Glutamate Receptor Channels in Rat DRG Neurons: Activation by Kainate and Quisqualate and Blockade of Desensitization by Con A

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Summary

Primary afferent C fibers in rat dorsal roots are depolarized by the excitatory amino acids kainate and domoate. Under whole-cell voltage clamp, kainate and domoate increase membrane conductance in a subpopulation of freshly dissociated DRG neurons. In contrast to kainate currents observed in CNS neurons, responses to kainate and domoate in DRG cells desensitize with prolonged agonist exposure. Half-maximal activation is achieved with much lower concentrations of kainate and domoate in sensory neurons than in CNS neurons from cerebral cortex. Rapid applications of glutamate, guisgualate, and AMPA evoke a transient current in DRG neurons and desensitize cells to subsequent applications of kainate or domoate. Brief incubation with the lectin concanavalin A eliminates desensitization to excitatory amino acids; after treatment with concanavalin A, all five agonists gate sustained currents of similar amplitude via the same receptor.

Introduction

Glutamate receptors mediate fast excitatory synaptic transmission in many areas of the vertebrate central nervous system (Mayer and Westbrook, 1987). From the action of selective agonists and antagonists, it is clear that most neurons in the brain and spinal cord express two or more distinct subtypes of glutamate receptor that are directly linked to ion channels (Watkins and Evans, 1981; Dingledine et al., 1988). One receptor class, selective for the synthetic agonist N-methyl-p-aspartate (NMDA), controls the activity of channels permeable to Ca2+ and monovalent cations (Jahr and Stevens, 1987; Mayer et al., 1987; Ascher and Nowak, 1988b) and appears to play a role in modulating synaptic strength and stability (Cline and Constantine-Paton, 1990; Collingridge et al., 1983). Less well defined are receptors specific for the agonists kainate and quisqualate (often termed non-NMDA receptors).

In neurons throughout the CNS, both kainate and quisqualate increase membrane conductance to monovalent cations, but the current activated by kainate has a time course very different from that of the current gated by quisqualate. Rapid applications of quisqualate or glutamate elicit a transient peak of current that quickly decays to a much lower steady-state level. Currents gated by kainate in CNS neurons, on the othe, nd, do not desensitize, but maintain their initial amplitude for the full duration of exposure to agonist. Early pharmacological experiments suggested that kainate and guisgualate activate two separate receptors (Watkins and Evans 1981). However, interactions between glutamate, quisqualate, and kainate, such as cross-desensitization and lack of additivity (Kiskin et al., 1986; O'Brien and Fischbach, 1986; O'Dell and Christensen, 1989), as well as recent biochemical efforts to purify glutamate receptors (Henley et al., 1989) have raised the possibility that non-NMDA receptors may consist of a single class of ion channels with one or more binding sites for kainate and quisqualate (see Discussion). Although the exact relationship between kainate and guisgualate receptors is not yet certain, it is fairly well established that some form of non-NMDA receptor mediates the early component of excitatory postsynaptic current (Mayer and Westbrook, 1987).

Much less is known about excitatory amino acid receptors in the peripheral nervous system. Indeed, it is widely assumed that glutamate receptors are absent from the periphery of vertebrates (De Groat et al., 1972; Ransom et al., 1977; Wolf and Keilhoff, 1983). One line of evidence for expression of excitatory amino acid receptors by peripheral neurons, however, comes from Evans and his colleagues, who report that kainic acid and several other compounds depolarize sensory nerve fibers in the dorsal roots of newborn rats (Davies et al., 1979). This effect appears to be unique to primary afferent C fibers (Agrawal and Evans, 1986) and may be due to a specific receptor, since it can be blocked by a number of glutamate receptor antagonists (Evans et al., 1987; Lewis et al., 1987).

In the present study, kainate and domoate were found to activate a conductance increase to monovalent cations in a subset of freshly isolated rat dorsal root ganglion (DRG) neurons. Glutamate and quisqualate gated a transient current in the freshly dispersed cells and caused desensitization to kainate and domoate; prolonged applications of kainate and domoate also produced desensitization. The plant lectin concanavalin A (Con A), which inhibits desensitization of glutamate receptors expressed by invertebrate muscle (Mathers and Usherwood, 1976) and by vertebrate CNS neurons (Mayer and Vyklicky, 1989; O'Dell and Christensen, 1989), completely eliminated desensitization of glutamate receptors in the DRG cells. Single-channel recordings and analysis of wholecell current fluctuations indicated that in DRG neurons both kainate and quisqualate activate the same population of channels.

Results

Figures 1A and 1B show whole-cell currents evoked by $4 \mu M$ domoate and 200 μM kainate in a freshly isolated DRG neuron. Cells that respond to kainate and domo-



Figure 1. Whole-Cell Currents Evoked by Domoate, Kainate, and Glutamate

(A) Domoate (4 μ M) and (B) kainate (200 μ M) were applied to the cell by local perfusion. Holding potential, -80 mV. Rapid applications of (C) 500 μ M kainate and (D) 1 mM glutamate. Holding potential, -80 mV. The two vertical spikes in (C) and (D) are electronic artifacts due to the valves that controlled solution flow (see Experimental Procedures). Smooth curves superimposed on the desensitizing phase in (C) and (D) represent least squares fits of two exponentials plus a constant. For kainate, $\tau_1 = 35$ ms, $\tau_2 = 400$ ms, with relative amplitudes $\tau_1 = 74\%$, $\tau_2 = 17\%$, steady-state current = 9%. For glutamate, $\tau_1 = 16$ ms (97%), $\tau_2 = 150$ ms (3%).

ate represent a subpopulation of neurons with small to intermediate cell diameter; current gated by kainate or domoate was detected in 218 of 326 cells smaller than 30 µm, whereas none of 18 neurons larger than 30 µm responded. Of 71 cells tested with both agonists, 26 did not respond to either kainate or domoate and the rest were sensitive to both compounds. In most neurons, saturating doses of kainate and domoate (see below) evoked currents with approximately equivalent peak amplitudes, although some cells exhibited a slightly larger response to domoate than to kainate. The current amplitude in sensitive cells ranged from barely detectable to a maximum of 400 pA, with a mean (\pm SEM) of 103 \pm 10 pA for kainate (n = 72) and 119 \pm 8 pA for domoate (n = 86), at -70 to -80 mV. In addition, saturating levels of kainate and domoate were not additive; current recorded during simultaneous application of both agonists did not exceed the response to a saturating concentration of domoate alone. These results, together with several of the properties described below, suggest that kainate and domoate both interact with the same receptor on the surface of DRG cells.

