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Research report

Quisqualate induces an inward current via mGluR activation in neocortical pyramidal neurons

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Abstract

Activation of metabotropic glutamate receptors (mGluRs) has multiple effects on the excitability of pyramidal neurons in rat frontal neocortex. Synaptic transmission and intrinsic excitability are both affected. During studies of the effects of quisqualate on synaptic activity, it was observed that quisqualate also induced a slow inward current. Whole-cell patch clamp recordings were obtained from layer II/III pyramidal neurons of neocortical slices in vitro. The bath solution contained APV, CNQX and bicuculline to block ionotropic glutamate and $GABA_A$ receptors. At a holding potential of −70 mV, quisqualate (2 μM) induced an inward current of about 60 pA. The response was reversible upon washing. This current was associated with an increase in membrane conductance and was still seen in the presence of TTX (0.5 μM). Bath application of the nonselective mGluR antagonist, (*R,S*)-α-methyl-4-carboxyphenylglycine (MCPG, 200–500 μM) reduced the current by 70%. Other mGluR agonists (ACPD, DHPG, L-CCG-1 and L-AP4) did not induce a significant inward current at the concentrations tested. The current–voltage relation of the quisqualate-induced current was linear with a reversal potential near 0 mV suggesting involvement of nonselective cation channels. The quisqualate-induced inward current was markedly reduced (72%) with 200 μM GDP-β-S in the pipette solution, indicating that it is a postsynaptic phenomenon mediated by a G-protein dependent mechanism. These results suggest that mGluRs can directly increase the postsynaptic excitability of pyramidal cells.

Keywords: Quisqualate; Inward currents; Neocortex; mGluRs; G-proteins

1. Introduction

Glutamate is the principle excitatory neurotransmitter in the neocortex. It activates both ionotropic and metabotropic glutamate receptors (mGluRs). The former is involved in fast synaptic transmission whereas the latter play a modulatory role. Several excitatory amino acid (EAA) analogs, particularly quisqualate, also can activate mGluRs. Molecular cloning studies have demonstrated the existence of at least eight different mGluRs, termed mGluR1 to mGluR8. Depending on their sequence homology, signal transduction pathway and pharmacological properties, mGluRs have been divided into three distinct groups [14]. Group I includes mGluR1 and mGluR5 which are coupled to increased phosphoinositide hydrolysis and are highly sensitive to quisqualate. Group II (containing mGluR2 and mGluR3) and Group III (composed of mGluR4 and mGluR6–8) are both negatively linked to adenylate cyclase and are preferentially activated by (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl) glycine (L-CCG-I) and L-2-amino-4-phosphonobutyric acid (L-AP4), respectively. Each mGluR has a unique distribution in the CNS that likely reflects a diversity of function in normal and pathological processes [14].

In the rat neocortex, mGluRs have multiple effects both on synaptic transmission [3,6] and intrinsic neuronal excitability [4,18]. Activation of Group II and III mGluRs depresses neocortical EPSPs and IPSPs via a presynaptic mechanism [3] whereas Group I receptors enhance spontaneous IPSPs [6,18]. Group I agonists, particularly quisqualate, have direct postsynaptic effects on membrane excitability in neocortex and many other brain structures [14]. The principal effect is to increase excitability. ACPD reduces spike frequency adaptation in neocortical pyramidal [4] but the mGluR subtype and ionic mechanism involved is not known. In neocortical interneurons, quis-

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qualate and ACPD induce an inward current associated with a conductance increase [18]. Similar inward currents have been reported in hippocampus [10] and dorso-lateral septal nucleus neurons [17].

In the present of study, we have used whole-cell patch clamp recording to investigate the direct postsynaptic effects of mGluR activation on layer II/III rat frontal neocortical pyramidal neurons. Our results demonstrated that quisqualate induces a novel, G-protein mediated inward current. Some of the data have been presented in abstract form [5].

2. Materials and methods

Neocortical brain slices were prepared as described previously [6]. Sprague–Dawley rats (16–26 day-old) were anesthetized with ketamine (100 mg/kg) and decapitated. The brains were quickly removed and immersed in ice-cold oxygenated saline, bubbled with 5% CO_2 and 95% O_2. Six to eight coronal slices (300 μM thick) of frontal neocortex were prepared on a Vibratome and immediately transferred to a storage chamber. After a recovery period of at least 1 h, individual slices were transferred to a recording chamber mounted on the stage of a Zeiss Axioskop FS microscope. The chamber was continuously perfused with oxygenated saline at a rate of 2–4 ml/min. All experiments were conducted at room temperature (21–23°C).

