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FUNCTIONAL SIMILARITIES AND DIFFERENCES OF AMPA AND KAINATE RECEPTORS EXPRESSED BY CULTURED RAT SENSORY NEURONS

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Abstract—Dorsal root ganglion neurons express functional AMPA and kainate receptors near their central terminals. Activation of these receptors causes a decrease in glutamate release during action potential evoked synaptic transmission. Due to differences in kinetic properties and expression patterns of these two families of glutamate receptors in subpopulations of sensory neurons, AMPA and kainate receptors are expected to function differently. We used embryonic dorsal root ganglion (DRG) neurons maintained in culture to compare functional properties of kainate and AMPA receptors. Most DRG neurons in culture expressed kainate receptors and about half also expressed AMPA receptors. Most AMPA and kainate receptor-expressing DRG neurons were sensitive to capsaicin, suggesting involvement of these glutamate receptors in nociception. When activated by kainate, AMPA receptors were capable of driving a sustained train of action potentials while kainate receptors tended to activate action potential firing more transiently. Glutamate elicited more action potentials and a larger steady-state depolarization in neurons expressing both AMPA and kainate receptors than in neurons expressing only kainate receptors. Adding to their more potent activation properties, AMPA receptors recovered from desensitization much more quickly than kainate receptors. Activation of presynaptic receptors by low concentrations of kainate, but not ATPA, caused a tetrodotoxin-sensitive increase in the frequency of spontaneous EPSCs recorded in dorsal horn neurons. By recording synaptic pairs of DRG and dorsal horn neurons, we found that activation of presynaptic kainate and AMPA receptors decreased evoked glutamate release from terminals of DRG neurons in culture. Our data suggest that the endogenous ligand, glutamate, will cause a different physiological impact when activating these two types of non-NMDA glutamate receptors at central or peripheral nerve endings of sensory neurons.

Key words: dorsal root ganglion, action potential, presynaptic receptors, synaptic transmission, spontaneous EPSCs, capsaicin.

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Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ConA, concanavalin A; CTZ, cyclothiazide; DRG, dorsal root ganglion; EPSCs, excitatory postsynaptic currents; NGF, nerve growth factor; SEM, standard error of mean; sEPSCs, spontaneous EPSCs; TTX, tetrodotoxin.

Nociceptors are a heterogeneous group of sensory neurons that sense noxious, damage-causing stimuli in the periphery and transmit those signals to the CNS. They extend their processes peripherally and centrally from cell bodies that are located in the dorsal root ganglion (DRG). Nociceptive fibers innervate peripheral tissues such as skin and visceral organs and detect noxious thermal, chemical and mechanical stimuli. Centrally, fibers from these nociceptors enter the spinal cord through the dorsal root entry-zone and form synapses with dorsal horn neurons located in the superficial lamina of the spinal cord.

Nociceptors release glutamate from their central terminals. The glutamate activates postsynaptic ionotropic glutamate receptors for rapid excitatory communication with target dorsal horn neurons (Schneider and Perl, 1988; Yoshimura and Jessell, 1990). All three classes of ionotropic glutamate receptors, NMDA, AMPA, and kainate receptors, are expressed by dorsal horn neurons and mediate fast synaptic transmission at postsynaptic sites (Yoshimura and Jessell, 1990; Li et al., 1999).

Accumulating evidence demonstrates that not only spinal cord dorsal horn neurons but also peripheral sensory DRG neurons express ionotropic glutamate receptors. Functional kainate receptors are expressed by acutely dissociated, small diameter, DRG neurons (Huettner, 1990) in a defined population of IB4 positive nociceptors (Lee et al., 2001). Functional AMPA receptors are preferentially expressed near the presynaptic terminals of a subpopulation of peripherin positive nociceptors (Lee et al., 2002). Activation of presynaptic AMPA and kainate receptors causes a decrease in evoked release of glutamate from central terminals of nociceptors (Lee et al., 2002; Kerchner et al., 2001). Glutamate receptors also have been proposed to be transducers at the peripheral endings of nociceptors (Carlton et al., 1995; Jackson et al., 1995). Kainate and glutamate applied to exposed skin was shown to cause spinal reflexes in rat spinal cord-tail preparation (Ault and Hildebrand, 1993) and ultrastructural studies showed that AMPA and kainate receptor subunits are expressed in the peripheral axons of small diameter fibers in the rat and human skin (Coggeshall and Carlton, 1998; Kinkelin et al., 2000).

Because AMPA and kainate receptors display a distinct distribution and expression pattern (Lu et al., 2002; Chambille and Rampin, 2002; Lee et al., 2001, 2002), it is predicted that they would be engaged in different