Desensitization

As shown in Figures 1A, 1B, and 1C, steady application of kainate or domoate did not elicit a maintained response, but instead produced an initial peak of current that decayed to a much lower level over an interval of several seconds. During prolonged applications, lasting several minutes, the currents appeared to reach steady-state levels of roughly 10% of the peak response to kainate and 15% of the peak response to domoate. There was clear cross-desensitization between kainate and domoate; cells exposed to one of the two agonists became desensitized to both.

With relatively slow agonist applications ($\tau > 50$ ms) the decay of current during desensitization appeared approximately mono-exponential, as seen in Figures 1A and 1B. However, when the onset of agonist exposure was sufficiently rapid (τ of solution exchange < 30 ms), desensitization could be seen to proceed in two phases. Figure 1C shows the current evoked by rapid application of a saturating dose of kainate. The smooth curve represents a least squares fit with the sum of two exponentials. In this cell, the faster time constant was 35 ms and the slower one 400 ms. In general, the rapid phase of desensitization was more prominent for kainate than for domoate, and the time constant of the slower phase was significantly longer for domoate than for kainate. With agonist doses below saturation, both phases of desensitization proceeded more slowly.

Other Agonists

In addition to kainate and domoate, freshly dissociated DRG neurons were tested with several other excitatory amino acids, including glutamate, aspartate, NMDA, quisqualate, and AMPA (α-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid). Because responses to glutamate, quisqualate, and AMPA in CNS neurons are biphasic, with a very rapid peak and a much smaller maintained plateau (Kiskin et al., 1986), particular care was taken to apply these compounds to the DRG cells as rapidly as possible. Figure 1D shows that rapid exposure to 1 mM glutamate activates a transient current that decays with an initial time constant of 16 ms. Quisqualate and AMPA also gated transient currents when applied rapidly enough (data not shown). In contrast to kainate and domoate, there was no detectable steady-state current in freshly dissociated cells exposed to glutamate, quisqualate, or AMPA, and there was no response at all to these compounds when the onset of exposure was relatively slow (τ of exchange > 50 ms; Huettner, 1989, Soc. Neurosci., abstract).

The desensitization produced by glutamate, quisqualate, and AMPA suppressed responses to subsequent applications of kainate or domoate. As shown in Figure 2A, exposure to 50 μ M glutamate for 2–3 min produced complete desensitization of the current gated by domoate. Recovery from desensitization began as soon as glutamate was removed, but full recovery to the initial response amplitude took approxi-



Figure 2. Recovery from Desensitization by Glutamate

(A) Control current activated by 2 µM domoate is shown on the left, followed by a family of traces recorded at various time intervals after exposure to 50 µM glutamate for 2-3 min. In the first experimental trace the cell was switched directly from glutamate into domoate: in succeeding traces the cell was washed with control solution from the end of each glutamate exposure to the onset of domoate.

(B, inset) In a different cell, the onset of current upon switching from glutamate to domoate. The smooth curve superimposed on the trace is the best fit of a single exponential (τ = 18 s). The dashed line represents 15% of the peak amplitude of a control response to domoate in this cell.

mately 5 min. A similar time course was observed for recovery from desensitization produced by guisgualate and AMPA and from the steady-state level of desensitization achieved with prolonged applications of kainate and domoate. With all of these compounds it took 3-6 min for full recovery from desensitization. Figure 2B shows that when a cell is switched directly from glutamate into domoate, the current relaxes fairly rapidly to the level expected for steady-state desensitization after a long exposure to domoate. This result suggests that molecules of glutamate do not remain bound to the receptor for more than a few seconds after glutamate has been removed from the external solution. Therefore, recovery from desensitization, which is much slower, most likely involves the slow equilibration of unliganded receptors be-

It was difficult to determine the relation between

steady-state desensitization and glutamate concentration because the rate of onset of desensitization became very slow with low doses of glutamate. Qualitatively, there was almost no desensitization after 5 min exposures to 1 µM glutamate. Full desensitization required application of 50 µM glutamate for 1-2 min, whereas desensitization was complete following a few seconds in 500 µM glutamate.

Aspartate and NMDA, both tested at 500 µM in Mg^{2+} -free Tyrode's solution with 1 μ M glycine, evoked current in only 2 of 33 neurons. Kainate and domoate activated current in 20 of these 33 cells, including 1 of the 2 cells that responded to NMDA and aspartate. For these experiments, cells were dissociated with protease from Aspergillus oryzae (Sigma type XXIII), which preserves responsiveness to NMDA in CNS neurons (Akaike et al., 1988; Chizhmakov et al., 1989). Currents gated by kainate and domoate were not af-

> Figure 3. Dose-Response Relations for Kainate and Domoate in Cells from Dorsal Root Ganglia and Cerebral Cortex

> (A) Currents activated by 1-250 µM kainate in a DRG neuron. Holding potential, -70 mV.

> (B) Currents activated by 16 nM to 63 μ M domoate in a different DRG cell. Holding potential, -70 mV. The cells were exposed to control solution for 2-3 min between agonist applications.

> (C) Dose-response data for kainate from 7 DRG neurons (squares) and 3 cortical cells (circles).

