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ACCELERATED COMMUNICATION

Differential Modulation by Cyclothiazide and Concanavalin A of Desensitization at Native α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid- and Kainate-Preferring Glutamate Receptors

LINDA A. WONG and MARK L. MAYER

Laboratory of Cellular and Molecular Neurophysiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

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SUMMARY

Concanavalin A, cyclothiazide, and aniracetam, ligands that modulate desensitization at glutamate receptors, were tested for their actions on responses at kainate-preferring receptors in dorsal root ganglion (DRG) neurons and at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-preferring receptors in hippocampal neurons. In DRG neurons concanavalin A blocked desensitization produced by either kainate or 5-chlorowillardiine and strongly potentiated the peak amplitude of responses to both agonists. However, in hippocampal neurons concanavalin A produced only weak potentiation of responses to kainate and 5-chlorowillardiine, and after treatment with lectin responses to 5-chlorowillardiine remained strongly desensitizing. In contrast, cyclothiazide completely blocked desensitization produced by 5-chlorowillardiine in hippocampal neurons and strongly potentiated responses to kainate; the action of aniracetam was similar but much weaker. In DRG neurons cyclothiazide and aniracetam had no effect on desensitization and instead produced weak inhibition of responses to kainate. The different sensitivities of native AMPA- and kainate-preferring glutamate receptors to cyclothiazide and concanavalin A should prove useful for the differentiation of glutamate receptor subtypes in other areas of the central nervous system.

The isolation of multiple gene families for glutamate receptors and analysis of their functional responses after expression *in vitro* suggest the existence in central nervous system tissue of multiple subtypes of glutamate receptor (1). AMPA-preferring receptors are assembled from the subunits GluR-1 through GluR-4 (2, 3), whereas kainate-preferring receptors are assembled from the subunits GluR-5 through GluR-7 (4–6), most likely in combination with KA-1 or KA-2 (7, 8). The subunit composition of native glutamate receptor subtypes has not yet been established, but two preparations are available that appear to express predominantly only a single receptor subtype. These are hippocampal neurons, which generate functional responses comparable to those observed upon transfection of cell lines with cDNA for AMPA-preferring receptors (9–12), and DRG neurons, which generate functional responses comparable to those observed upon transfection of cell lines with cDNA for kainate-preferring receptors (8, 13, 14).

Agonist responses at both AMPA-preferring and kainate-preferring glutamate receptors show strong desensitization (10, 13), but it is not known whether modulators of desensitization act equally on both receptor families. It is well established that the lectin concanavalin A irreversibly inhibits desensitization at both vertebrate and invertebrate glutamate receptors (10, 13, 15–17), such that treatment with lectins has been used as an experimental tool to facilitate the study of agonist responses at glutamate receptor subtypes with strong desensitization (5, 18, 19). Recent studies have shown that the drugs aniracetam, diazoxide, and cyclothiazide reversibly inhibit desensitization of responses to glutamate in hippocampal neurons (20–26). To date there have been no systematic studies performed to compare the effects of these drugs and of lectins that inhibit desensitization on responses recorded from AMPA- versus kainate-preferring glutamate receptors. The results of our study reveal that cyclothiazide and aniracetam act with complete

ABBREVIATIONS: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DRG, dorsal root ganglion; NMDA, *N*-methyl-D-aspartate; DMSO, dimethylsulfoxide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; GluR-1 to GluR-7, glutamate receptor subunits 1 to 7; KA-1 and KA-2, kainate receptor subunits 1 and 2.

selectivity at native AMPA-preferring glutamate receptors expressed in hippocampal neurons and have no effect on desensitization of native kainate-preferring glutamate receptors expressed in DRG neurons. In contrast, concanavalin A strongly inhibits desensitization in DRG neurons but has much weaker effects in hippocampal neurons.

