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Ca^{2+} permeability and Joro spider toxin sensitivity of AMPA and kainate receptors on cerebellar granule cells

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Abstract

We have investigated the Ca^{2+} permeability of native kainate- and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate- (AMPA) receptors in cultured rat cerebellar granule cells. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) increases and Mn^{2+} quench of fura-2 (a measure of Ca^{2+} entry) mediated by kainate receptors were completely dependent on the presence of extracellular Na^+. Kainate receptor-mediated $[Ca^{2+}]_i$ rises were reduced 37% by the L-type voltage-gated Ca^{2+} channel blocker nifedipine (1 μM). AMPA receptor-mediated $[Ca^{2+}]_i$ rises observed in Na^+-free buffer were sensitive to Joro spider toxin (500 nM) blockade showing a 65% reduction, while kainate receptor-mediated $[Ca^{2+}]_i$ responses were largely insensitive. These results suggest that a component of AMPA receptor-mediated $[Ca^{2+}]_i$ increases occurs through Ca^{2+} permeable receptors which lack the GluR2 subunit and are Joro spider toxin sensitive. In contrast, kainate receptors do not appear to directly gate significant Ca^{2+} but raise $[Ca^{2+}]_i$ through activation of voltage-gated Ca^{2+} channels and seem largely insensitive to Joro spider toxin.

Keywords: GluR2 subunit; GluR5 subunit; GluR6 subunit; Ca^{2+}, intracellular; GYKI 53655; Ca^{2+} channel, voltage-gated

1. Introduction

The majority of excitatory neurotransmission between synapses in the mammalian central nervous system (CNS) is mediated by ionotropic glutamate receptors. These cation selective receptor channels are divided into three major types named after the specific agonists *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate (Collingridge and Lester, 1989). AMPA and kainate receptors have often been referred to collectively as non-NMDA receptors, as the pharmacological distinction between them has been blurred by the agonist action of kainate at AMPA receptors (Fletcher and Lodge, 1996).

While all subtypes of NMDA receptor appear to directly gate calcium ions, the Ca^{2+} permeability of the non-NMDA receptors is not as clear cut. Initially, activation of AMPA or kainate receptors was thought to raise intracellular Ca^{2+} ($[Ca^{2+}]_i$) indirectly by activation of voltage-gated Ca^{2+} channels as a result of depolarisation caused by Na^+ influx through the receptor (Mayer and Miller, 1990). However, following cloning of the subunits for AMPA and kainate receptors, it emerged that certain subtypes of these receptors have appreciable Ca^{2+} permeability (Fletcher and Lodge, 1996).

In the case of AMPA receptors, the presence of an edited GluR2 subunit restricts the Ca^{2+} permeability of recombinant receptors (Burnashev et al., 1992, 1995). The low Ca^{2+} permeability of receptors containing GluR2 is due to RNA editing which changes a single amino acid in the GluR2 subunit from glutamine (Q) to arginine (R) at the Q/R site (Sommer et al., 1991). Consistent with data from recombinant AMPA receptors, cells which lack GluR2 express native AMPA receptors which are Ca^{2+} permeable (Bochet et al., 1994). However, even in cells where GluR2 is present, the expression of Ca^{2+} permeable AMPA receptors may still occur, for example in cultured rat Purkinje cells (Brorson et al., 1992). It seems that it is the level of expression of GluR2 relative to the other receptor subunits GluR1, 3 and 4 that determines the overall Ca^{2+} permeability of AMPA receptors in a given cell (Geiger et al., 1995).