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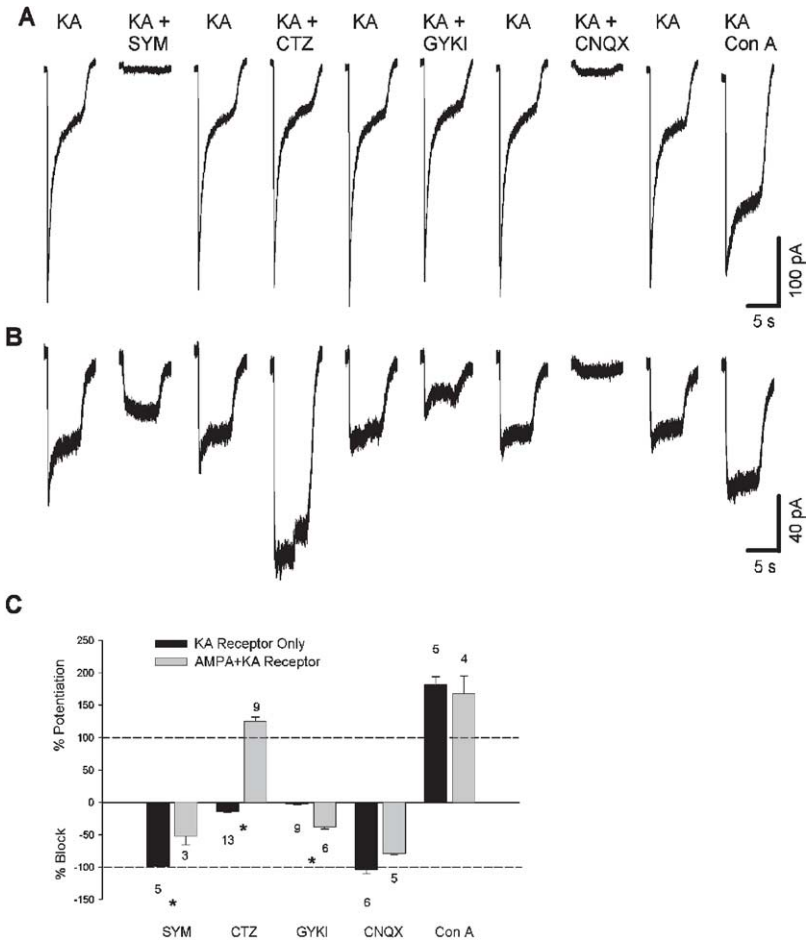

Fig. 1. Some DRG neurons in culture express AMPA and kainate receptors and some express only kainate receptors. (A) Whole-cell current responses to 100 μM kainate (KA) in this DRG neuron display a pharmacological profile suggesting activation of kainate receptors, with complete suppression by 3 μM SYM 2081, no potentiation by 100 μM CTZ, no block by 10 μM GYKI 53655, almost complete block with 250 μM CNQX, and potentiation with 5 min 25 μg/ml ConA pretreatment. (B) Responses to KA in a different DRG neuron show a pharmacological profile indicating the presence of both AMPA and KA receptors, with incomplete block by 3 μM SYM 2081, potentiation by 100 μM CTZ, partial block with 10 μM GYKI 53655, almost complete block with 250 μM CNQX, and potentiation after 250 μg/ml ConA pretreatment. Cells in this series of experiments were recorded using gramicidin D perforated patch configuration and held at −60 mV under voltage clamp; 0.5 μM TTX was present at all times. KA was applied at 100 μM, for 5 s, every 7–10 min, with or without indicated drugs. All the other drugs were applied at least 1 min prior to and during KA applications. (C) Results are summarized and expressed as a percent block or percent potentiation. Two types of DRG neurons were grouped based on their potentiation by CTZ (see Experimental Procedures). Asterisk (*) indicates a statistical significance by Student's *t*-test ($P<0.05$). Error bars indicate S.E.M. The number on top of a bar indicates the number of DRG neurons tested.

nociceptive processing in central terminals as well as in peripheral nerve endings. In addition, the known kinetic properties of AMPA and kainate receptor channels differ greatly (reviewed in Dingledine et al., 1999). Therefore, we have characterized the functional properties of these two types of receptors expressed by nociceptive DRG neurons. We have utilized the microisland co-culture system (Gu and MacDermott, 1997), in which DRG neurons and dorsal horn neuron are cultured together in isolation. The advantages of this system include fast drug access and precise identification of presynaptic and postsynaptic neurons. We have found robust expression of functional AMPA and kainate receptors in these cultured DRG neurons and observed clear differences in the functional properties of AMPA and kainate receptors expressed by cultured DRG neurons.

EXPERIMENTAL PROCEDURES

Preparation of microisland co-cultures

DRG and dorsal horn neurons were isolated from rat embryos age 16 days obtained in a manner approved by the Columbia University Institutional Animal Care and Use Committee. All experiments conform to the "Guide for the Care and Use of Laboratory Animals" published by the US Public Health Service. Briefly, pregnant rats were killed by CO_2 asphyxiation followed by cervical dislocation, and the embryos were removed and transferred to ice-cold Leibowitz-15 medium (Gibco, Grand Island, NY, USA). Isolated DRG and dorsal horns were mechanically dissociated after enzymatic digestion with 0.25% trypsin for 20 min. Dorsal horn and DRG neurons were plated together on glass coverslips previously prepared with rat cortical astrocytes (Albuquerque et al., 1999) on microislands (Segal and Furshpan, 1990; Gu and MacDermott, 1997). 2.5S nerve growth factor (NGF; 50 ng/ml) and 5-fluoro-2′-