Materials and Methods

Cell preparation. Rat DRG neurons were dissociated based on a protocol described previously (27). In brief, cervical and thoracic dorsal root ganglia were dissected from P8–P13 Sprague-Dawley rat pups and incubated at 35° (15–20 min) in an enzyme solution consisting of 82 mM Na_2SO_4, 30 mM K_2SO_4, 5 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, 0.001% phenol red, and 0.6 mg/ml Sigma type XXIII protease. After enzyme digestion, the tissue was rinsed and triturated in minimum essential medium (GIBCO no. 320-1090) containing 15 mM glucose, 10 mM HEPES, 1 mg/ml trypsin inhibitor (Sigma), and 1 mg/ml bovine serum albumin fraction V (Sigma), titrated to pH 7.4 with NaOH. The freshly dissociated cells were then left to settle in this solution at room temperature for >12 hr. Four hours before recording, the cells were plated onto poly-L-lysine-coated dishes containing minimum essential medium supplemented with 0.7% methylcellulose, 2 mM L-glutamine (GIBCO), 10 mM D-glucose (Sigma), 10% fetal bovine serum (GIBCO), and 0.03% gentamicin (Sigma) and were maintained at 35° in a humidified chamber (5% CO_2 95% air). Rat hippocampal cultures were prepared from E22/23 Sprague-Dawley rat fetuses according to previously published protocols (28). The hippocampi were dissected, incubated in a solution containing 100 units of papain (Worthington), and triturated. Hippocampal neurons were then plated on a confluent glial cell feeder layer.

Recording. Experiments were conducted at room temperature on small diameter (20–30-μm) DRG neurons and on hippocampal neurons grown in primary culture for 3–10 days. Voltage-clamp was performed using the whole-cell patch-clamp technique (29) and Axon Instruments amplifiers; an Axopatch-1C was used for experiments on DRG neurons and an Axoclamp-2A operating in discontinuous voltage-clamp mode (switching at approximately 10 kHz) was required for accurate measurement of the much larger amplitude currents in hippocampal neurons. Fire-polished glass pipettes had tip resistances of 4–7 MΩ when filled with 125 mM $CsCH_3SO_3$, 15 mM CsCl, 5 mM Cs-BAPTA, 10 mM HEPES, 3 mM $MgCl_2$, 0.5 mM $CaCl_2$, 2 mM Mg-ATP (titrated to pH 7.2 with CsOH). The holding potential was set at −60 mV for both preparations; series resistance for experiments on DRG neurons was usually between 8 and 15 MΩ and was not compensated. Agonist-activated currents were filtered at 1.0 kHz (eight-pole Bessel filter) and digitized at intervals of 0.5–1 msec using an Instrutech ITC-16 interface and a Macintosh IIfx computer controlled by software written by J. W. Nash (Synergistic Research Systems, Silver Spring, MD); data analysis was performed using the same system. The external solution consisted of (in mM): 160 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose, with 0.001% phenol red (titrated to pH 7.3 with NaOH); tetrodotoxin (500 nM) was used to inhibit sodium channel currents and, for experiments on hippocampal neurons, 5 μM bicuculline methochloride was added to block γ-aminobutyric acid receptor-mediated inhibitory synaptic currents.

Drug application. Agonists were applied through a linear array of nine glass barrels (outer diameter, 400 μm; Garner Glass), positioned 100–150 μm from the cells. Rapid solution exchange was attained as described previously, using computer-controlled solenoid valves to switch the flow between adjacent barrels immediately upon completion of movement to the desired position by a stepper motor (10). Kainate was obtained from Sigma; 5-chlorowillardiine, cyclothiazide, and aniracetam were gifts from Professor J. C. Watkins (Bristol, UK), Eli Lilly (Indianapolis, IN), and Hoffman La Roche (Nutley, NJ), respectively. All other chemicals were obtained from Sigma. Stock solutions of agonists were prepared by dissolving the drugs in extracellular recording solution and adding NaOH as needed to maintain neutral pH and were stored at −20°. Cyclothiazide and aniracetam were dissolved in DMSO at 20 and 500 mM, respectively, and were diluted with extracellular recording solution before use; DMSO was added to control solutions at the same concentration as used for application of aniracetam or cyclothiazide, to control for a weak inhibitory action of DMSO on glutamate receptor responses.

Unless stated otherwise, data values are presented as mean ± standard deviation. Statistical analysis was performed using a two-tailed paired t test (InStat version 1.01; GraphPAD). Measurements of the degree of desensitization were quantified using the ratio I_{ss}/I_{peak}, where I_{ss} represents the agonist-activated current at steady state and I_{peak} the peak amplitude measured before the onset of desensitization. For DRG neurons desensitization develops with both rapid and slow components (13), with the result that true steady state measurements are not possible until 15–30 sec after the start of the application of agonist[1]; because agonist applications for shorter periods of time facilitated the completion of experiments with the rapid perfusion techniques used in the present study, we routinely applied agonists for 2 sec, at which time desensitization reached 60 and 90% of the equilibrium value achieved with longer applications of kainate and 5-chlorowillardiine, respectively. The time course of onset of desensitization was measured using a Simplex algorithm to fit exponential functions to agonist responses; rise times were determined automatically from measurements of the sample number between data points representing 10 and 90% of the peak response.

