (Fig. 1F) was 94 ± 5\% \((n = 6)\) of control [control: 8.0 ± 1.2 nS; glutamate (300 \(\mu\)M): 7.4 ± 1.2 nS; \(P > 0.05\); \(n = 6\)]. The (R,S)-CHPG-induced inward current was also not accompanied by a change in slope conductance (Fig. 1, G and H; \(n = 7\)).

Both phenylglycine agonists were blocked by group I antagonist, AIDA (\(n = 2\)).

Concentration-response relationships for group I mGlu agonists

Concentration-response curves obtained for QUIS, (S)-DHPG, (R,S)-CHPG, and glutamate are illustrated in Fig. 2. Comparison of the curves for these agonists showed a rank order of potency of QUIS > (S)-DHPG ≥ GLU > (R,S)-CHPG. Nonlinear (sigmoid) regression analyses of the agonist-evoked current amplitudes show apparent EC\(_{50}\) of 19 \(\mu\)M \((n = 3–14)\), 57 \(\mu\)M \((n = 4–17)\), 0.6 mM \((n = 3–9)\), and 2.6 mM \((n = 5–9)\) with maximum current amplitudes of 109 ± 30 pA (100 \(\mu\)M), 54 ± 6 pA (1 mM), 157 ± 15 pA (1 mM), and 103 ± 15 pA (4 mM) for QUIS, (S)-DHPG, glutamate, and (R,S)-CHPG, respectively. These results also suggest that QUIS, glutamate, and (R,S)-CHPG are full agonists for this current, whereas (S)-DHPG may be a partial agonist.

Changes in mGlu receptor-activated inward currents recorded in kindled animals

The concentration-response relationship for (S)-DHPG-induced inward currents (Fig. 3A) shows that the effect of this agonist is enhanced by kindling-induced epileptogenesis. The curve for the (S)-DHPG inward current in kindled animals is shifted upward relative to that obtained from control animals. In kindled neurons, (S)-DHPG has an apparent EC\(_{50}\) of 160 \(\mu\)M. Two-way ANOVA indicated a significant effect due to the kindling treatment \((P < 0.01)\) as well as a significant effect \((P < 0.0001)\) of (S)-DHPG concentration in both control and kindled conditions. (R,S)-CHPG also induced activation of the Na\(^{+}\)-Ca\(^{2+}\) exchanger (Fig. 3B) with an apparent EC\(_{50}\) in kindled neurons of 1.8 mM; a two-way ANOVA indicated a significant effect due to the (R,S)-CHPG concentration \((P < 0.0001)\) in control and kindled animals but no significant effect due to the kindling treatment \((P < 0.26)\). A similar lack of effect was observed with QUIS (kindled EC\(_{50}\) = 19 \(\mu\)M). The enhanced response to (S)-DHPG is consistent with up-regulation of an mGlu receptor-activated Na\(^{+}\)-Ca\(^{2+}\)-exchange current in kindled BLA neurons.

DISCUSSION

The main findings of this study are 1) the Na\(^{+}\)-Ca\(^{2+}\) exchange current is produced by activation of a group I mGlu receptor, possibly mGlu5, and 2) kindling-induced epilepsy up-regulates the mGlu-activated exchange current.

Pharmacology of mGlu receptor-activation of Na\(^{+}\)-Ca\(^{2+}\) exchange

The pharmacology of the mGlu agonist response suggests that the receptor underlying the inward current is a group I mGlu receptor, possibly mGlu5. Glutamate and (R,S)-DHPG reportedly activate mGlu1a and mGlu5a expressing cells with similar potency (Brabet et al. 1995) and (R,S)-CHPG is selective agonist for mGlu5 (Doherty et al. 1997). Previous evidence in ventral medial hypothalamic neurons suggested that group I mGlu receptors mediate activation of the exchange current (Lee and Boden 1997). Agonist potency in BLA neurons was QUIS > (S)-DHPG ≥ glutamate > (R,S)-CHPG, data consistent with activation of mGlu5 subtype.
QUIS and glutamate activate the Na\(^{+}\)-Ca\(^{2+}\) exchange current in a manner consistent with that of a complete agonist, whereas (S)-DHPG in control BLA neurons evoked currents with a maximum amplitude about 50% of that produced by QUIS, glutamate, or (R,S)-CHPG. These data suggest that (S)-DHPG may be acting as a partial agonist at the group I receptor mediating the inward exchange current. This characteristic of DHPG has been reported by others (Brabet et al. 1995; Joly et al. 1995; Schoepp et al. 1994).