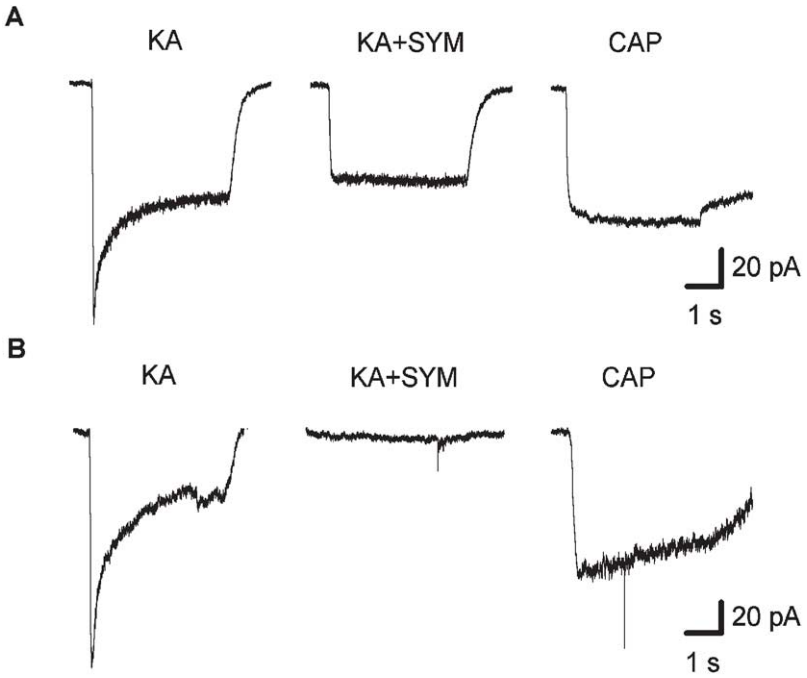

Fig. 2. Cultured DRG neurons expressing either kainate (KA) receptors only or AMPA and kainate receptors, also show sensitivity to capsaicin. (A) Partial block of the response to an 8 s application of 100 μM KA by 3 μM SYM 2081 indicates that this neuron expressed both KA and AMPA receptors; 10 μM capsaicin (CAP) induced an inward current indicating that this neuron is nociceptor-like (n=14). (B) The response to 100 μM KA by this neuron was completely blocked by SYM 2081 indicating that only KA receptors were present; 10 μM CAP induced an inward current (n=4). Currents were recorded using the whole cell patch configuration and membrane voltage was held at −60 mV. The bath contained 0.5 μM TTX throughout the recording period.

deoxyuridine (10 μM) were added at the time of plating. 2.5S NGF was added once every week when cells were fed with fresh media. The usual dorsal horn neuron density used was 10,000–30,000 neurons per dish and DRGs were plated at 30,000–50,000 neurons per dish. Neurons were used between 6 and 13 days in culture. DRG neurons were easily distinguished from dorsal horn neurons by their morphology; DRG neurons are larger in diameter with round, phase bright soma and their neurites are much finer than those of dorsal horn neurons (see Fig. 5A).

Electrophysiology and data analysis

Recordings from cultured DRG neurons were made with patch pipettes either in the gramicidin perforated-patch or whole-cell configurations. For perforated-patch voltage clamp recordings, internal solution contained 25 μg/ml gramicidin D, 75 mM Cs_2SO_4, 10 mM NaCl, 0.1 mM $CaCl_2$, and 10 mM HEPES, pH adjusted to pH 7.1 with CsOH and osmolarity adjusted to 310 mOsm with sucrose. For perforated-patch current clamp recordings, internal solution contained 25 μg/ml gramicidin D, 75 mM K_2SO_4, 10 mM KCl, 0.1 mM $CaCl_2$, and 10 mM HEPES, pH adjusted to pH 7.1 with KOH and osmolarity adjusted to 310 mOsm with sucrose. For whole-cell voltage clamp recordings, internal solution contained 130 mM $CsMeSO_3$, 10 mM NaCl, 0.5 mM $CaCl_2$, 5 mM EGTA, and 10 mM HEPES, pH adjusted to pH 7.3 with CsOH and osmolarity adjusted to 310 mOsm with sucrose. Pipette resistances ranged from 3 to 5 MΩ. For perforated patch, it took 20–30 min to achieve acceptable perforation, with final series resistances ranging from 15 to 40 MΩ. Drug solutions were applied to cells by local perfusion through a capillary tube (1.1 mm inner diameter) positioned near the cell of interest. The solution flow was driven by gravity and controlled by miniature solenoid valves (The Lee Company, Westbrook, CT, USA). The speed of drug application was measured at around 20–50 ms. Membrane currents or voltages were recorded using Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA), filtered at 2 kHz and digitized at 2–5 KHz with pClamp 6 acquisition software (Axon Instruments). External bath solution contained 145 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 10 mM HEPES, 2 mM $MgCl_2$, and 5.5 mM glucose at pH 7.3 and 325 mOsm. In whole-cell current recordings, 0.5 μM tetrodotoxin (TTX) was used to block any unclamped action potentials.