Results

DRG and hippocampal neurons show differential sensitivity to concanavalin A. The experiments described were performed using two agonists to activate non-NMDA receptors in DRG and hippocampal neurons. 5-Chlorowillardiine was chosen as a potent agonist that produces strong desensitization in both cell types (30, 31); although similar results were obtained with AMPA, use of 5-chlorowillardiine was economically advantageous due to the higher potency in DRG neurons of 5-chlorowillardiine (EC_{50}, 2 μM), compared with AMPA (EC_{50}, 260 μM).[1] Kainate was chosen as an agonist that, under whole-cell recording conditions, produces strongly desensitizing responses in DRG but not hippocampal neurons (9, 10, 13, 25, 32); EC_{50} values for hippocampal and DRG neurons were 142 and 12 μM, respectively (25, 31). Agonists were applied at concentrations known to produce near-maximal responses (equal to or greater than 100 times the EC_{50} for agonists producing strong desensitization and 1 mM or higher for responses to kainate in hippocampal neurons). Preliminary experiments revealed that pretreatment of DRG neurons with concanavalin A (300 μg/ml for 1 min) blocked desensitization evoked by both 1.2 mM kainate and 300 μM 5-chlorowillardiine. When responses to these agonists were recorded from individual DRG neurons before and after treatment with lectin (Fig. 1A), not only was desensitization markedly reduced, from 57 ± 12% to 4 ± 2% for kainate (eight cells) and from 88 ± 6% to 18 ± 10% for 5-chlorowillardiine (four cells), but in addition the amplitude of the peak response to these agonists was enhanced, 1.96 ± 0.56- and 2.36 ± 0.47-fold, respectively; both effects were maintained for at least 20 min after the application of concanavalin A. The increase in amplitude of agonist-activated responses is likely to arise, at least in part, as a result of block of desensitization occurring during the rising phase of the response to kainate or 5-chlorowillardiine. The onset of

[1] L. Wong and M. L. Mayer, unpublished observations.