Whole-cell patch clamp recordings were obtained from visually identified layer II/III pyramidal neurons, as judged by neuronal morphology and position below the pial surface. Patch pipettes were pulled from Garner KG-33 glass capillaries using a Narishige Model PP-83 puller. Pipettes had resistances of 3–4 MΩ. Voltage-clamp recordings were made with an Axopatch-200 amplifier. Responses were filtered at 2–5 kHz, digitized at 10–20 kHz and stored on computer for later analysis.

The normal extracellular bath solution contained (in mM): 125 NaCl, 3.5 KCl, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$, and 10 D-glucose. The patch pipette solution contained (in mM): 135 CsCl, 0.5 EGTA, 10 HEPES, 2 MgATP, 0.2 NaGTP. pH was adjusted to 7.3 with 1 M NaOH and osmolarity was adjusted to 270 mOsm.

Quisqualate; 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D(-)2-amino-5-phosphonovaleric acid (D-APV) were obtained from Cambridge Research Biochemicals. ACPD, 3,5-dihydroxyphenylglycine (DHPG), L-AP4 and (*R*,*S*)-α-methyl-4-carboxyphenylglycine (MCPG) were purchased from Tocris Neuramin. Bicuculline methiodide (BIC) and tetrodotoxin (TTX) were obtained from Sigma and guanosine-5′-*o*-(2-thiodiphosphate) (GDP-β-S) from Boehringer Mannheim Biochemica. All compounds were bath applied except for GDP-β-S which was included in the patch pipette. Each neuron served as its own control.

Statistical analysis used paired *t* tests or one-way analysis of variance (ANOVA). Significance levels of 0.05 and 0.01 were used. All data are expressed as mean±S.E.M.

3. Results

Whole-cell recordings were obtained in the presence of D-APV (20 μM) and CNQX (20 μM) to block ionotropic glutamate receptors. Bath application of quisqualate (1–5 μM) induced a slow inward current in all neocortical layer II/III pyramidal neurons tested ($n=65$). At a holding potential of −70 mV, quisqualate-induced an inward current of 88.3±13.5 pA ($n=17$). As shown in Fig. 1A, this current was accompanied by an increase in baseline noise. The inset in Fig. 1A shows that the increase in noise was due to an augmentation of spontaneous inhibitory postsynaptic current (IPSC) frequency, as shown previously [6]. The inward current declined only slightly in the continued presence of quisqualate. The quisqualate-induced inward current was also observed in the presence of 40 μM CNQX, indicating that activation of ionotropic glutamate receptors was not involved. Quisqualate effects were dose dependent and reversible upon drug washout. In the presence of TTX, quisqualate still induced an inward current, as shown in Fig. 1B. Current amplitudes (88.6±21.3 pA; $n=14$) were not significantly different from those observed without TTX. In the presence of TTX, no increase in noise or synaptic activity was observed.

The conductance changes underlying the quisqualate-induced current were studied in the presence of 20 μM D-APV, 20 μM CNQX, 10 μM BIC and 0.5 μM TTX. Hyperpolarizing and depolarizing voltage steps (500 ms) from −90 mV to −30 mV were applied in the presence and absence of quisqualate. As shown in Fig. 2A, 2 μM quisqualate increased membrane conductance in neocortical pyramidal neurons, as indicated by increases in the response to voltage step commands. The voltage dependence of the quisqualate-inward current was also studied using voltage ramps (−70 to +30 mV). Fig. 2B shows the *I–V* relationship for the quisqualate-induced current. The current was determined by subtracting responses to ramps recorded in the presence and absence of quisqualate. The current showed a linear *I–V* relationship and reversed near 0 mV (−3.52±1.06; $n=4$).