mGlu receptor activation of Na\(^{+}\)-Ca\(^{2+}\) exchange is also observed in cerebellar Purkinje cells (Linden et al. 1994; Staub et al. 1992) and in ventral medial hypothalamus (Lee and Boden 1997) where DHPG is an agonist for the exchange current. In the BLA, more mGlu5 than mGlu1 is expressed (Romano et al. 1995) and the mGlu5 selective agonist (R,S)-CHPG activates the exchange current albeit at low potency. Thus receptor localization and response to CHPG support mGlu5 as the subtype underlying activation of the exchanger in BLA neurons.

The inward current evoked by (S)-DHPG is enhanced in kindled animals

In the BLA, (S)-DHPG behaves as a partial agonist with a peak current amplitude that was markedly increased in kindled animals relative to control. The mechanisms underlying partial agonism are not known and are beginning to be defined (Clark and Bond 1998; Clarke et al. 1999; Kenakin 1996). Several possible mechanisms may account for the reported observations in kindled animals. In ventral medial hypothalamus neurons, the mGlu-activated Na\(^{+}\)-Ca\(^{2+}\) exchange current has been shown to be inhibited by guanosine 5’-O-(2-thiodiphosphate) (GDP-\(\beta\)-S) and potentiated by guanosine 5’-O-(3-thiotriphosphate) (GTP-\(\gamma\)-S), indicating mediation by G proteins (Lee and Boden 1997). If the maximum response of a partial agonist is dependent on the efficiency of the coupling mechanism, then kindling may induce a more efficient second-messenger coupling, e.g., between the receptor and G protein or between the G protein and the effector system, and larger response amplitude to (S)-DHPG would be observed; this effect would not be obvious with agonists in which coupling is already highly efficient (Kenakin 1996). Group I mGlus can also dimerize (Robbins et al. 1999; Romano et al. 1996). If kindling increases dimerization, partial agonists may detect increases in the dimerized state, whereas full agonists may not. Some data suggest that partial agonists in one system may be full agonists in another due to the environment extrinsic to the receptor and may involve the stoichiometry/complement of G-proteins or organization/composition of the lipid bilayer (Ghanekar et al. 1997). A kindling-induced alteration in similar constituents could account for the selective enhancement of (R,S)-DHPG compared with the other agonists. Alternatively, it has been shown that in expression systems increasing the amount of mGlus density can increase the maximum response (Hermans et al. 1999). If the result of kindling is an increase in the number of mGlus, a partial agonist like (S)-DHPG that requires occupancy of all available receptors to produce its maximum response (Kenakin 1996) in normal animals would produce a larger response in kindled animals because of the increased number of receptors. Highly efficacious agonists may not be able to detect increased receptors except perhaps with excessive transmitter release that can be attained during epilepsy.

The mGlu receptor activation of Na\(^{+}\)-Ca\(^{2+}\) exchange may be a mechanism for removal of the excess intracellular Ca\(^{2+}\) that occurs during periods of high glutamate release; the Ca\(^{2+}\) extrusion and Na\(^{+}\) entry may contribute further to increasing cellular excitation and ictal-like bursting activity. Additionally, up-regulation of Na\(^{+}\)-Ca\(^{2+}\) exchange may reduce intracellular Ca\(^{2+}\) concentration such that inhibitory Ca\(^{2+}\)-activated potassium channels in the amygdala are unable to contribute to regulation of membrane excitability.

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