Whole-cell agonist-evoked currents were analyzed using Mini Analysis Program (Synaptosoft, Inc., Decatur, GA, USA). Peak amplitudes were calculated as the difference between baseline measured before drug application and the peak current amplitude during drug application. End-of-drug current amplitudes were calculated as the difference between the baseline and current amplitude just before the end of drug application. For calculations of percent block by antagonists or percent potentiation by cyclothiazide (CTZ) or concanavalin A (ConA), end-of-drug amplitudes of kainate-evoked currents in the presence of antagonists, CTZ or after exposure to ConA were compared with the control values and expressed as a percentage. In order to monitor and compensate for the effect of rundown, kainate was applied between each kainate plus drug application and was used for comparison. Each cell was put into one of two groups based on potentiation by CTZ, a drug that suppresses AMPA receptor desensitization (Wong and Mayer, 1993). If the kainate response was potentiated by CTZ, the neurons were assumed to express AMPA receptors and their data were placed in the AMPA and kainate receptor group (n=9/22). If the response to kainate was not potentiated by CTZ or even depressed as previously reported (Wong and Mayer, 1993), then the DRG neuron was assumed to express only kainate receptors and the data were placed in the kainate receptor only group (n=11/22). Two cells (n=2/22) had a small potentiation (10% and 21%) of the kainate response by CTZ, but they showed either insensitivity to GYKI or a complete block by SYM 2081 and thus were placed in the kainate receptor only group.

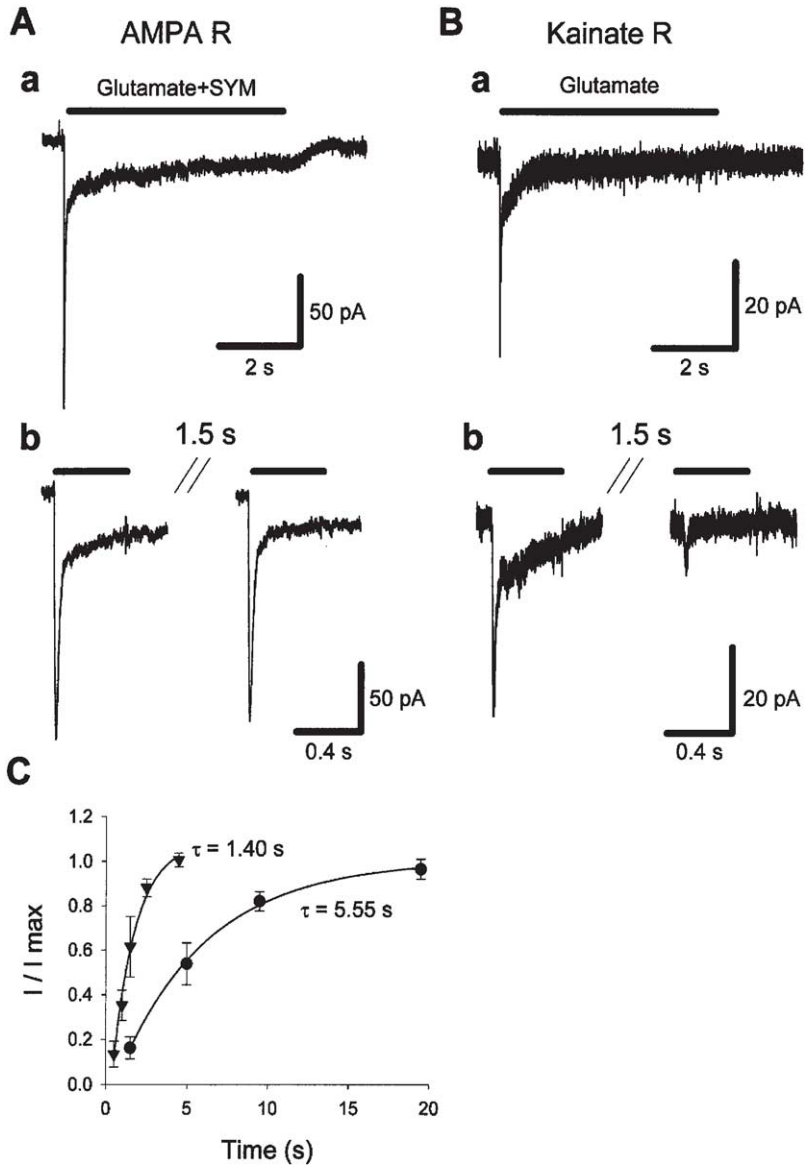

Fig. 3. The rapidly desensitizing response to glutamate mediated by AMPA receptors in DRG neurons has a faster recovery and larger steady state current than the response mediated by kainate receptors. (Aa) In this example, a 5 s application of 5 mM glutamate plus 3 μM SYM 2081 caused an inward current with a fast initial peak, rapidly desensitizing time course, and substantial steady-state current. (Ab) Using the same neuron as in Fig. 5Aa, glutamate plus SYM 2081 was applied for 500 ms two times with a time interval of 1.5 s from the end of the test pulse to the beginning of the second pulse. The second response recovered almost fully. (Ba) A different neuron expressed only kainate receptors as demonstrated by a complete block of kainate response with SYM 2081 (not shown). Glutamate was applied at 5 mM without SYM 2081. (Bb) Glutamate was applied twice for 500 ms with an interval of 1.5 s. The second response showed a small recovery at this interval. (C) Recovery from desensitization was assessed by varying the time interval between the two 500 ms glutamate applications. The degree of recovery was expressed as the fraction of the second peak over the peak of test pulse. Calculated fractions from DRG neurons at each interval were averaged across the AMPA receptor activation (triangles, $n=4-6$) and kainate receptor activation (filled circles, $n=4-5$). The error bars indicate the S.E.M. The resulting distributions were fitted with first order exponential function, $Y=1-Ae^{(-x/\tau)}$, where A is the initial amplitude and τ is the time constant of recovery. This and subsequent series of experiments are all done under gramicidin D perforated patch configuration. The bath contained 0.5 μM TTX throughout the recording period.