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Kainate receptor subunits comprise GluR5–7 which can form functional homomeric recombinant receptors or heteromeric receptors with distinct properties when combined with KA1 or KA2 subunits (Lerma, 1997). Studies of the Ca^{2+} permeability of kainate receptors has been limited to recombinant receptors. The Ca^{2+} permeability of recombinant homomeric GluR6 kainate receptors is influenced by RNA editing at a Q/R site in a similar way to GluR2 (Sommer et al., 1991). Homomeric receptors formed from unedited GluR6(Q) subunits have appreciable Ca^{2+} permeability, with the fractional Ca^{2+} current accounting for 2% of the whole cell current (Burnashev et al., 1995). Edited GluR6(R) homomeric receptors were initially thought to also have appreciable calcium permeability (Egebjerg and Heinemann, 1993; Kohler et al., 1993) but subsequent studies have shown that homomeric GluR6(R) receptors show a negligible fractional Ca^{2+} current (less than 0.2%; Burnashev et al., 1995) and virtually no permeability to Ca^{2+} (Burnashev et al., 1996). The edited form of GluR5 does not appear to form functional homomeric receptors, but may be incorporated into functional heteromeric receptors (Herb et al., 1992) and could therefore affect Ca^{2+} permeability of native kainate receptors. In contrast to the AMPA GluR2 subunit, which is almost completely edited in the adult rat CNS, there are significant levels of unedited GluR5 and GluR6 in the adult rat brain (Sommer et al., 1991). Although there has been a demonstration of the functional effects of RNA editing on native kainate receptors in cultured hippocampal neurons (Ruano et al., 1995), the Ca^{2+} permeability of these native kainate receptors was not investigated.

With a number of pharmacological tools now available to discriminate kainate receptors from AMPA receptors (for review, see Fletcher and Lodge, 1996), there has been an increasing interest in kainate receptors as a distinct subtype of ionotropic glutamate receptor. Indeed, several recent reports have revealed the involvement of kainate receptors in synaptic transmission (Castillo et al., 1997; Clarke et al., 1997; Vignes and Collingridge, 1997). We have previously identified $[Ca^{2+}]_i$ increases mediated by kainate receptors in cultured rat cerebellar granule cells, following selective blockade of AMPA receptors with (1-(4-aminophenyl)-3-methyl-carbamyl-4-methyl-3,4-dihydro-7, 8-methyl-endioxyl-5*H*-2,3 benzodiazepine (GYKI 53655 also known as LY 300168; Savidge et al., 1997). We have also shown that these cells express a proportion of AMPA receptors which are Ca^{2+} permeable (Savidge and Bristow, 1997a). In this study, we have extended these observations in two ways. First, we have examined the pathways by which kainate receptor activation raises $[Ca^{2+}]_i$ to determine if native kainate receptors in cerebellar granule cells directly flux significant amounts of Ca^{2+}. Secondly, we have used the AMPA/kainate subunit specific blocker Joro spider toxin (Blaschke et al., 1993; Iino et al., 1996) as a tool to investigate the relationship between Ca^{2+} permeability, toxin sensitivity, and possible subunit conformation and editing status of AMPA and kainate receptors in these cells.

2. Materials and methods

2.1. Cell culture

Primary cultures of rat cerebellar granule cells were prepared as previously described (Brown and Bristow, 1996). Cells (1.5×10^6) were seeded onto poly-D-lysine (2 $\mu g/cm^2$) coated glass coverslips. After 24 h, medium was replaced with serum-free Earle's Basal Medium containing 2 mM Glutamax-I, 10 mM Hepes, N2 supplement, 25 mM KCl and 10 μM cytosine β-D-arabinofuranoside. All experiments were carried out on cells at 6–8 days in vitro at room temperature (25 ± 1°C).

2.2. Measurement of intracellular calcium ($[Ca^{2+}]_i$)

Cells were washed in Locke's buffer (pH 7.2) of the following composition (in mM): 140 NaCl, 5 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.8 $CaCl_2$, 10 Hepes, 10 Glucose. After loading with 5 μM fura-2 acetoxymethyl ester (30 min, 25°C), cells were washed and incubated in buffer for a further 30 min. Coverslips were then placed in a laminar flow perfusion chamber and continually perfused at approximately 5 ml/min with Locke's buffer (Savidge and Bristow, 1997a). Drugs were diluted from stock solutions in Locke's buffer and applied by superfusion. In Na^+-free solutions, NaCl was replaced with 140 mM *N*-methyl-D-glucamine (pH 7.2 with HCl). For experiments investigating kainate receptor-mediated responses, cells were pretreated with concanavalin A (250 $\mu g/ml$) for 10–20 min to inhibit kainate receptor desensitisation. Tetrodotoxin (TTX, 500 nM) was included in all solutions.