> (D) Data for domoate from 3 DRG neurons (squares) and 6 cortical cells (circles), Points show the mean ± SD of the peak current amplitude plotted as a fraction of the maximal current. The smooth curves are least squares fits of

> $I = (1 + \alpha/\beta)/(1 + (\alpha/\beta) (1 + (2K_d/[Agonist])))$ + (K_d/[Agonist])²)



[Domoate], (M)

[Kainate], (M)

tween desensitized and activatable states.

в Α Domoate, (µM) Kainate. .016 .063 .25 30 pA 80 pA 15 sec 15 sec С D 1.0 1.0 Current (fraction of maximum) (fraction of maximum) DRG DRG 0.8 0. 8 EC50 $EC_{50} = 0.7 \,\mu M$ 15 *u*N 0.5 0.6 0.4 0.4 Cortex Corte Current $EC_{50} = 120 \mu M$ $EC_{50} = 32 \mu M$ 0.2 0.2 ۵ О 10-3 102 10-8 10 104 10³ 105 10 10 10 10 10 10



Figure 4. Current-Voltage Relation for 50 μM Kainate

(A) Currents activated by 3 s pulses of 50 μ M kainate at holding potentials from -100 to +60 mV. (B) Peak current plotted versus holding potential.

fected by prior exposure to NMDA or aspartate in any of the cells.

Dose-Response Relation

The dose-response relations shown in Figure 3 reveal that domoate is a significantly more potent agonist than kainate. Figure 3A shows currents evoked in a DRG neuron by seven concentrations of kainate ranging from 1 µM to 250 µM. Responses to increasing levels of domoate, from 16 nM to 63 μ M, that were recorded in a different cell are shown in Figure 3B. Concentrations of kainate lower than about 1 µM or of domoate less than 50 nM did not elicit current. Responses increased in amplitude with increasing agonist concentrations up to maximal levels at roughly 200 μ M kainate or 10 μ M domoate. As shown in Figures 3C and 3D, DRG cells exhibit higher sensitivity to kainate and domoate than do freshly dissociated neurons from rat cerebral cortex. Half-maximal activation was achieved with 15 µM kainate in DRG cells compared with 120 µM in cortical neurons. For domoate, the EC_{50} was 0.73 μM in DRG neurons and 32 μM in cortical cells.

The equation for 1-to-1 binding (Hill, 1909) provides a poor fit to the dose–response data in Figures 3C and 3D; the currents evoked by increasing concentrations of kainate and domoate grow more steeply than expected for a 1-to-1 relation. This observation suggests that more than one molecule of agonist must bind for efficient channel opening. The smooth curves shown in Figures 3C and 3D represent the best fits for a simple receptor scheme with two independent agonist binding sites, which is similar to the model of Colquhoun and Ogden (1988) for the nicotinic acetylcholine receptor.

Current-Voltage Relation

The current-voltage relation is approximately linear for both kainate (n = 7) and domoate (n = 9). Figure

4 shows the current-voltage relation for kainate from -100 mV to +60 mV, with Na⁺ as the principal external cation and Cs⁺ as the major internal cation. Currents activated by kainate or domoate reversed near 0 mV with Cs⁺ or K⁺ as the internal cation and Cl⁻ or methanesulfonate as the major internal anion. Substitution of 100 mM N-methyl-D-glucamine chloride for 100 mM external NaCl shifted the reversal potential to approximately -30 mV (n = 5). Taken together, these results suggest that the channels activated by kainate and domoate in DRG neurons are permeable to Na⁺, K⁺, and Cs⁺, with relatively little selectivity among these three cations.

Effects of Con A

Brief exposure to the lectin Con A eliminates desensitization of current gated by kainate and domoate. Figure 5A shows that after a 5 min incubation with Con A current is maintained throughout the period of agonist application. There was often a substantial increase in response amplitude following treatment with Con A, which may reflect elimination of an unliganded desensitized state or may represent failure to resolve the initial peak of current in many of the untreated cells. In 16 cells tested before and after Con A treatment, the peak current elicited by domoate or kainate increased by an average of about 2-fold. The maximum current recorded after Con A treatment was 840 pA; the mean current (\pm SEM) for kainate was 250 \pm 26 pA (n = 47); that for domoate, 196 \pm 21 pA (n = 41). Con A did not alter the current-voltage relation or ionic selectivity for currents gated by kainate and domoate (n = 10) and did not change the agonist concentrations required for half-maximal activation (Figure 5D). In Con A-treated neurons the EC_{50} values were 15 μ M for kainate (n = 5) and 0.50 μ M for domoate (n = 3).

Following treatment with Con A, both glutamate and quisqualate evoked currents with amplitudes



Figure 5. Con A Eliminates Desensitization (A) Currents evoked by 100 μ M kainate, 2 μ M domoate, and 500 μ M glutamate following exposure to Con A.

(B) Currents activated by 1 mM kainate alone and by kainate plus 4 mM glutamate, 4 mM quisqualate, or 40 μM domoate.

(C) Currents gated by 4 μ M to 16 mM glutamate and by 1 μ M to 4 mM quisqualate. Holding potential, -80 mV.