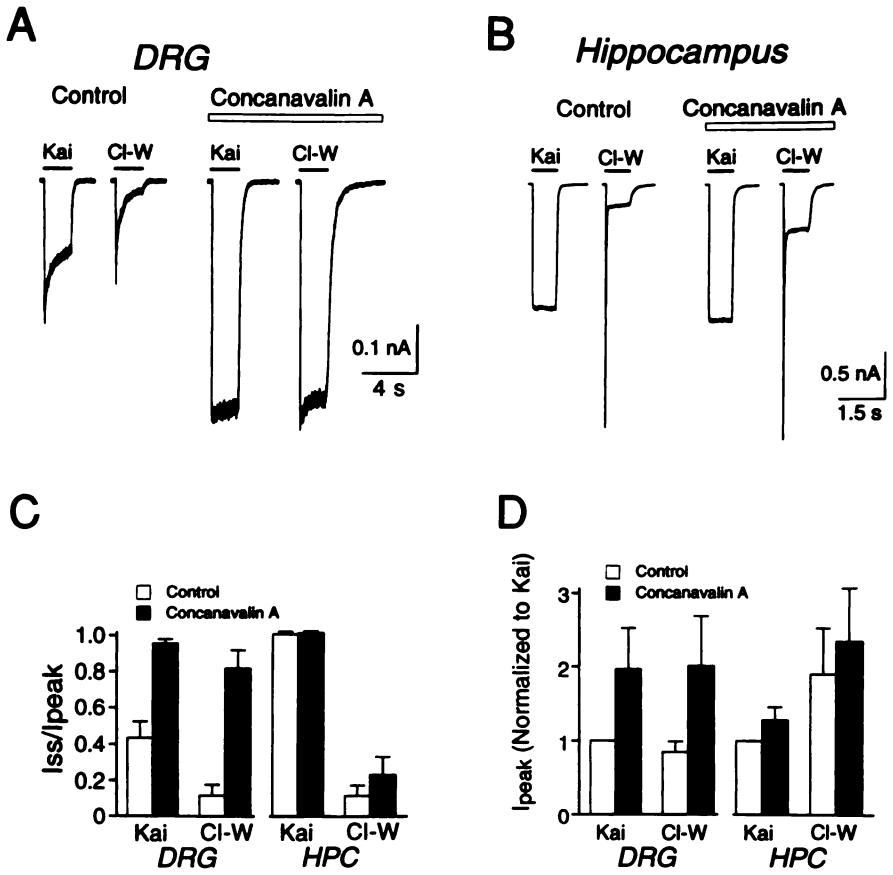

Fig. 1. Glutamate receptor desensitization in DRG but not hippocampal neurons is blocked by concanavalin A. A, Responses evoked by 1.2 mM kainate (*Kai*) and 300 μM 5-chlorowillardiine (*Cl-W*) in a single DRG neuron before and after treatment for 1 min with concanavalin A (300 μg/ml). B, Responses to 5 mM kainate and 875 μM 5-chlorowillardiine in a rat hippocampal neuron before and after a 7-min treatment with concanavalin A (300 μg/ml); note that concanavalin A has only a small effect on desensitization evoked by 5-chlorowillardiine. C, Mean values for the steady state to peak ratio (I_{ss}/I_{peak}) for responses to 5-chlorowillardiine ($n = 4$) and kainate ($n = 8$), measured at 2 sec for DRG neurons and at 1 sec for hippocampal neurons (*HPC*) ($n = 5$). *Error bars*, standard deviation. D, Peak amplitude of agonist-evoked responses normalized to the control response to kainate, before and after treatment with concanavalin A; note that potentiation was much greater for DRG than for hippocampal neurons.

desensitization evoked by either agonist and recorded before treatment with concanavalin A was best fit by the sum of two exponentials and occurred with a component sufficiently rapid to truncate the rising phase of agonist responses, because the extrapolated peak current was on average 22.5 ± 6.6% and 17.4 ± 2.5% (mean ± standard error) larger than the experimentally measured peak current for responses to 5-chlorowillardiine and kainate, respectively. The desensitization time constants and relative amplitudes were τ_{fast} = 55 ± 7.2 msec (47 ± 4%) and τ_{slow} = 508 ± 33 msec (53 ± 6%) for 5-chlorowillardiine and τ_{fast} = 61 ± 5 msec (31 ± 4%) and τ_{slow} = 695 ± 47 msec (69 ± 4%) for kainate. The 10–90% rise time for responses to 1.2 mM kainate increased from a control value of 7.4 ± 2.1 msec to 15.4 ± 2.3 msec (mean ± standard error) after treatment with concanavalin A; for 300 μM 5-chlorowillardiine, the increase was from 4.0 ± 0.34 msec to 16.1 ± 2.6 msec.

In contrast to its potent and irreversible effects on DRG neurons, concanavalin A (300 μg/ml for 7 min) had only weak effects on the desensitization and amplitude of responses of hippocampal neurons to kainate and 5-chlorowillardiine (Fig. 1B), even though the lectin was applied for much longer periods than for experiments on DRG neurons. In five hippocampal neurons, desensitization evoked by 5-chlorowillardiine decreased slightly, from 88 ± 5.7% to 77 ± 9.9% after treatment with lectin (significant at $p < 0.005$, paired t test), whereas there was no rapid desensitization for responses to 5 mM kainate either before or after treatment with concanavalin A (Fig. 1, B and C). The peak amplitude of responses to maximally effective doses of 5-chlorowillardiine and kainate showed a much smaller increase than observed in DRG neurons after treatment with concanavalin A; this increase was significant for 5-chlorowillardiine (1.31 ± 0.10 times control) but not kainate (1.22 ± 0.22 times control) ($p < 0.05$, paired t test), reflecting the observation that in hippocampal neurons the degree of potentiation of responses to kainate was more variable than that to 5-chlorowillardiine. Similarly to results obtained in DRG neurons, the much weaker effects of concanavalin A on hippocampal neurons were irreversible during the 10-min period over which electrophysiological experiments were conducted.