Intracellular calcium levels were imaged as previously described (Zamani and Bristow, 1996). During cell stimulation images were captured at 2-s intervals. Data is expressed as the peak increase of $[Ca^{2+}]_i$ over initial basal levels.

2.3. Mn^{2+} quench of fura-2 fluorescence as a measure of Ca^{2+} entry

These experiments were carried out as described elsewhere (Savidge and Bristow, 1997b). Briefly, cells were continuously perfused with nominally Ca^{2+} and Mg^{2+}-free buffer containing 100 μM $MnCl_2$ and excited at 360 nm. Data is expressed as the % decline in fluorescence at 360 nm/s.

2.4. Materials

GYKI 53655 (LY 300168) and cyclothiazide were generously provided by Dr. David Bleakman, Lilly Research

Centre (Windlesham, UK). (*S*)-AMPA and kainate were obtained from Tocris Cookson (Bristol, UK). Joro spider toxin was from Research Biochemicals. Concanavalin A (Type IV), TTX, DNase, and poly-D-lysine were obtained from Sigma. Fura-2 acetoxymethyl ester was from Calbiochem-Novabiochem (Nottingham, UK). Cell culture reagents were from Gibco (Paisley, UK). All other reagents were of analytical grade and Elga purified water was used throughout. Fura-2, cyclothiazide and GYKI 53655 stock solutions were prepared in dimethyl sulphoxide. Final dimethyl sulphoxide concentrations never exceeded 0.2% (v/v).

2.5. Data analysis

All data are expressed as mean ± S.E.M. with *n* representing the total number of cells tested on at least three separate occasions from different cultures. Statistical analysis was carried out using Instat (GraphPad Software) using non-parametric paired or unpaired tests as appropriate.

3. Results

3.1. Blockade of L-type voltage-gated Ca^{2+} channels reduces kainate receptor-mediated $[Ca^{2+}]_i$ increases

We isolated $[Ca^{2+}]_i$ rises mediated by kainate receptors by applying kainate in the presence of the selective AMPA receptor antagonist GYKI 53655 to cells pre-treated with concanavalin A (Savidge et al., 1997). Nineteen of forty-one cells tested responded to initial application of kainate (100 μM) in the presence of GYKI 53655 (50 μM) with a $[Ca^{2+}]_i$ rise of over 100 nM. The peak $[Ca^{2+}]_i$ increase to kainate/GYKI 53655 in these cells in the presence of nifedipine (1 μM) was reduced by 37% when compared with a subsequent response in the absence of nifedipine ('recovery'; Fig. 1). Responses in the presence of nifedipine are compared to subsequent responses following wash-off to exclude any effect due to rundown of the kainate receptor-mediated response.

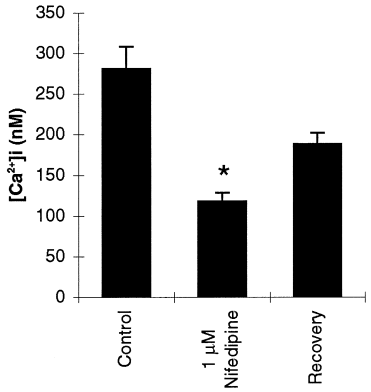

Fig. 1. Average peak $[Ca^{2+}]_i$ increases in cells responding to kainate (100 μM) in the presence of GYKI 53655 (50 μM) following concanavalin A treatment (Con A; 250 μg/ml, 10 min). A subsequent response to kainate/GYKI 53655 in the same cells was significantly reduced ($P < 0.05$) by nifedipine (1 μM) compared to the response following wash-off of nifedipine ('recovery'). Nifedipine and GYKI 53655 were present in the perfusion buffer for at least 30 s before challenge with kainate. Cells were perfused with buffer alone (wash-off) for at least 5 min between kainate stimulations. Data is from 19 cells in 3 separate experiments using 3 different cultures.