For current clamp recordings, all of the action potentials were detected using Mini Analysis Program (Synaptosoft). The numbers of action potentials for each second of stimulation were counted for each cell, normalized to the total number of action potentials, averaged for each group, and then plotted. Recordings of spontaneous synaptic activity were also analyzed by using Mini Analysis Program v5.6. The amplitude threshold was set at 10 pA for detection. Control baseline recordings were from 5 to 3 min for each cell and the average frequency and amplitude during drug application were compared with the baseline frequency and amplitude. The changes in frequency and amplitude were expressed as percent of control.

Synaptic responses from DRG and DH pairs were recorded after sequentially patching both cells under current clamp for DRG neuron and voltage clamp for DH neuron. Once the series resistance for each patch reached under 50 MΩ, DRG neuron was stimulated by injecting current ranging from 1 nA to approximately 10 nA for 1 ms to evoke an action potential. Appearance of a synaptic response in

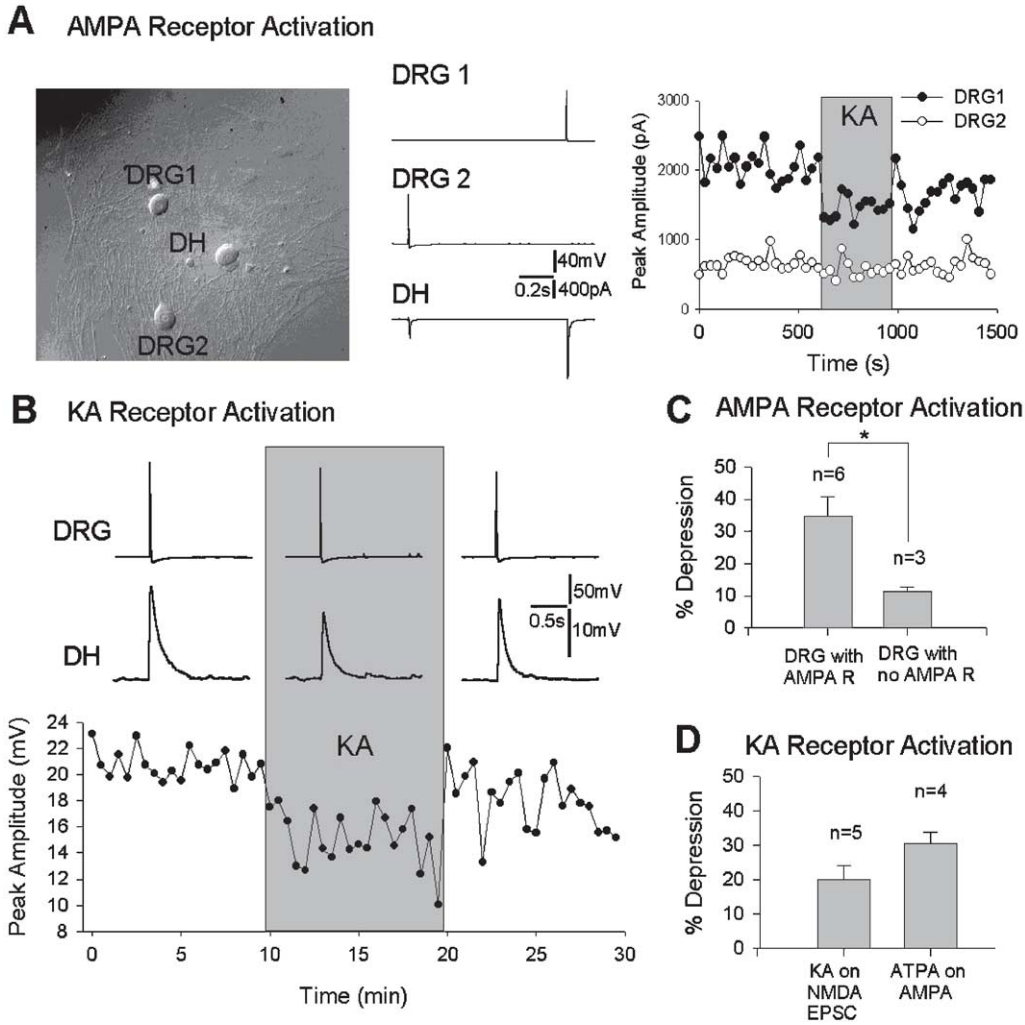

Fig. 5. Activation of presynaptic AMPA and kainate receptors reduces evoked EPSC amplitudes in pairs of DRG and dorsal horn neurons grown on microisland co-cultures. (A) Selective activation of presynaptic AMPA receptors was achieved by adding 3 μM SYM 2081. In this example, three neurons were patched and recorded simultaneously. DRG1 and DRG2 shown in the picture were stimulated at different times and EPSCs were recorded from the dorsal horn neuron (DH). The middle panel shows the raw traces of DRG1 and DRG2 under current clamp and DH under voltage clamp. Peak amplitude vs. time plot shows that 5 μM kainate applied in the presence of SYM 2081 caused a significant depression of EPSC amplitude from DRG1 (27% depression) but not from DRG2 (7% depression). Both DRG neurons were then tested for the presence AMPA receptors by applying 100 μM kainate; DRG2 did not express AMPA receptors while DRG1 did. (B) Selective activation of presynaptic kainate receptors was achieved by adding 0.5 μM kainate in the presence of 100 μM GYKI53655. Bath solution contained 0 Mg^{2+}, 10 μM bicuculline, 5 μM strychnine, and 0.5 μM TTX. The raw traces are shown for both the DRG and DH neurons. Amplitude vs. time plot shows that 0.5 μM kainate caused reduction in the amplitude of postsynaptic responses by 28%. (C) Results for AMPA receptor activation are summarized for nine cell pairs including DRG neurons with and without AMPA receptors recorded in the presence of SYM 2081. The average depression of the EPSC amplitudes from pairs with AMPA receptor-expressing DRG neurons ($n=6$) was significantly greater than from those without ($n=3$) as indicated by the (* $P<0.05$). (D) Results for kainate receptor activation are summarized. The EPSC amplitude from each pair tested was significantly depressed in the presence of kainate and ATPA ($P<0.05$).

DH neuron within 10 ms of latency after the stimulus ensured a synaptic connection between the two cells. Then DRG neuron was stimulated periodically with a 10–20 s interval for AMPA receptor mediated EPSCs and 20–30 s interval for NMDA receptor-mediated synaptic responses to ensure full recovery. At least 10 baseline responses were obtained before application of kainate or ATPA for activation of presynaptic kainate receptor and kainate in the presence of SYM 2081 for activation of presynaptic AMPA receptors. Percent depression was calculated by averaging the amplitude of last five synaptic responses during the drug application and comparing it to the baseline average. Synaptic pairs displaying no recovery were discarded from analysis. To determine the presence of AMPA receptors in DRG neurons, 100 μM kainate was applied in the presence of 3 μM SYM 2081 after the synaptic recordings, and the amount of depolarization from DRG neuron was assessed similarly as in Fig. 4.

All the average values are expressed as mean±S.E.M.

Materials

GYKI 52466, and CTZ were purchased from RBI (Natick, MA, USA). Kainate, CNQX, ATPA and SYM 2081were purchased from Tocris Cookson, (Ballwin, MO, USA). NGF was from Boehringer Mannheim (Germany). Glutamate, ConA, gramicidin D, and other chemicals were from Sigma (St. Louis, MO, USA). GYKI 53655 was kindly provided by Eli Lilly (Indianapolis, IN, USA)