(D) Dose-response data for domoate (3 neurons, 8 applications), kainate (5 neurons, 18 applications), glutamate (3 neurons, 8 applications), and quisqualate (3 neurons, 8 applications). Points show the mean \pm SD of the peak current amplitude plotted as a fraction of the maximal current. The smooth curves are least squares fits of

 $l = (1 + \alpha/\beta)/(1 + (\alpha/\beta) (1 + K_d/[Agonist])) \\ in which <math display="inline">\beta$ is the rate constant for channel opening, α is the rate constant for channel closing, and K_d is the microscopic dissociation constant. α/β was set to 1 to be consistent with current fluctuations. For domoate, $K_d = 0.99 \ \mu$ M; kainate, $K_d = 30 \ \mu$ M; for glutamate (shown), $K_d = 115 \ \mu$ M; for quisqualate, $K_d = 99 \ \mu$ M.

and properties similar to those of currents gated by kainate and domoate. In some cells, exposure to Con A did not totally overcome desensitization to glutamate and quisqualate. The residual desensitization appeared as a slow decay in the current obtained with high doses of glutamate or quisqualate (see Figures 5A and 5C). After exposure to Con A, the maximal currents obtained with saturating doses of kainate, domoate, glutamate, and guisgualate were nearly identical and were not additive, although submaximal doses of kainate and glutamate did sum together. Figure 5B shows that application of 4 mM glutamate, 4 mM quisqualate, or 40 µM domoate together with 1 mM kainate did not evoke any more current than kainate alone. The full dose-response relation for glutamate and quisqualate in Con A-treated cells is shown in Figures 5C and 5D. Half-maximal activation was obtained with 50 μ M guisqualate and 58 μ M glutamate. AMPA also activated sustained current in Con A-treated cells, but fairly high concentrations were required; preliminary dose-response experiments indicate a relatively low affinity for AMPA (EC₅₀ approximately 520 µM).

Con A had an additional effect on currents gated by excitatory amino acids. In Con A-treated neurons the dose-response data for kainate and domoate, and for glutamate and quisqualate, are best fitted by the equation for 1-to-1 binding (Figure 5D). Schemes that require binding of two agonist molecules, with either independent or cooperative binding sites, do not fit the data well. Thus it appears that after incubation with Con A, channels can be opened by the binding of a single agonist molecule. In addition to Con A, several other lectins reduced desensitization to excitatory amino acids; these include wheat germ agglutinin (Thio et al., 1989, Soc. Neurosci., abstract), isolectin B_4 from Griffonia simplicifolia (Murphy and Goldstein, 1977), and succinyl Con A, which exists as a dimer and does not cause aggregation of membrane proteins as does native, tetravalent Con A (Gunther et al., 1973).

Current Fluctuations and Single-Channel Properties

There was a clear increase in current variance during responses to excitatory amino acids. Figure 6A shows sample currents activated by several concentrations of glutamate in a Con A-treated cell. In Figure 6B the increase in variance relative to control is plotted as a function of the mean current. For a homogeneous population of independent channels, the data should fall along a parabolic trajectory, reaching a maximum for a probability of opening (po) equal to 1/2 (Sigworth, 1980). The results in Figure 6 indicate that even with a saturating dose of glutamate, the maximum average po is roughly 1/2 or less (the parabolic fit is not well constrained unless p_o actually exceeds 1/2). The initial slope in Figure 6B provides an estimate of the single-channel current of 0.38 pA. Given the holding potential of -80 mV, and assuming reversal at 0 mV, this suggests a unitary conductance of 4.8 pS. Table 1 summarizes the properties of current fluctuations observed with kainate, domoate, guisqualate, and glutamate. For each agonist, the maximal p_o was $\leq 0.5-0.6$ and the mean estimated unitary conductance ranged from 2 to 4 pS (individual values ranged from 1.6 to 5.1 pS). The covariance functions (Neher and Stevens, 1977) for all the agonists exhibited both a fast ($\tau = 1$ ms) and a slow ($\tau = 10-20$ ms; Figure 6C) component.

Unitary currents activated by kainate, domoate, glutamate, and quisqualate were recorded in 24 of 39 out-



Figure 6. Fluctuations in Whole-Cell Current Evoked by Glutamate

(A) Sample traces in 0, 16 μ M, 63 μ M, and 1 mM glutamate following exposure to Con A. Straight lines are drawn at the mean current level. Numerical values present the mean current and current variance for each 205 ms trace. Holding potential, -80 mV. (B) Glutamate-induced current variance versus mean evoked current (same cell as [A]). Points show the mean \pm SD of current variance, which was calculated in 5 intervals of 1.6 s (after subtracting a straight line to correct for steady DC changes). The smooth curve is the best fit of $\sigma^2 = i \cdot I - I^2/N$, in which I is the mean macroscopic current, i is the unitary current amplitude, 0.38 pA, and N is the number of channels, 1460 (see Sigworth, 1980). The straight line with slope of i is tangent to the curve at I = 0.

(C) The covariance function (Neher and Stevens, 1977) calculated from 4.6 s of current evoked by 16 μ M glutamate. The smooth curve represents the sum of two exponentials: $\tau_1 = 1.1$ ms (40%); $\tau_2 = 18.8$ ms (60%). Current was filtered at 3 KHz (-3 dB, 8-pole Bessel) and digitized at 10 KHz.

side-out patches, obtained from cells that responded to kainate in the whole-cell recording mode. Several different current amplitudes were observed with each agonist. Figure 7A shows single-channel events gated by kainate in a patch from a freshly dissociated cell that had not been exposed to Con A. In this patch, the predominant unitary conductance was 8 pS (Figure 7B, inset) with occasional brief openings of 15-18 pS. The open time histogram for this patch, shown in Figure 7B, was best fitted by a sum of two exponentials with time constants of 1.1 and 4 ms. Figure 7C shows the current-voltage relations for unitary events of 8 pS activated by kainate and domoate in a different patch from a cell that had been exposed to Con A. In addition to channels of 8 and 15-18 pS, kainate and the other agonists also gated channels of approximately 4-5 pS. In 13 patches the majority of openings were 8 pS, in 5 patches a roughly equivalent proportion of 4 and 8 pS events was displayed, and in 6 patches the principal conductance level was 4 pS. Events of 15-18 pS were a minor fraction of most patches. Figure 7D shows 4 pS channels activated by kainate, glutamate, quisqualate, and domoate in a patch obtained from a cell treated with Con A.