3.2. Kainate receptor-mediated $[Ca^{2+}]_i$ rises are abolished by removal of extracellular sodium

Having established the involvement of one subtype of voltage-gated Ca^{2+} channel in kainate receptor-mediated $[Ca^{2+}]_i$ rises, we investigated if activation of a number of different voltage-gated Ca^{2+} channel subtypes was solely responsible for kainate receptor-mediated $[Ca^{2+}]_i$ elevations. To prevent activation of all voltage-gated Ca^{2+} channel subtypes as a result of depolarisation due to Na^+ influx through kainate receptors, we applied kainate/GYKI 53655 in the nominal absence of extracellular Na^+ by replacing NaCl with *N*-methyl-D-glucamine (NMDG) in Na^+-free buffer.

In cells pre-treated with concanavalin A, kainate (100 μM) (in the presence of 50 μM GYKI 53655) produced an $[Ca^{2+}]_i$ rise of >100 nM in 28 of 69 cells tested (average peak $[Ca^{2+}]_i$ increase 203 ± 19 nM). Removal of extracellular Na^+ completely abolished the response in these cells (Fig. 2A).

In order to confirm that removal of extracellular Na^+ was not exerting a non-specific inhibitory effect on kainate evoked responses, we applied kainate under Na^+-free conditions in the absence of the AMPA receptor selective antagonist GYKI 53655. We have shown previously that AMPA, together with cyclothiazide, activates Ca^{2+} permeable AMPA receptors in the absence of extracellular Na^+ (Savidge and Bristow, 1997a). Since kainate acts as a non-desensitising agonist at AMPA receptors (Fletcher and Lodge, 1996), kainate should also produce a $[Ca^{2+}]_i$ rise in the absence of GYKI 53655 under Na^+-free conditions that is mediated by activation of Ca^{2+} permeable AMPA receptors. Consistent with this hypothesis, kainate (100 μM) applied alone in the absence of extracellular Na^+ evoked a peak $[Ca^{2+}]_i$ increase of >100 nM in 26/49 cells (average peak $[Ca^{2+}]_i$ rise of 189 ± 30 nM), which was reversibly blocked by GYKI 53655 (Fig. 2B).

3.3. Kainate induced Ca^{2+} entry measured by Mn^{2+} quench of fura-2 fluorescence

We confirmed the results obtained with conventional ratiometric fura-2 imaging of $[Ca^{2+}]_i$ by using Mn^{2+} quench of fura-2 fluorescence, a more specific assay of Ca^{2+} entry (Simpson et al., 1995; Savidge and Bristow, 1997b). The data obtained with this technique were entirely consistent with the results from $[Ca^{2+}]_i$ measure-