In order to verify that quisqualate was acting on mGluRs, the effect of the mGluR antagonist MCPG was tested. MCPG (200–500 μM) reduced the amplitude of the quisqualate-induced current by 70% ($n=14$), as shown in Fig. 3. The effects of mGluR activation are G-protein mediated [14,15] although in some circumstances G-protein involvement has been hard to demonstrate. We included GDP-β-S (200 μM), a non-hydrolyzable GDP analog, in the pipette to prevent G-protein activation [8]. When neurons were recorded with GDP-β-S (200 μM) in

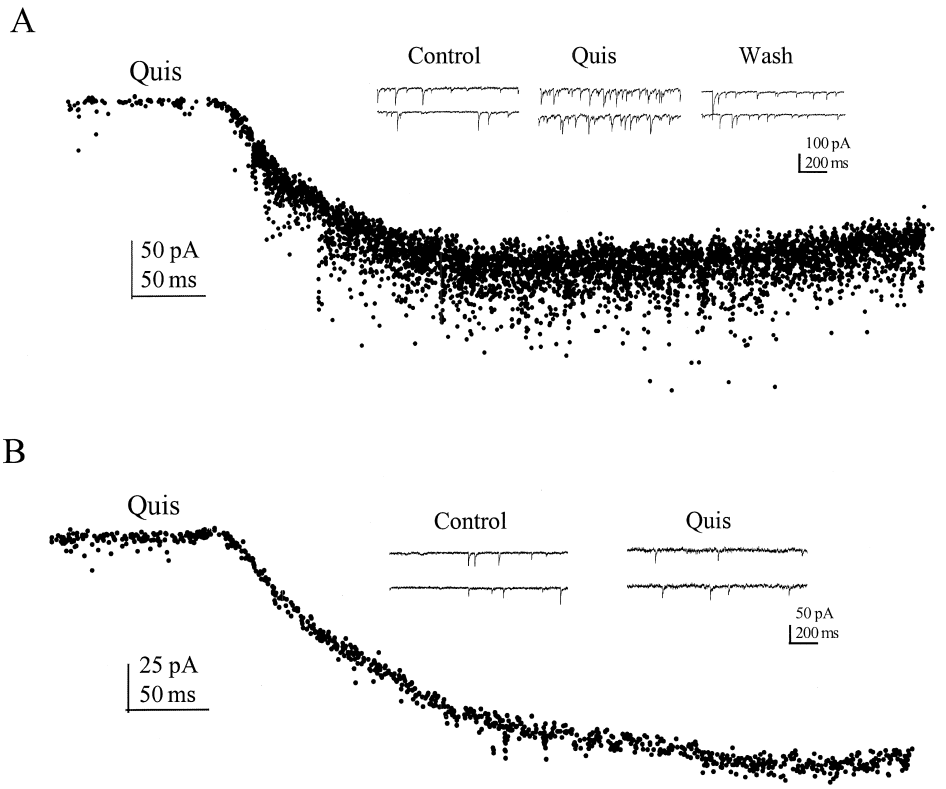

Fig. 1. Quisqualate induces a slow inward current in neocortical pyramidal cells. (A) Effect of quisqualate on a neuron bathed in 20 μM D-APV and 20 μM CNQX. At a holding potential of −70 mV, quisqualate application produced a slow inward current. An increase in baseline noise was observed. The inserts show recordings at a faster time scale. Quisqualate application was associated with an increase in spontaneous synaptic activity. (B) Quisqualate was applied to another cell after addition of TTX to the D-APV and CNQX containing saline. A similar inward current was observed. As shown in the inserts, no increase in synaptic activity was observed.

the pipette, quisqualate induced a current of only 16.50±2.39 pA (a 72% reduction from control, $n=34$), as shown in Fig. 3. These data indicate that the quisqualate-induced inward current is dependent on postsynaptic mGluR activation of G-proteins.

Group specific mGluR agonists were used to determine the type of mGluR inducing the inward current. The results are shown in Fig. 4. L-CCG-I and L-AP4 are agonists at Group II and Group III mGluRs, respectively. Bath application of L-CCG-I (20–50 μM) and L-AP4 (100 μM) did not induce a significant current in layer II/III pyramidal neurons, consistent with their predominant presynaptic inhibitory action. DHPG (100 μM), a specific agonist for Group I mGluRs did not induce a significant current. Low concentrations of 1S,3R-ACPD (30 μM) were also ineffective. These results suggest a novel mGluR-mediated quisqualate response in neocortical neurons.

4. Discussion

The present results indicate that quisqualate can induce a non-desensitizing inward current in layer II/III pyramidal cells. This response is dependent upon activation of G-protein coupled mGluRs.