Antagonists

Responses to kainate and domoate, both before and after exposure to Con A, were blocked by several different antagonists of excitatory amino acid receptors, including kynurenate, (\pm) -*cis*-2,3-piperidine dicarboxylic acid, γ -D-glutamylglycine (Watkins and Evans, 1981; Dingledine et al., 1988), and the quinoxalinediones, CNQX and DNQX (Honore et al., 1988; Verdoorn et al., 1989). Both the onset of blockade and the recovery from inhibition by these antagonists were relatively rapid, being complete within a few hundred milliseconds. Currents evoked by kainate and domoate were not affected by glutamate dimethylesther, 2-amino-4-phosphonobutyrate, or 2-amino-5-phosphonovaleric acid.

In freshly dispersed cells, fairly high doses of CNQX (160 μ M), DNQX (160 μ M), and kynurenate (1 mM) blocked the transient current evoked by glutamate and completely prevented glutamate from inducing desensitization of responses to kainate and domoate. After Con A treatment, these antagonists inhibited the maintained currents gated by glutamate and quis-

Table 1. Estimates of Single-Channel Properties Derived from Agonist-Induced Fluctuations in Whole-Cell Current									
Agonist	Con A	iª (pA)	p _o max	Covariance Function					
				τ ₁	τ ₂	n			
Kainate	-	0.27 ± 0.05	<0.4	1.6 ± 0.3 (46% ± 8%)	11.3 ± 5.4 (54% ± 8%)	5			
	+	$0.28~\pm~0.06$	<0.6	$1.3 \pm 0.4 \; (39\% \pm 11\%)^{ m b}$	16.1 \pm 2.7 (61% \pm 11%) ^b	5			
Domoate	-	0.21 ± 0.05	<0.5	$1.3 \pm 0.2 \ (68\% \pm 4\%)^{\circ}$	16.5 ± 3.7 (32% ± 4%) ^c	6			
	+	$0.16 \pm 0.03^{\circ}$	<0.5	$1.3 \pm 0.6 \ (61\% \pm 6\%)$	16.5 ± 1.7 (39% ± 6%)	6			
Glutamate	+	0.33 ± 0.06^{d}	<0.5	$1.3 \pm 0.4 (49\% \pm 14\%)$	16.4 ± 2.6 (51% ± 14%)	7			
Quisqualate	+	$0.27~\pm~0.03$	<0.6	$1.8 \pm 0.4 \ (49\% \pm 3\%)$	12.6 \pm 3.1 (51% \pm 3%)	5			

^a Unitary current at -80 mV, mean \pm SD of n determinations. Differences among means are significant at P < 0.05 for i and for the covariance amplitudes (ANOVA, F statistics).

The differences between means were assessed by confidence intervals (P < 0.05):

^b Significantly different from domoate (+).

^c Significantly different from kainate (+/-), glutamate, and quisqualate.

d Significantly different from domoate (-).



Figure 7. Single-Channel Activity in Outside-Out Patches from DRG Neurons

(A) Unitary currents activated by 1 mM kainate in a patch from a fresh cell not exposed to Con A. Holding potential, -80 mV. Three continuous traces are shown approximately 10 s after the onset of kainate application.

(B) Open time histogram constructed using a half-amplitude threshold criterion for 1682 events in the patch in (A). The smooth curve is the best fit of two exponentials: $\tau_1 = 1$ ms (81%); $\tau_2 = 4$ ms (19%). (Inset) Amplitude histogram for the same patch; mean unitary current = 0.65 pA.

(C) Current-voltage relation for channels activated by kainate (circles) and domoate (squares) in a patch from a cell exposed to Con A. Points show the mean \pm SD of amplitude histograms constructed from unitary events recorded at each potential. For clarity, points for kainate and domoate have been shifted by -1 and +1 mV, respectively. Unitary conductance = 8 pS.

(D) Channel activity gated by kainate, glutamate, gluisqualate, and domoate, which were applied in that order (separated by 1-2 min in control) to a patch from another cell that was exposed to Con A. For each agonist, three continuous traces show activity recorded several seconds after the onset of agonist exposure. Holding potential, -80 mV. The mean unitary amplitude of wellresolved events is 0.35 pA. All currents were filtered at 1 KHz (-3 dB, 8-pole Bessel) and digitized at 10 KHz.

qualate. Figure 8 shows the dose-response relation for blockade by CNQX of current evoked by 100 μ M glutamate in a cell that was exposed to Con A. Table 2 presents a summary of the IC₅₀ values (antagonist concentrations that produced 50% block) obtained from freshly isolated cells and from cells treated with Con A, for several different pairings of agonist with antagonist. As shown in Table 2, exposure to Con A produced little change in the IC₅₀ for antagonism of domoate by CNQX. In addition, concentrations of domoate and glutamate that gave roughly equivalent



Figure 8. Dose-Response Relation for Antagonism of Glutamate by CNQX

(A) Currents activated by 100 μM glutamate plus 0–160 μM CNQX. Holding potential, –80 mV.

(B) Dose-response data for 3 neurons (9 applications). Points show the mean \pm SD current amplitude plotted as a fraction of control (no CNQX). The curve is a least squares fit of 1/(1 + [CNQX]/IC₅₀), with IC₅₀ = 2 μ M.

fractional channel activation were blocked by CNQX with very similar affinity. These observations provide further evidence that the same population of receptors is responsible for the currents observed before and after exposure to Con A, and they suggest that both domoate and glutamate gate current by binding to the same receptor site.

Discussion

Whole-cell recordings from freshly dissociated rat DRG neurons revealed that a subpopulation of cells expresses a unique type of excitatory amino acid receptor. Several properties distinguish this receptor from those previously described in physiological ex-

Table 2. Antagonist Concentrations Producing 50% Inhibition of Peak Current Gated by 1 µM Domoate or 100 µM Glutamate								
Agonist	Antagonist	Con A	IC ₅₀ ^a (μM)	n				
Domoate	DNQX	_	2.7 ± 0.4	3 (2)				
Domoate	CNQX		0.9 ± 0.1	3 (2)				
Domoate	CNQX	+	1.4 ± 0.1	6 (3)				
Glutamate	CNOX	+	2.1 + 0.1	9 (3)				

 $^{\rm a}$ Mean \pm SD of n trials with a series of six antagonist concentrations.