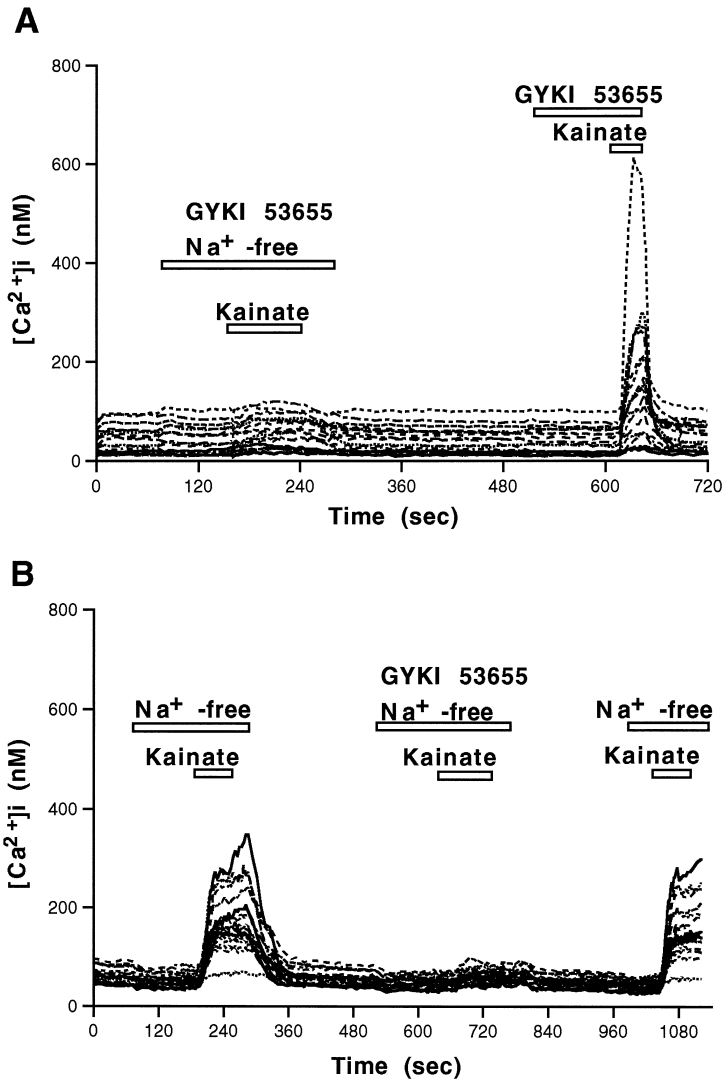

Fig. 2. Representative responses following concanavalin A treatment (Con A; 250 μg/ml, 10 min) in groups of cells in a single experiment. Experiments were carried out in at least 3 separate occasions on cells from at least 3 separate cultures. Cells were continuously perfused with Locke's buffer. Horizontal bars indicate time and duration of perfusion with drugs and/or Na^+-free buffer. (A) Kainate receptor mediated responses (100 μM kainate applied in the presence of 50 μM GYKI 53655) are not detectable in the absence of extracellular Na^+ (Na^+-free), but are restored in the presence of extracellular Na^+. (B) Kainate (100 μM) applied in the absence of extracellular Na^+ increases $[Ca^{2+}]_i$ presumably by activation of Ca^{2+} permeable AMPA receptors. Again, in the presence of GYKI 53655 (50 μM), kainate responses mediated by kainate receptors in Na^+-free conditions are not detected.

ments and is shown in Table 1. The results are expressed as the % decline in 360 nm fluorescence per second. Each experiment was carried out on a separate coverslip, but coverslips were from the same culture and experiments carried out on the same day. Basal quench rates were typically around 0.1%/s. As with kainate receptor-media-

Table 1
Mn^{2+} quench rate of fura-2 fluorescence stimulated by 100 μM kainate under the conditions indicated

Con A	GYKI 53655 (50 μM)	Extracellular Na^+	Quench rate (% decline/s)	Receptor mediating response
−	−	+	1.05 ± 0.05 ($n = 54$)	AMPA
+	+	+	0.44 ± 0.03 ($n = 49$)	Kainate
−	+	+	0.19 ± 0.01 ($n = 54$)	Kainate (desensitised)
−	−	−	0.63 ± 0.07 ($n = 49$)	Ca^{2+} permeable AMPA
+	+	−	0.14 ± 0.01 ($n = 45$)	Ca^{2+} permeable kainate

Quench rates for desensitised kainate receptors and Ca^{2+} permeable kainate receptors were not significantly increased above basal. All quench rates showed a significant ($P < 0.001$) difference from each other except kainate receptors vs. Ca^{2+} permeable AMPA receptors and desensitised kainate receptors vs. Ca^{2+} permeable kainate receptors. Each experimental condition was carried out on a separate coverslip of cells from the same culture on the same day, and repeated on 3 separate occasions on different cultures. Results are expressed as mean ± S.E.M., n indicates total cells tested.

ted $[Ca^{2+}]_i$ rises, kainate receptor-mediated Mn^{2+} quench (i.e., in the presence of GYKI 53655) was only detectable following incubation of cells with concanavalin A to inhibit kainate receptor desensitisation. Kainate applied in the presence of the AMPA selective antagonist GYKI 53655 to cells not pre-treated with concanavalin A produced no significant increase in quench rate. This was also true of kainate/GYKI 53655 applied to concanavalin A pre-treated cells in the absence of extracellular Na^+, indicating no permeation of Mn^{2+} directly through the kainate receptor channel. However, kainate applied in the absence of GYKI 53655 induced a significant quench in Na^+-free buffer, indicating the activation of Ca^{2+} permeable AMPA receptors.