Quisqualate-induced currents were associated with a conductance increase and had a linear current –voltage relation. These findings suggest activation of a non-specific cation conductance.

Quisqualate is a potent agonist at ionotropic glutamate receptors. It is unlikely, however, that the inward current observed here was due to activation of ionotropic receptors. The concentrations of quisqualate employed were relatively low and the concentrations of the ionotropic antagonists present were high (20 μM D-APV and 20–40 μM CNQX). Furthermore, the quisqualate-induced postsynaptic response was blocked by the mGluR antagonist MCPG and almost abolished by including GDP-β-S in the recording pipette. These results suggest quisqualate activated a mGluR-dependent current.

Specific agonists for group II and III mGluRs failed to evoke a significant inward current, suggesting that these mGluRs are not involved in this postsynaptic response. This is consistent with results from electrophysiological studies showing a predominantly presynaptic effect of these receptors [3].

Quisqualate is a potent agonist for group I mGluRs. Group I receptors are know to be located postsynaptically [13], suggesting a role for these receptors in generating the observed inward current. In addition, the sensitivity to

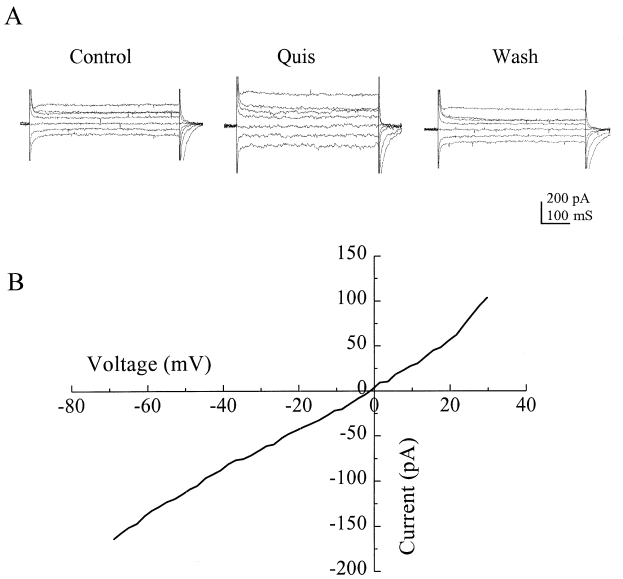

Fig. 2. Conductance changes and current–voltage relations for quisqualate-induced currents in a layer II/III pyramidal neuron. (A) Currents evoked by step commands from a holding potential of −70 mV. In the presence of quisqualate (middle traces) conductance was increased. (B) Control bathing solution contained 0.5 μM TTX, 20 μM D-APV, 20 μM CNQX and 10 μM BIC. Intracellular pipette solution contained 135 mM CsCl. Currents were evoked by ramping the cell from −70 mV to 35 mV in the absence and presence of 1 μM quisqualate. The difference current, obtained by subtracting the control current from the current evoked in the presence of quisqualate, represents the agonist-induced current. The current was linear and reverses polarity near 0 mV.

MCPG suggests that activation of mGluR1 is involved since MCPG is more potent at antagonizing mGluR1 versus mGluR5-mediated responses [11]. Direct evidence in support of this must await the development of a selective mGluR1 antagonist.

An inward current was not seen in response to 1*S*,3*R*-ACPD. The lack of effect of 1*S*,3*R*-ACPD may have been

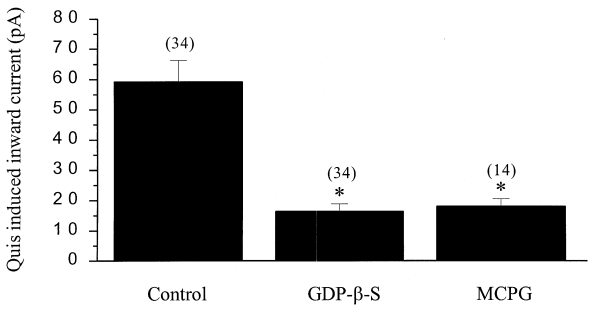

Fig. 3. Quisqualate induced currents are dependent upon activation of G-proteins and mGluRs. Bar graph shows the amplitude of the quisqualate induced current in cells recorded under control conditions (left), in cells recorded with pipettes containing 200 μM GDP-β-S to prevent G-protein (middle) and in slices bathed in the mGluR antagonists MCPG (200–500 μM). Prevention of G-protein activation and inhibition of mGluRs significantly reduced quisqualate responses. The number of cells tested is indicated in parentheses. * Represents a $P<0.05$ compared to control values.