The number in parentheses indicates the number of cells.

periments on CNS neurons and glia. Most strikingly, the receptor on DRG cells has a much higher affinity for kainate and domoate than the receptor expressed by CNS neurons. For DRG cells, activation is halfmaximal with 0.73 μ M domoate and 15 μ M kainate compared with 32 μ M domoate and 120 μ M kainate in cortical neurons. In both cell types, domoate is a more potent agonist than kainate; however, the separation in EC₅₀ values for kainate and domoate is much wider in DRG cells (~20-fold) than in cortical neurons (~ 4-fold).

A second major difference between the receptors expressed by central and peripheral neurons is that the currents gated by kainate and domoate in DRG cells undergo desensitization during prolonged agonist applications; the steady-state current recorded after several minutes of exposure to kainate or domoate represents only 10%-15% of the initial peak. In contrast, CNS neurons exhibit maintained currents that do not diminish during continuous application of kainate or domoate (Kiskin et al., 1986; Mayer and Vyklicky, 1989; O'Dell and Christensen, 1989). Glutamate and quisqualate activate transient currents in both DRG cells and CNS neurons, provided that the onset of agonist exposure is sufficiently rapid. Responses to glutamate and quisqualate in freshly dissociated DRG cells desensitize completely, whereas the currents in CNS neurons decay from an initial peak to a lower steady-state level of maintained current.

The currents gated by kainate and quisqualate in CNS neurons are not directly additive; high concentrations of guisgualate, glutamate, or AMPA invariably reduce the current activated by kainate rather than adding to it (Ishida and Neyton, 1985; Kiskin et al., 1986; O'Brien and Fischbach, 1986; Zorumski and Yang, 1988; Perouansky and Grantyn, 1989). Upon removal of guisgualate or glutamate, the current gated by kainate recovers to its maximal amplitude within a few hundred milliseconds (Kiskin et al., 1986). A similar rapid recovery is observed for the transient peak of responses to glutamate, quisqualate, and AMPA (Tang et al., 1989; Trussell and Fischbach, 1989). In some CNS cell types the interaction between kainate and quisqualate does not appear to be competitive (Ishida and Neyton, 1985), whereas in other cases, such as spinal motoneurons (O'Brien and Fischbach, 1986), increasing concentrations of kainate can overcome inhibition by quisqualate. One interpretation of these results (Kiskin et al., 1986; O'Brien and Fischbach, 1986) is that kainate and quisqualate both operate the same channels and that quisqualate and glutamate produce cross-desensitization of responses to kainate. A second possibility (Tang et al., 1989) is that quisqualate and glutamate activate a distinct, separate class of channels, but serve as antagonists (or weak partial agonists) at the kainate receptor. More elaborate models are also possible.

The results from DRG neurons are best explained by the first type of model. The inhibition brought on by glutamate and quisqualate clearly involves desensitization and not simply competitive blockade, because it persists long after the receptors are unoccupied. The lack of additivity in Con A-treated cells and the similarities in single-channel properties suggest that all the agonists operate the same population of channels. At present, there is not sufficient data to decide conclusively whether kainate and quisqualate both interact with the same binding sites or with distinct sites on the same receptor-channel complex.

In addition to the activation of CNS receptors by kainate that has been considered above, biochemical studies (Monaghan and Cotman, 1982; Foster and Fagg, 1984) have demonstrated binding sites with high affinity for kainate in several regions of the brain, including the striatum, the deep cortical layers, and field CA3 of the hippocampus. Neuronal responses to 10–100 nM kainate have been reported in these areas (Robinson and Deadwyler, 1981; Westbrook and Lothman, 1983); however, the physiological properties of the high-affinity CNS kainate receptor have not been characterized in detail, and its relation to the receptor of DRG cells is not clear.

Single-Channel Properties

In DRG cells and in CNS neurons (Jahr and Stevens, 1987; Ascher and Nowak, 1988a, 1988b; Cull-Candy et al., 1988) and glia (Usowicz et al., 1989), excitatory amino acids activate single-channel events with several distinct amplitudes. The two predominant conductance levels in DRG neurons are 8 pS and approximately 4 pS, with infrequent openings of 15-18 pS. Direct transitions between these levels were rare, and there were no high-conductance openings (40–50 pS) typical of channels gated by NMDA (Nowak et al., 1984). These results are in fairly close agreement with previous work (Jahr and Stevens, 1987; Ascher and Nowak, 1988a; Cull-Candy et al., 1988, 1989a, 1989b) on CNS cells in which kainate and quisqualate were found to gate unitary events of 4-6 pS, 8-10 pS, and 15–35 pS (Zorumski and Yang, 1988; Tang et al., 1989; Trussell and Fischbach, 1989).

Analysis of whole-cell current fluctuations in DRG cells provides further evidence that kainate, quisqualate, glutamate, and domoate activate the same channels, although complete interpretation of the fluctuations is made difficult by the presence of multiple single-channel conductance levels. The estimated unitary conductance (2–4 pS) and maximal p_o (\sim 0.5–0.6) were similar for all four agonists, and for each agonist, the covariance of current fluctuations displayed two exponential components. The faster time constant (τ = 1 ms) is likely to arise from the opening and closing reaction (see Figure 7B); the slower component (τ = 10-20 ms) may reflect the tendency for openings to occur in clusters or bursts (Figure 7A) or may be due to differences in the kinetics of the two principal conductance levels. Currents gated by kainate and quisqualate in CNS neurons also usually exhibit two kinetic components with time constants of 1–2 ms and >10 ms (Ascher and Nowak, 1988a; Cull-Candy et al., 1988, 1989a, 1989b).