3.4. Joro spider toxin blocks Ca^{2+} permeable AMPA receptors but not kainate receptors

Joro spider toxin exhibits remarkable subunit specificity in its blocking action on AMPA and kainate receptors. It blocks native and recombinant AMPA receptors which lack the edited GluR2 subunit (and are therefore Ca^{2+} permeable) and unedited homomeric recombinant GluR6 kainate receptors (Blaschke et al., 1993; Iino et al., 1996).

We examined the effect of Joro spider toxin on Ca^{2+} permeable AMPA receptors in order to test our hypothesis that these receptors lacked edited GluR2 and would therefore be blocked by Joro spider toxin. We also examined the effect of Joro spider toxin on kainate receptor-mediated

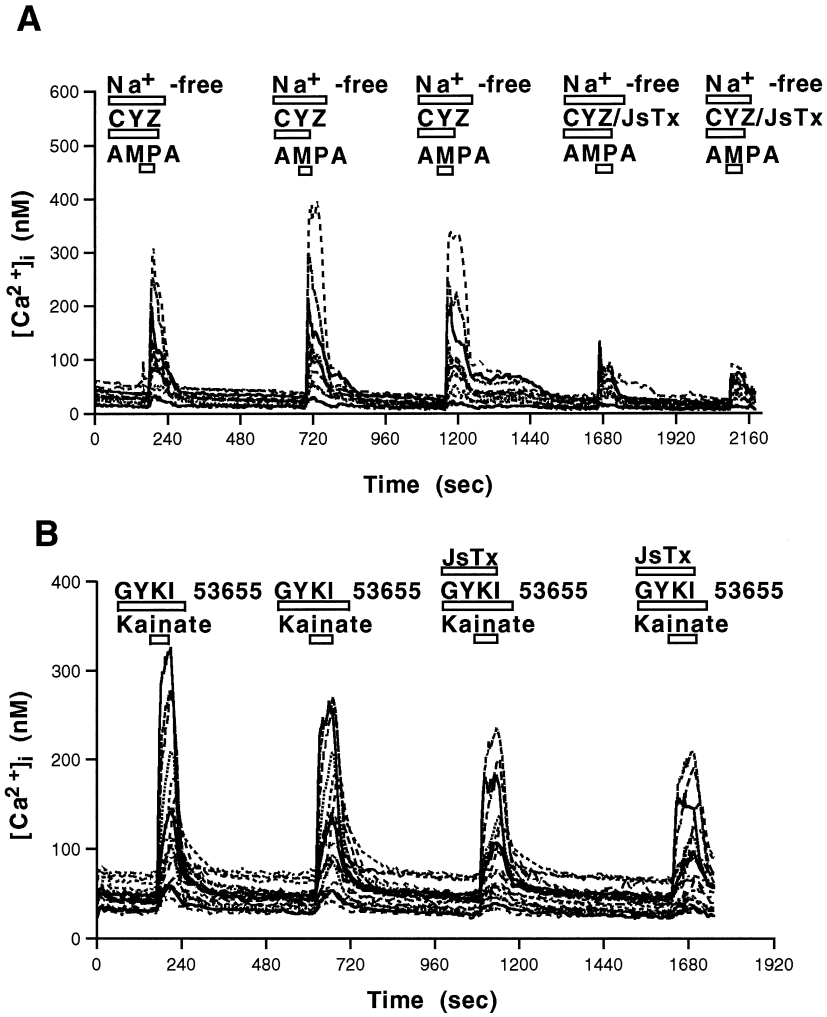

Fig. 3. (A) Representative responses in cells from a single experiment showing the effect of Joro spider toxin (JsTx, 500 nM) on AMPA (100 μM)/cyclothiazide (CYZ, 100 μM) induced $[Ca^{2+}]_i$ rises in the absence of extracellular Na^+ (Na^+-free). Similar results were obtained from a total of 4 separate experiments carried out on different cultures. (B) Representative responses in cells from a single experiment showing the effect of Joro spider toxin (JsTx, 500 nM) on kainate (100 μM) induced $[Ca^{2+}]_i$ rises in the presence of GYKI 53655 (50 μM) following treatment with concanavalin A (Con A; 250 μg/ml, 10 min). Similar results were obtained from a total of 4 separate experiments carried out on different cultures. Cells were continuously perfused with Locke's buffer. Horizontal bars indicate time and duration of perfusion with drugs and/or Na^+-free buffer.

responses to characterise its action at native kainate receptors that, as we have demonstrated in this study, do not appear to directly gate Ca^{2+}.