Cyclothiazide blocks desensitization produced by 5-chlorowillardiine at AMPA but not kainate receptors. Cyclothiazide and aniracetam, drugs that modulate responses to glutamate at non-NMDA receptors (20–26), were tested for their effects on the strongly desensitizing responses to 5-chlorowillardiine recorded in both DRG and hippocampal neurons. Fig. 2 shows that 100 μM cyclothiazide virtually abolished desensitization evoked by 5-chlorowillardiine in hippocampal neurons (control, 86.8 ± 6.2% desensitization; with cyclothiazide, 7.3 ± 4.4%; $n = 6$), but there was no reduction in the degree of desensitization for responses to 5-chlorowillardiine recorded from DRG neurons (control, 87.3 ± 8.0% desensitization; with cyclothiazide, 87.8 ± 7.1%; $n = 5$). In contrast to the strong potentiation of the peak amplitude of responses to 5-chlorowillardiine produced by cyclothiazide in hippocampal neurons (cyclothiazide/control, 2.2 ± 0.3), potentiation was not observed in DRG neurons (cyclothiazide/control, 1.1 ± 0.1), providing additional evidence that cyclothiazide is inactive at kainate receptors expressed in sensory neurons. The increase in peak amplitude of responses to 5-chlorowillardiine recorded from hippocampal neurons in the presence of cyclothiazide is likely to occur in part as a result of a block of desensitization

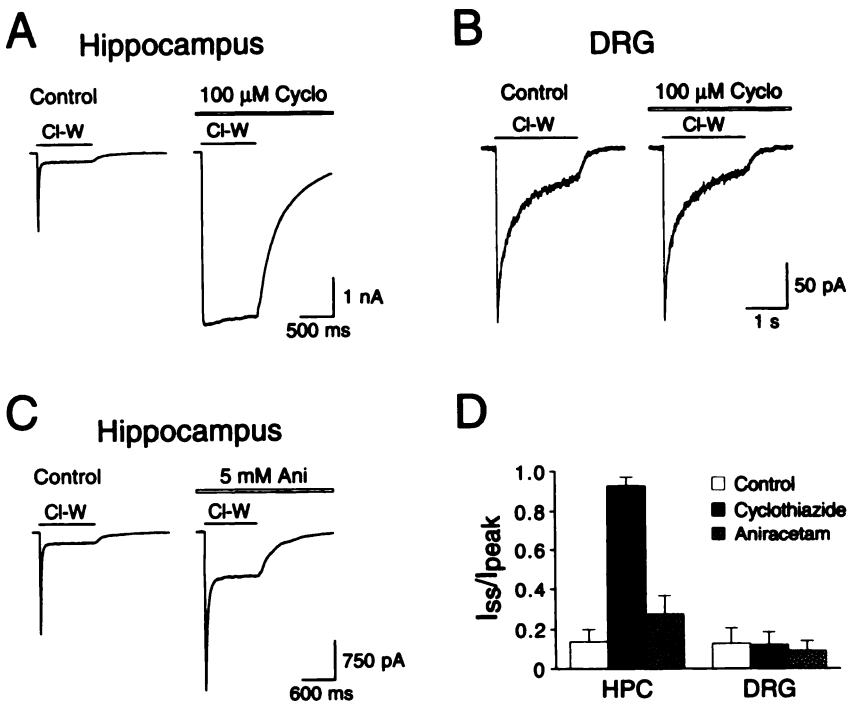

Fig. 2. Rapid desensitization to 5-chlorowillardiine (*Cl-W*) is inhibited by cyclothiazide (*Cyclo*) and aniracetam (*Ani*) in hippocampal but not DRG neurons. A, Responses of a hippocampal neuron to 875 μM 5-chlorowillardiine before and during treatment with 100 μM cyclothiazide. B, Responses of a DRG neuron to 300 μM 5-chlorowillardiine before and during treatment with 100 μM cyclothiazide. C, Responses of a hippocampal neuron (same cell as A) to 5-chlorowillardiine before and during treatment with 5 mM aniracetam. D, Mean values for the steady state to peak ratio (I_{ss}/I_{peak}) for responses to 5-chlorowillardiine in hippocampal neurons (*HPC*) ($n = 6$) and DRG neurons ($n = 5$), before and during application of either 100 μM cyclothiazide or 5 mM aniracetam. *Error bars*, standard deviation. Desensitization to 5-chlorowillardiine is greatly reduced by cyclothiazide in hippocampal but not DRG neurons, whereas aniracetam is weakly effective in hippocampal neurons and inactive in DRG neurons.

during the rising phase of the response to agonist; when a piezoelectric device giving solution exchange within 300 μsec was used to apply L-glutamate to outside-out patches taken from hippocampal neurons (25), cyclothiazide did not produce increases in peak amplitude comparable to those observed with whole-cell recording, for which solution exchange can take up to 10–20 msec.