Effects of Con A

In DRG cells Con A virtually abolishes desensitization to glutamate and the other agonists. Following a brief incubation with the lectin, agonist-evoked currents rise monotonically to a maintained level. In CNS neurons from the hippocampus (Mayer and Vyklicky, 1989) and retina (O'Dell and Christensen, 1989), Con A does not affect the amplitude of currents gated by kainate or domoate. Treatment with Con A leads to an increase in the steady-state current activated by glutamate and quisqualate but does not completely eliminate desensitization in CNS cells-there there is still a transient peak of current at the onset of agonist exposure (Mayer and Vyklicky, 1989; O'Dell and Christensen, 1989). The molecular mechanism by which Con A inhibits desensitization of glutamate receptors has not yet been determined. Con A may bind directly to sugar residues on the extracellular domain of the receptor, or it may act by a second messenger pathway, as in the modulation by Con A of K⁺ (Lin et al., 1989) and Ca²⁺ (Kuno et al., 1986) channel activity. Recent biochemical work (Henley et al., 1989) has shown that binding sites for kainate and guisgualate, solubilized from Xenopus brain, are retained on a wheat germ agglutinin affinity column; this finding supports the possibility of a direct interaction with the receptor. Aggregation of membrane proteins does not appear to be required for Con A to inhibit desensitization in DRG neurons, since the succinylated, dimeric form of Con A was also effective (see Kehoe, 1978).

The principal effect of Con A on channel gating in DRG cells appears to be the maintenance of a relatively high frequency of opening in the continued presence of agonist (see Mathers, 1981). For kainate and domoate, exposure to Con A did not change the whole-cell current fluctuations or the amplitude of unitary currents in outside-out patches. However, it was difficult to analyze the gating behavior of the 4–5 pS events, which often could not be resolved as clear step functions; thus, subtle effects of Con A on channel lifetime cannot be ruled out.

Although Con A is an exogenous protein, obtained from the jack bean, neurons in both the CNS and the PNS express endogenous lectins (Jessell et al., 1990) that could serve to regulate receptor desensitization in vivo. Endogenous lactose binding lectins have been demonstrated (Regan et al., 1986) in a subset of small diameter DRG neurons and in their central axon terminals. Nearly all cells in this subpopulation also express lactoseries glycoconjugates on their surfaces (Regan et al., 1986). Isolectin B_4 from G. simplicifolia (Murphy and Goldstein, 1977), which binds to lactosecontaining carbohydrates, inhibits desensitization to excitatory amino acids in freshly dissociated DRG cells.

Comparison with Other Receptors

The glutamate receptor expressed by DRG cells shares a number of properties with the nicotinic acetylcholine receptor. Both receptors undergo desensitization in two phases, with roughly similar time constants, to reach low (but non-zero) steady-state currents (Feltz and Trautmann, 1982; Cachelin and Colquhoun, 1989). Although the exact nature of the desensitized states is not well understood for either receptor, it seems likely that some form of cyclic reaction scheme (Katz and Thesleff, 1957) will be required to account for the phenomenology of glutamate receptor desensitization. In particular, it appears that recovery from desensitization in DRG cells involves the slow conversion of unliganded, desensitized receptors to the resting, activatable state. As mentioned above, recovery from desensitization to glutamate and quisqualate proceeds much more rapidly in CNS neurons than in DRG cells. Trussell and Fischbach (1989) have shown that the time constant of recovery in chick spinal cord neurons depends upon the duration of exposure to agonist. Although this point was not investigated in DRG cells, their results (Trussell and Fischbach, 1989) together with the biphasic onset of desensitization in both central and peripheral cells suggest the presence of at least two desensitized states (see Feltz and Trautmann, 1982).

Con A binds directly to the nicotinic acetylcholine receptor of muscle and has been reported to decrease desensitization to acetylcholine in cat adrenal gland (Kirpekar and Prat, 1978). However, in cultured muscle cells the chief effect of Con A is to reduce the frequency of channel openings gated by acetylcholine (Young and Poo, 1983). In DRG cells, which express nicotinic acetylcholine receptors as well as GABA_A receptors (Choi and Fischbach, 1981) and receptors for extracellular ATP (Krishtal et al., 1983; Bean, 1990), exposure to Con A reduces the whole-cell current evoked by acetylcholine, but does not change the properties of currents activated by ATP or GABA (J. E. H., unpublished data).

Responses to Glutamate In Vivo

It seems very likely that the glutamate receptor found in a subset of freshly isolated DRG neurons underlies the depolarization of primary afferent nerve fibers by kainate and domoate (Davies et al., 1979). The EC_{50} values obtained for kainate (15 μ M) and domoate (0.73 μ M) in dissociated DRG cells agree well with the results of Evans and colleagues (Davies et al., 1979; Agrawal and Evans, 1986), who found that 8-9 µM kainate produced half-maximal depolarization of isolated dorsal root preparations and that domoate was 20- to 40-fold more potent than kainate. In dorsal roots, as well as peripheral nerves, sensitivity to kainate appears to be restricted primarily to C fibers, because application of kainate causes a selective reduction in the C fiber volley evoked by electrical stimulation (Agrawal and Evans, 1986). C fibers arise from

small to intermediate diameter (<30 μ m) sensory neurons, and most axons in the C fiber range convey nociceptive or thermoreceptive information. The subset of freshly dissociated cells that was sensitive to kainate contained exclusively cells with diameters of less than 30 μ m; approximately 65% of neurons smaller than 30 μ m responded to kainate. Additional experiments to correlate kainate sensitivity with cell type-specific markers, such as neuropeptides or surface carbohydrate moieties (Dodd and Jessell, 1985), should help to identify which population of cells expresses the kainate receptor.

Primary afferents respond to glutamate and quisqualate with weak, transient depolarizations, but glutamate consistently desensitizes nerves to kainate (Davies et al., 1979). During prolonged exposures to glutamate, there is relatively little effect on the electrically evoked C fiber volley (Agrawal and Evans, 1986), which suggests that there must be little or no steadystate activation by glutamate in situ. Similarly, in dissociated DRG neurons, glutamate and quisqualate gate transient currents that quickly decay to undetectable levels. These agonists also cross-desensitize the cells to kainate and domoate, with full recovery from desensitization taking 3–6 min.