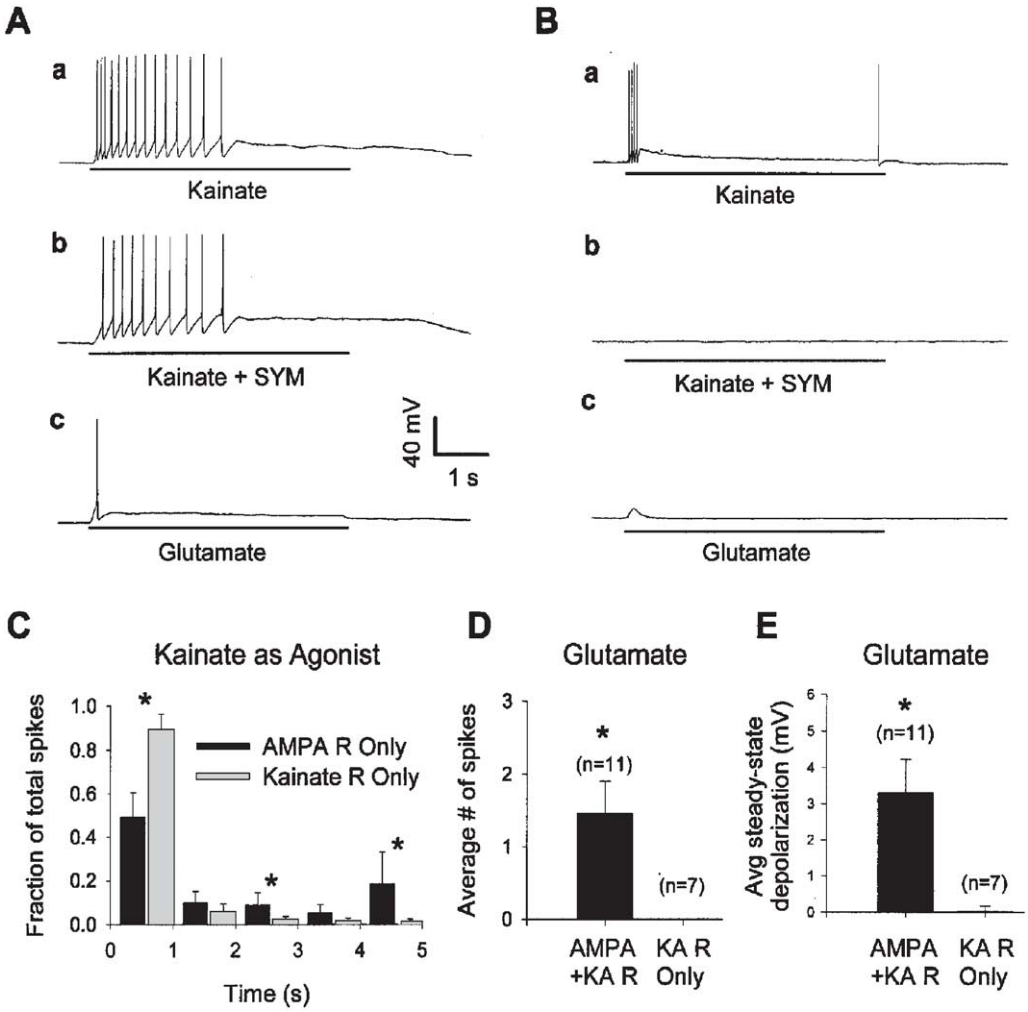

Fig. 4. Selective activation of AMPA and kainate receptors induces different action potential firing patterns in cultured DRG neurons. (A) In this DRG neuron showing expression of both AMPA and kainate receptors, (a) 100 μM kainate induced action potential firing (b) also in the presence of 3 μM SYM 2081; (c) 5 mM glutamate also induced action potential firing from this cell. (B) In another DRG neuron showing the expression of only kainate receptors, (a) kainate induced a burst of action potential firing at the beginning of kainate application. (b) This response was completely blocked by 3 μM SYM 2081. (c) Glutamate did not induce action potential firing. All the drugs were applied for 5 s. Membrane voltages were recorded under perforated-patch, current clamp configuration. (C) Each kainate response was grouped into one of two categories; one for kainate receptor activation ($n=6$) and the other for AMPA receptor activation ($n=6$; See Experimental Procedures and Results). Each bar represents the average number of action potentials during each second of drug application expressed as the fraction of the total number of action potentials. The error bars represent S.E.M. The asterisk (*) indicates significant difference ($P<0.05$) when tested with Student's *t*-test. (D) Each cell was categorized as expressing both AMPA and kainate receptors (AMPA+KA R) or kainate receptors only (KA R) and the total number of action potentials evoked by glutamate were counted and averaged across each group. The neurons that expressed AMPA+KA R showed a significantly larger number of action potentials (1.45±0.48, $n=11$) than neurons that expressed KA R only, which did not show any action potentials (mean=0, $n=7$). (E) Glutamate-induced steady-state depolarization was measured at the end of 5 s glutamate applications for each neuron and then averaged for each group. AMPA+KA R group (3.31±0.48 mV, $n=11$) showed significantly greater degree of steady-state depolarization than KA R Only group (0.03±0.14 mV, $n=7$). The error bars indicate S.E.M. The asterisk (*) indicates significant difference ($P<0.005$) when tested with Student's *t*-test.

RESULTS

Expression of AMPA and kainate receptors by cultured embryonic DRG neurons

Kainate, like glutamate, is an effective agonist for both the AMPA and kainate receptor families. Kainate has half-maximal activation (EC50) for AMPA receptors ranging from 23 to 142 μM as determined in a wide variety of preparations (Egebjerg and Heinemann, 1993; Hollmann and Heinemann, 1994; Huettner, 1990; Patneau et al., 1993; Reichling and MacDermott, 1991; Wong and Mayer, 1993; Yoshimura et al., 1991). The