Aniracetam, another compound that has been reported to enhance excitatory synaptic transmission and to modulate responses to glutamate in hippocampal neurons (20–23), was also tested for an effect on desensitization in hippocampal and DRG neurons (Fig. 2, C and D). Aniracetam, applied at 5 mM, the limit of solubility in the solutions used for our experiments, was much less effective than cyclothiazide in blocking desensitization of responses to 5-chlorowillardiine in hippocampal neurons (control, 85.7 ± 7.0% desensitization; with aniracetam, 72.7 ± 9.1%; significant at $p < 0.005$, paired t test) and, like cyclothiazide, aniracetam failed to block desensitization of responses to 5-chlorowillardiine in DRG neurons (control, 87.3 ± 8.0% desensitization; with aniracetam; 91.0 ± 5.0%).

Cyclothiazide and aniracetam have opposite effects on responses to kainate in DRG and hippocampal neurons. At 100 μM, cyclothiazide produced a small but reproducible inhibition of the peak amplitude of responses to kainate in DRG neurons (Figs. 3A and 4C), to 94 ± 6% of control (significant at $p < 0.05$, paired t test, $n = 5$). Similarly to observations made for 5-chlorowillardiine, cyclothiazide failed to reduce desensitization of responses to kainate in DRG neurons (control, 68.5 ± 6.5% desensitization; with cyclothiazide, 66.5 ± 4.9%). Although in hippocampal neurons 100 μM cyclothiazide is a maximally effective dose for block of desensitization (25), for experiments on DRG neurons increasing the concentration of cyclothiazide to 300 μM failed to alter the degree of desensitization evoked by kainate, and there was no potentiation of the peak amplitude of agonist responses; the effect of higher concentrations could not be tested because at 1 mM cyclothia-

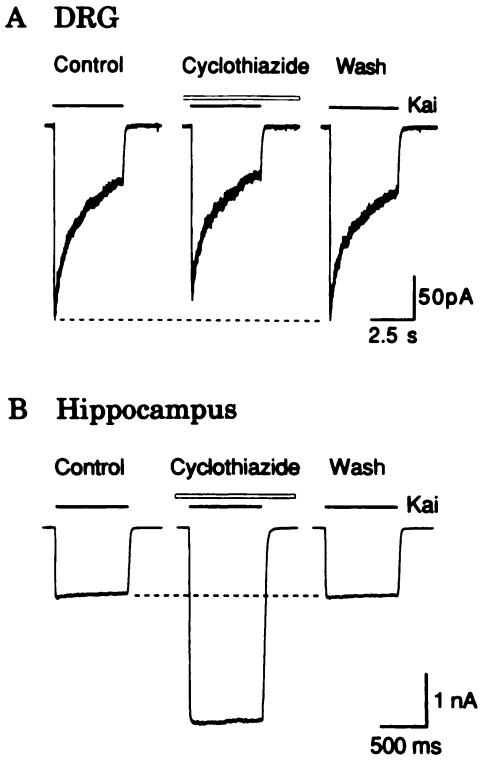

Fig. 3. Selective potentiation by cyclothiazide of responses to kainate (*Kai*) in hippocampal but not DRG neurons. A, Responses of a DRG neuron to 1.2 mM kainate before, during, and after application of 100 μM cyclothiazide; DMSO was present at 0.5% in all solutions and thus did not contribute to the block produced by cyclothiazide. B, Responses to 1.2 mM kainate recorded from a hippocampal neuron before, during, and after application of 100 μM cyclothiazide; in contrast to results obtained in DRG neurons, cyclothiazide strongly potentiated responses to kainate.