We isolated responses mediated by Ca^{2+} permeable AMPA receptors by applying (*S*)-AMPA (100 μM) together with cyclothiazide (100 μM) in the absence of extracellular Na^+. Out of 62 cells, 27 responded with an $[Ca^{2+}]_i$ rise of >100 nM. The average peak $[Ca^{2+}]_i$ rise to an initial AMPA/cyclothiazide application in these cells was 155 ± 11 nM. After a 5-min interval, the AMPA/cyclothiazide response was reproducible (peak $[Ca^{2+}]_i$ rise 158 ± 13 nM, Fig. 3A). Application of Joro spider toxin (500 nM) produced a marked inhibition of the AMPA/cyclothiazide induced $[Ca^{2+}]_i$ rises in these responding cells, reducing the peak $[Ca^{2+}]_i$ increase to 55 ± 4 nM (Fig. 3A).

Kainate receptor-mediated response evoked by kainate (100 μM) in the presence of GYKI 53655 (50 μM) in cells pre-treated with concanavalin A were also reproducible at 5-min intervals. In the 18/50 cells which responded with a $[Ca^{2+}]_i$ rise >100 nM, first and second responses to kainate/GYKI 53655 were 322 ± 69 nM and 294 ± 64 nM, respectively, which are not significantly different. Addition of Joro spider toxin (500 nM) caused a small but statistically significant ($P < 0.05$) reduction of kainate receptor-mediated $[Ca^{2+}]_i$ rises of around 15% to 251 ± 61 nM (Fig. 3B). The slight reduction following Joro spider toxin addition could be due to a small number of receptors sensitive to blockade. However, considering the variability of kainate receptor-mediated responses and the size of this reduction compared with the effect of Joro spider toxin on responses mediated by Ca^{2+} permeable AMPA receptors, this may simply reflect a slight rundown of responses rather than a specific action of Joro spider toxin.

4. Discussion

The present study has demonstrated that the pathway responsible for kainate receptor-mediated $[Ca^{2+}]_i$ increases in cerebellar granule cells appears to be solely through activation of voltage-gated Ca^{2+} channels. This suggests that native kainate receptors in these cells do not directly gate Ca^{2+}. We have also shown that responses mediated by Ca^{2+} permeable AMPA receptors in these cells show a large degree of blockade by Joro spider toxin, indicating that most of these receptors lack the GluR2 subunit. In contrast, the native kainate receptors appear largely insensitive to Joro spider toxin blockade.

We have previously shown that many cerebellar granule cells show an appreciable $[Ca^{2+}]_i$ increase in response to AMPA/cyclothiazide in Na^+-free buffer, indicating $[Ca^{2+}]_i$ elevations mediated by Ca^{2+} permeable AMPA receptors (Savidge and Bristow, 1997a). Here, we have also shown that kainate stimulates $[Ca^{2+}]_i$ increases in Na^+-free buffer demonstrating that kainate also activates Ca^{2+} permeable AMPA receptors on these cells. To investigate these observations further, we have taken advantage of the highly subunit specific blocking action of Joro spider toxin on AMPA receptors to provide information on the possible subunit conformations of these native Ca^{2+} permeable AMPA receptors. The specificity of Joro spider toxin appears to be determined by a single amino acid at the Q/R site, such that only receptors made up of subunits with glutamine at this site are susceptible to block (Blaschke et al., 1993). This site also controls the Ca^{2+} permeability of AMPA receptors, which is markedly reduced by the presence of edited GluR2 which contains arginine at this site (Fletcher and Lodge, 1996). The fact that responses mediated by Ca^{2+} permeable AMPA receptors could be extensively inhibited by Joro spider toxin suggests that most of these receptors lack edited GluR2 subunits. This is consistent with a study by Iino et al. (1996) showing selective blockade of Ca^{2+} permeable AMPA receptors in hippocampal neurons by Joro spider toxin. It is also consistent with the fact that AMPA receptors lacking GluR2 are Ca^{2+} permeable (see review by Fletcher and Lodge, 1996). However, sensitivity to Joro spider toxin blockade does not necessarily indicate Ca^{2+} permeability of AMPA receptors since Meucci et al. (1996) have detected AMPA receptors in cortical glial cells that are insensitive to Joro spider toxin block and therefore apparently contain edited GluR2, but still appear to have appreciable Ca^{2+} permeability.