EC_{50} of kainate for native kainate receptors expressed by DRG neurons is around 15 μM (Huettner, 1990; Wong and Mayer, 1993). Used together with other compounds known to show high selectivity for AMPA or kainate receptors, kainate is a good agonist to use to test for the expression of AMPA and kainate receptors because it induces less desensitization of both receptors than glutamate (Huettner, 1990; Patneau and Mayer, 1991; Wilding and Huettner, 1997). Kainate at 100 μM was able to induce whole cell currents from almost all of the embryonic DRG neurons maintained in culture

that were tested (two examples in Fig. 1A, B). The kainate-induced currents recorded at a holding potential of −60 mV displayed a wide range of peak amplitudes ranging from −35 to −337 pA (n=23).

Pharmacological tools were used to probe the identity of the receptors underlying the kainate-induced currents. The experiments revealed two types of responses to kainate by subpopulations of cultured DRG neurons. One type of response was mediated solely by kainate receptors and is illustrated by the data shown in Fig. 1A. In this example, the response to 100 μM kainate was blocked by pre-application of 3 μM SYM 2081, a strongly desensitizing kainate receptor agonist (Jones et al., 1997; Zhou et al., 1997; Lee et al., 2001). SYM 2081 was bath applied for at least 1 min prior to application of 100 μM kainate together with SYM 2081 (KA/SYM), resulting in loss of kainate response. Subsequent application of kainate in the presence of 100 μM CTZ, an AMPA receptor selective modulator (Wong and Mayer, 1993), did not increase the kainate-induced current amplitude, suggesting that there was no detectable AMPA receptor-mediated component in the kainate-induced current in those neurons (n=11; Fig. 1A). GYKI 53655, a selective AMPA receptor non-competitive antagonist, produced little effect on the current amplitude or profile, also suggesting that there was no AMPA receptor-mediated component (n=9). At 10 μM, GYKI 53655 should produce more than 90% block of AMPA receptors but less than 5% inhibition of kainate receptors (Wilding and Huettner, 1997). The non-NMDA receptor antagonist, CNQX (250 μM), blocked most of the kainate-induced current, although not completely (n=6). Finally, pretreatment of the same neuron with 250 μg/ml ConA caused the current response to kainate to be much less desensitizing due to its irreversible block of desensitization of kainate receptors (n=5; Wong and Mayer, 1993). Because ConA acts selectively on kainate receptors, this is further support for the interpretation of the data in Fig. 1A as showing only kainate receptor expression by the DRG neuron under study.