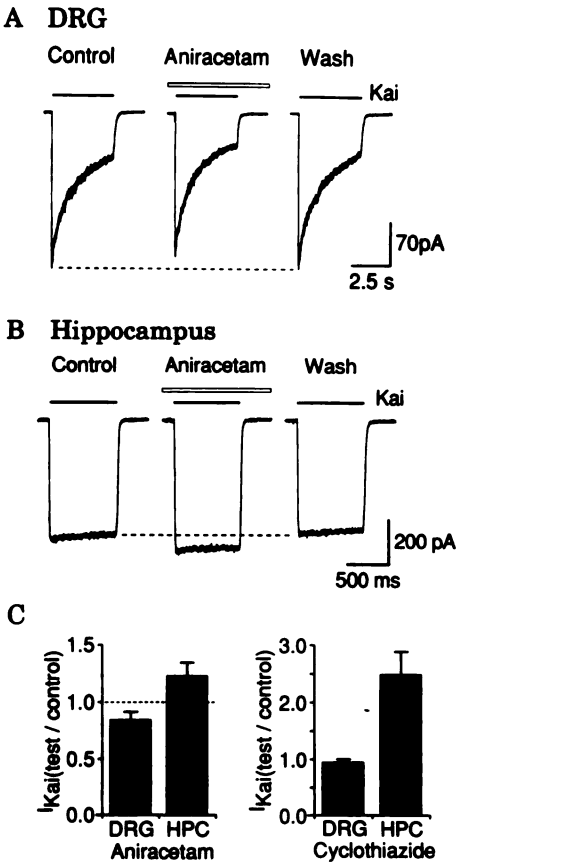

Fig. 4. Aniracetam potentiates kainate-evoked responses in hippocampal but not DRG neurons. A, Responses of a DRG neuron to 1.2 mM kainate (*Kai*) before, during, and after application of 5 mM aniracetam; DMSO was present at 1% in all solutions and thus did not contribute to the block produced by aniracetam. B, Similar experiments on a hippocampal neuron, revealing weak potentiation by 5 mM aniracetam of responses to 1.2 mM kainate. C, Mean amplitude of responses to kainate in the presence of either aniracetam or cyclothiazide for DRG and hippocampal (*HPC*) neurons, normalized to control (*error bars*, standard deviation; seven cells for each preparation and drug); on the scale used, inhibition by cyclothiazide of responses to kainate in DRG neurons, although statistically significant, is not clearly visible.

zide did not remain in solution under the conditions used for our experiments. Preliminary studies showed that the weak depressant action of cyclothiazide on responses to kainate in DRG neurons was voltage independent and was not associated with any change in the kinetics of desensitization or deactivation of responses to kainate. In contrast to the weak effects of cyclothiazide on responses to kainate in DRG neurons, when 100 μM cyclothiazide was applied to hippocampal neurons (Fig. 3B) the amplitude of the apparently nondesensitizing response to 1.2 mM kainate was reversibly increased, by 249 ± 40% (n = 6). Because, when applied to patches from hippocampal neurons using a perfusion system capable of submillisecond solution exchange, kainate produces very rapidly desensitizing responses (25), the increase in amplitude produced by cyclothiazide is likely to be due in part to block of desensitization.

Similarly to the action of cyclothiazide, responses to kainate in DRG neurons were reversibly inhibited by 5 mM aniracetam (Fig. 4), to 84 ± 8% of control ($p < 0.001$, paired t test, $n = 7$). This inhibitory action of aniracetam on responses to kainate was not due to the presence of 1% DMSO used to dissolve aniracetam, because DMSO was added to all solutions used for this experiment. Like cyclothiazide, aniracetam did not reduce desensitization evoked by kainate (Fig. 4A). In contrast to its inhibitory action on DRG neurons, aniracetam (5 mM) produced a small, reversible, but significant degree of potentiation of kainate-activated responses in hippocampal neurons (Fig. 4B), to 123 ± 11% of control ($p < 0.001$, paired t test, $n = 7$). This effect is, however, much smaller than that produced by cyclothiazide (Fig. 4C) but consistent with results describing potentiation by aniracetam of responses to kainate at AMPA-preferring glutamate receptors expressed in oocytes (33).

Discussion

The classification of glutamate receptor subunits into AMPA-, kainate-, and NMDA-preferring families by sequence homology has not yet led to an understanding of how many subtypes of native glutamate receptor exist in central nervous system tissue. The preparations used in our study, hippocampal and DRG neurons, show striking differences in their functional properties, which are likely to reflect receptor assembly in hippocampal neurons primarily from subunits of the AMPA-preferring family of glutamate receptors, whereas, for DRG neurons assembly would be expected from subunits of the kainate-preferring families. This pattern of assembly is also predicted by regional differences in the expression of mRNAs for individual glutamate receptor subunits in sensory ganglia and the hippocampus; high levels of the kainate-preferring subunits GluR-5 (4) and KA-2 (8) are expressed in DRG neurons, whereas AMPA-preferring subunit mRNAs show high levels of expression in the hippocampus (2, 3). The distinct functional properties of DRG and hippocampal neurons described here strongly suggest that these preparations express predominantly a single family of receptors and that there is unlikely to be significant expression of functionally active AMPA-preferring receptors in the soma of DRG neurons or of kainate-preferring receptors in the cell body and proximal dendrites of hippocampal neurons in the cultures used for our present experiments. The evidence for this includes profound differences in EC_{50} values for agonist responses recorded in DRG versus hippocampal neurons, differences in the kinetics of onset of and recovery from desensitization for agonist responses in the two preparations, and the different sensitivity to modulators of desensitization described here. Despite this, *in situ* hybridization reveals that mRNAs for receptor subunits from other glutamate receptor gene families are expressed in both tissues (4, 8), and it is unclear at present whether there are additional, cell-specific differences in the assembly and expression of glutamate receptor proteins in DRG and hippocampal neurons, other than assembly from either AMPA- or kainate-preferring receptor subunits, that contribute to their unique physiology. Further analysis of glutamate receptor composition in specific cell types will be greatly facilitated by the isolation of plasma membrane preparations from neurons in the appropriate tissues, the immunoprecipitation of glutamate receptor complexes, and the subsequent identification of their subunit composition with selective antibodies.