In contrast to AMPA receptor-mediated responses, kainate receptor-mediated $[Ca^{2+}]_i$ responses (those produced by kainate in the presence of GYKI 53655 following concanavalin A treatment) were totally dependent on the presence of extracellular Na^+. This suggests that kainate receptor-mediated $[Ca^{2+}]_i$ rises occur entirely as a result of voltage-gated Ca^{2+} channel activation and therefore, that native kainate receptors on rat cerebellar granule cells do not flux significant amounts of Ca^{2+}. This conclusion is supported by the observation that blockade of L-type voltage-gated Ca^{2+} channels produces a significant reduction in peak $[Ca^{2+}]_i$ increases following kainate receptor activation. The fact that a substantial residual response to kainate receptor activation remains following L-type voltage-gated Ca^{2+} channel blockade is not surprising given the diversity of voltage-gated Ca^{2+} channels in these cells (Randall and Tsien, 1995).

Previous studies of the Ca^{2+} permeability of kainate receptors have been limited to recombinant receptors rather than native receptors. Unedited homomeric GluR6(Q) receptors appear to have significant Ca^{2+} permeability, while edited GluR6(R) homomeric receptors are impermeable to Ca^{2+} (Burnashev et al., 1996). Since native kainate receptors may be heteromeric constructs containing various combinations of different subunits, it is difficult to draw conclusions about the Ca^{2+} permeability of native receptors simply from studies on recombinant homomeric recep-

tors. The present study provides important information on the Ca^{2+} permeability of native kainate receptors, but we are unable to relate this to the precise subunit construction of native kainate receptors in cerebellar granule cells as these are not fully characterised. However, the results presented here indicating the native kainate receptors in these cells do not appear to flux significant Ca^{2+} suggests there are unlikely to be significant numbers of unedited homomeric GluR6(Q) receptors. The fact that kainate receptor-mediated responses in these cells appear to be largely unaffected by Joro spider toxin is consistent with this conclusion. We have recently examined the effect of Joro spider toxin on kainate receptor-mediated currents in cerebellar granule cells using whole-cell voltage clamp electrophysiology. These studies have confirmed that kainate receptors on these cells are resistant to blockade by Joro spider toxin (J.R. Savidge, unpublished observations). In addition, our previous results using the subtype specific kainate receptor antagonist LY293558 have indicated the kainate receptor-mediated $[Ca^{2+}]_i$ responses are likely to be mediated predominantly via the GluR5 subtype of kainate receptor, rather than via receptors containing GluR6 subunits (Savidge et al., 1997). Future investigations of kainate receptor subunit proteins present in these cells, together with further pharmacological characterisation of kainate receptor-mediated responses will provide more definitive information on the subunit make-up of the kainate receptors they express.

In this study we have characterised the $[Ca^{2+}]_i$ responses mediated by non-NMDA receptors and have related these findings to the subtypes of native receptors that are likely to be present. The Ca^{2+} permeable AMPA receptors expressed on these cells appear to lack the edited GluR2 subunit whereas native kainate receptors on these cells do not appear to directly gate Ca^{2+} but raise $[Ca^{2+}]_i$ by activation of voltage-gated Ca^{2+} channels. The differences in Ca^{2+} permeability between these receptors is also reflected in their differential sensitivity to blockade by Joro spider toxin.

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