NMDA and aspartate do not cause significant depolarization of isolated dorsal root preparations (Agrawal and Evans, 1986). However, a recent report (Lovinger and Weight, 1988) indicates that a substantial proportion of DRG neurons from adult rats express conventional NMDA receptors, which show voltagedependent block by Mg²⁺ (Nowak et al., 1984) and potentiation by glycine (Johnson and Ascher, 1987). In the present study, NMDA and aspartate activated current in only a small percentage of early postnatal cells and did not affect responses to kainate and domoate.

The currents activated by kainate and glutamate in the cell bodies of dissociated DRG neurons are relatively small when compared with other transmitterand voltage-gated currents found in these cells (Bean, 1990). Given a unitary current amplitude of 0.32-0.65 pA and a maximal p_o of 0.5, the average whole-cell current of 250 pA (after Con A) suggests that freshly dissociated cells possess roughly 770-1500 individual channels. Agrawal and Evans (1986) recorded strong depolarizations to kainate in dorsal root fibers, but found little or no response when kainate was applied directly to the ganglia. The response in peripheral nerves was also weak; however, kainate did block conduction in peripheral C fibers. Taken together, their results from semi-intact preparations indicate that kainate receptors are preferentially distributed to the central branch of primary sensory fibers, with a much lower density on the cell soma and peripheral axon. Although the function of these receptors is not yet known, this selective localization raises the possibility that the kainate receptors may play a role in presynaptic modulation of primary afferent transmission.

Experimental Procedures

Cell Preparation

Lumbar and thoracic dorsal root ganglia taken from Long Evans rats were collected in oxygenated Ca2+-free Tyrode's solution, trimmed of nerve roots and connective tissue, and dissociated as described by Bean (1990). The isolated ganglia were minced with fine scissors, then gently stirred at 37°C in 10 ml of Ca2+free Tyrode's solution containing collagenase (15 mg; Sigma type I) and dispase (30 mg; Boehringer-Mannheim). After 40 min, this solution was replaced with 10 ml of Ca2+ free Tyrode's solution containing dispase alone (30 mg). The tissue was incubated at 37°C for another 40-50 min with mild trituration through a firepolished Pasteur pipette every 15 min. The resulting suspension of dissociated neuron was centrifuged for 10 min at 70 x g. Cells were resuspended in 2 Ca2+-Tyrode's solution (see below) and kept at room temperature for 1-10 hr until used. Most experiments were performed with DRG cells from young rats, 1-3 weeks of age, but comparable results were obtained in neurons from newborn, juvenile, and fully adult animals. For some experiments neurons were dissociated with papain (Worthington) as described for cerebral cortical tissue by Huettner and Baughman (1986) or with protease from A. oryzae (Sigma type XXIII; Chizhmakov et al., 1989). There was no difference in the responses to excitatory amino acids of cells treated with dispase and collagenase, with papain, or with protease from A. oryzae.

Electrical Recording and Drug Application

Tight-seal recordings were performed in the whole-cell and outside-out patch configurations described by Hamill et al. (1981). Pipette resistance ranged from 1 to 10 $M\Omega$ with an internal solution of 140 mM CsCH₃SO₃, 10 mM HEPES, 10 mM EGTA, 5 mM CsCl, 4 mM MgATP, 1 mM Na2GTP. Membrane potentials have been corrected for a junction potential of -10 mV between the internal and external solutions. Cell size was measured visually and calculated from the whole-cell capacitance, assuming 1 μ F/cm². The capacitance of cells with diameter <30 μ m ranged from 10 to 25 pF. Control or agonist-containing solutions were applied by local perfusion from a linear array of microcapillary tubes, which were mounted on a hydraulic manipulator. For most experiments, solution flow was driven by gravity; in some cases, the solutions were driven by a pump and flow was controlled by electronic valves (3-way isolatch; General Valve Corp.), as described by Mayer and Vyklicky (1989). Con A was applied at 2 µM for 5-10 min.

Currents were filtered at 1 KHz (-3 dB, 8-pole Bessel) and digitized at 5 KHz. During prolonged agonist applications, the data were compressed on-line for storage and display by averaging 3 ms of data at 0.1–1.0 s intervals. Most experiments were also recorded on video tape and filtered at 5–10 KHz (-3 dB, 4-pole Bessel) by the patch-clamp amplifier (Dagan 3900). Currents on tape were refiltered at 1 KHz (-3 dB, 8-pole Bessel) for single-channel recordings or at 3 KHz for analysis of whole-cell current fluctuations and were digitized at 10 KHz. Other whole-cell experiments were digitized from tape at 50 Hz to 5 KHz while filtering at 1 KHz.

Solutions

The Ca²⁺-free Tyrode's solution for cell preparation was composed of 150 mM NaCl, 4 mM KCl, 2 mM MgCl₂, 10 mM glucose, and 10 mM HEPES (pH 74). For storage of dissociated neurons and bulk perfusion of the recording chamber, 2 mM CaCl₂ was added (2 Ca²⁺-Tyrode's). The external solution for drug applications contained 160 mM NaCl, 10 mM HEPES (pH 74), and 1 μ M tetrodotoxin. For most experiments, CaCl₂ and MgCl₂ were added at 2 mM and 1 mM, respectively.

Materials

(-)-Domoate (Ohfune and Tomita, 1982) was the kind gift of Dr. Y. Ohfune, Suntory Institute for Bioorganic Research, Osaka, Japan. Comparable results were obtained with domoate acid from Sigma, which was used for several experiments. Other agonists and antagonists were purchased from Cambridge Research Biochemicals or from Sigma. Lectins were obtained from Sigma and from E. Y. Labs.

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