The results presented here extend the well established observation that lectins inhibit desensitization at both vertebrate and invertebrate non-NMDA receptors (10, 15–17) but suggest receptor subunit-specific differences in the efficacy of this effect. Previous work indicated that desensitization at both AMPA-preferring and kainate-preferring glutamate receptors

is modulated by lectins (5, 10, 13, 16, 17, 34), but the results of our study reveal that desensitization at kainate-preferring glutamate receptors in DRG neurons is considerably more sensitive to inhibition by lectins than is desensitization at AMPA-preferring glutamate receptors in hippocampal neurons. Although no direct comparison between glutamate receptor subtypes has been made previously, our experiments extend and are in good agreement with the results of two previous studies in which it was observed that, even after treatment of hippocampal neurons with concanavalin A for 30 min, responses to quisqualate continued to show substantial desensitization (10), whereas in DRG neurons treatment with concanavalin A for only 5 min eliminated desensitization in response to quisqualate (13). Amino acid sequences predicted from analysis of cDNAs for glutamate receptor subunits reveal potential *N*-linked glycosylation sites in the predicted amino-terminal extracellular domain for both the AMPA-preferring family GluR-1 through GluR-4 (four to six sites/subunit) and the kainate-preferring families GluR-5 through GluR-7 (six sites/subunit) and KA-1 plus KA-2 (eight to 10 sites/subunit); it is possible that lectins interact directly with glutamate receptors at these sites. Shifts in the apparent molecular mass of GluR-1 through GluR-4 and GluR-6 after treatment with *N*-glycosidase but not *O*-glycosidase (35–37) confirm that glutamate receptor subunits are glycosylated, primarily at asparagine residues. The 13-kDa shift in molecular mass observed for GluR-6 (37), compared with shifts of only 5–6 kDa for GluR-1 through GluR-4 (35, 36), suggests that at least some kainate-preferring glutamate receptor subunits are more extensively glycosylated than their AMPA-preferring counterparts; this, together with the greater abundance of potential *N*-linked glycosylation sites in the subunits KA-1 and KA-2, may contribute to the higher sensitivity to lectins of desensitization in DRG versus hippocampal neurons.

The recent discovery that the drugs aniracetam (20–23), diazoxide (24), and cyclothiazide (25, 26) modulate desensitization of responses to glutamate in hippocampal neurons provides a powerful new tool for the analysis of glutamate receptor physiology. Our finding that kainate-preferring glutamate receptors in DRG neurons are completely insensitive to the potentiating actions of aniracetam and cyclothiazide, whereas responses at AMPA-preferring receptors in hippocampal neurons show strong modulation by these drugs, suggests that cyclothiazide may be a useful tool for the differentiation of non-NMDA receptor subtypes. To test this we are currently performing experiments with expression of glutamate receptor subunits from different gene families; preliminary results (38) suggest that the action of cyclothiazide is completely selective for AMPA- versus kainate-preferring glutamate receptors expressed in oocytes. The weak block of responses to kainate produced by cyclothiazide and aniracetam in DRG neurons also occurs for responses at AMPA-preferring glutamate receptors and is independent of the reduction of desensitization (25). In both DRG and hippocampal neurons block by cyclothiazide is not voltage dependent, and the mechanism is currently unknown. However, this effect of cyclothiazide is weak, compared with the strong potentiation of responses produced at AMPA-preferring glutamate receptors, and is unlikely to seriously interfere with the use of cyclothiazide as an experimental tool.

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Send reprint requests to: M. L. Mayer, Building 49, Room 5A78, National Institutes of Health, Bethesda, MD 20892.