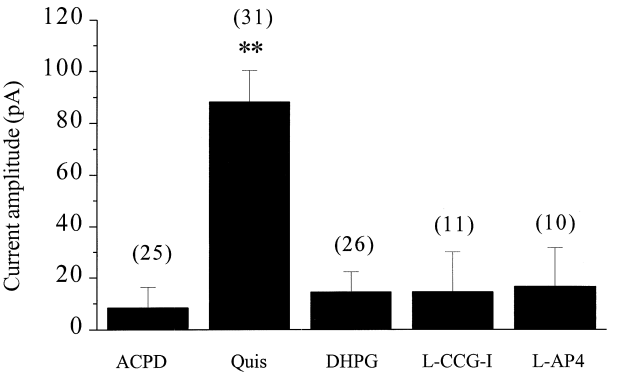

Fig. 4. Histogram summarizing the ability of mGluR agonists to induce an inward current. All recordings were made in the presence of 20 μM D-APV, 10 μM CNQX and 10 μM BIC. Experiments with and without 0.5 μM TTX were combined since similar results were obtained. The number of cells tested is indicated in parentheses. Quisqualate was the only agonist to induce a significant inward current.

due to the low concentration (30 μM) employed. Previous studies in neocortical neurons demonstrated that 100 μM 1*S*,3*R*-ACPD was needed to produce significant changes in resting membrane potential and input resistance [4]. The group I specific agonist DHPG was also ineffective. This may be due to the lower potency of this agonist at group I mGluRs. In our previous study of quisqualate effects on spontaneous IPSCs in neocortex, the largest increases in sIPSC frequency were observed with quisqualate [6]. DHPG was less effective. Consistent with this finding are the results obtained in the CA1 region of the hippocampus showing that the rank order of agonist potency for group I mGluR-mediated inhibition of afterhyperpolarizations was quisqualate>glutamate>ACPD≅DHPG [9]. Thus, the mGluR responsible for the inward current recorded here appears to be highly sensitive to quisqualate.

An inward current in response to group I mGluR activation has been reported in a number of brain regions, including hippocampus [7], dorsolateral septal nucleus [17], basolateral amygdala [12] and cerebellum [16]. Various mechanisms have been implicated, including reductions in several K^+ currents [16], induction of an Ca^{2+}-activated nonselective cationic current [7] and activation of a Na^+–Ca^{2+} exchange current [12]. The inward current observed in the present experiments is not attributable to blockade of K^+ currents or activation of a Na^+–Ca^{2+} exchange current since these mechanisms are associated with a decrease or no change in conductance. An increase in conductance was observed here. Voltage-sensitive Na^+ channels do not appear to be involved since quisqualate was effective in inducing an inward current in the presence of TTX.

Activation of group I mGluRs (mGluR1 and mGluR5) can activate phospholipase C. This results in phosphoinositide hydrolysis and the formation of IP3 and diacylglycerol. IP3 stimulates release of Ca^{2+} from intracellular stores [1,2,15]. Ca^{2+} and diacylglycerol stimu-

late protein kinase C. The downstream ionic mechanism responsible for the quisqualate induced inward current is unclear. The amplitude of the quisqualate-induced inward current was not significantly reduced in the presence of 200 μM Cd^{2+}, suggesting that Ca^{2+} entry via voltage-dependent Ca^{2+} channels is not needed (data not shown). The observed linear *I–V* relationship and reversal potential around 0 mV are consistent with a non-specific cation conductance activated by release of Ca^{2+} from intracellular stores.

In summary, quisqualate, presumably through group I mGluRs, induces an inward current in layer II/III rat neocortical pyramidal cells. This quisqualate-induced inward current might be expected to increase neuronal excitability and potentiate glutamate release. Postsynaptic mGluRs can thus serve as conditional potentiators of excitatory synaptic transmission. When a pyramidal neuron is brought to near action potential threshold by ionotropic glutamate receptors, mGluRs activation may bring the neuron to above the firing threshold.

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