In contrast, Fig. 1B illustrates the second type of response recorded from cultured embryonic DRG neurons. In this case, responses to kainate include two components, one mediated by kainate receptors and the other by AMPA receptors. Again this was demonstrated pharmacologically. Only part of the kainate-induced current, including the rapidly desensitizing component, was blocked by 3 μM SYM 2081. The remaining current in kainate plus SYM 2081 showed a non-desensitizing time course that is typical of kainate-induced AMPA receptor-mediated currents (n=3; see also Fig. 2A; Wong and Mayer, 1993). Both ConA (n=4) and CTZ (n=9) greatly enhanced the steady state current and 10 μM GYKI 53655 partially blocked the kainate-induced current (n=6). All of these data are consistent with the interpretation that this neuron expressed both AMPA and kainate receptors.

To analyze the population responses to kainate by cultured DRG neurons, we grouped the neurons into two groups based on their sensitivity to CTZ (see Experimental Procedures). The summary of the actions of SYM 2081, GYKI 53655, CNQX and ConA grouped in this way are shown in Fig. 1C. As expected, SYM 2081 produced a significantly greater block of the kainate-induced current in the kainate receptor only group while GYKI 53655 was significantly more potent at inhibiting kainate-induced currents from the AMPA and kainate receptor group. Both groups showed a strong potentiation with ConA indicating the expression of kainate receptors by both sets of DRG neurons.

In a separate series of experiments, we tested for capsaicin-induced currents in cultured DRG neurons to assess whether nociceptor-like neurons (Gold et al., 1996) express kainate or AMPA receptors. Twenty-seven DRG neurons were tested for expression of kainate receptors, AMPA receptors and capsaicin sensitivity. Of the DRG neurons tested, 89% expressed sensitivity to 1 or 10 μM capsaicin (n=24/27). Of the capsaicin positive DRG neurons in culture, 100% expressed kainate receptors (n=24/24) and 71% expressed AMPA receptors (n=17/24). In these experiments, kainate receptors were identified by full or partial block of kainate current by SYM 2081 or by a direct response to SYM 2081 (Fig. 2A, B). AMPA receptors were identified by observing kainate-evoked current in the presence of SYM 2081 (Fig. 2A, middle panel). Three cells were not responsive to capsaicin although they were sensitive to kainate. These data indicate that many of the embryonic DRG neurons expressing functional AMPA and kainate receptors in culture are likely to be nociceptors.

Differential effects of glutamate on activation of AMPA and kainate receptors

Because glutamate is the most likely endogenous ligand for AMPA and kainate receptors, we have tested the effect of glutamate on activation of AMPA and kainate receptors. Glutamate, at the saturating concentration of 5 mM for both AMPA and kainate receptors (Patneau and Mayer, 1990; Huettner, 1990) induced whole cell currents with a fast rise to peak and rapid desensitization kinetics (Fig. 3). Selective activation of AMPA receptors was achieved by exposing neurons to glutamate in the presence of 3 μM SYM 2081 (Fig. 3Aa). Selective activation of kainate receptors was specified when a glutamate response (Fig. 3Ba) was later completely suppressed by SYM 2081 (not shown). Glutamate activation of AMPA receptors induced a substantial steady-state current after 5 s of glutamate application (Fig. 3Aa; 15.5±3.6 pA, n=4) compared with the activation of kainate receptors, which showed almost complete desensitization (Fig. 3Ba; 1.6±0.6 pA, n=4). The presence of steady-state current at the end of a 5 s application indicates that glutamate action on AMPA receptors is more sustained than on kainate receptors.

Next we examined whether there is a difference between AMPA and kainate receptors in the time required to recover from desensitization to glutamate. In previous reports, kainate receptors expressed by DRG neurons had biphasic recovery kinetics with time constants of 36 ms and 4.7 s (Swanson and Heinemann, 1998). Trussell and Fischbach (1989) reported a recovery time constant of 89 ms for AMPA receptors on spinal cord neurons. Based on

these reports, we expected to see a substantial difference in recovery time between AMPA and kainate receptors after desensitization to glutamate. Fig. 3 Ab and Bb shows the responses to 500 ms applications of glutamate in two different DRG neurons. When two glutamate applications were separated by a 1.5 s interval and AMPA receptors were selectively activated by glutamate in the presence of SYM 2081, the current recovered substantially after 1.5 s (n=4; Fig. 3Ab), suggesting that most of the AMPA receptors readily recovered. However, when kainate receptors were activated by glutamate, only a small fraction of the current recovered within 1.5 s (n=4; Fig. 3Bb), indicating that most of the kainate receptors were still in the desensitized state. To assess the time constant of recovery, the time interval between the conditioning pulse and the test pulse was varied, and the resulting currents were expressed as a fraction of test current over the conditioning current. The resulting plot in Fig. 3C shows that recovery of AMPA receptors from activation by glutamate was much faster than recovery of kainate receptors. The peak response to AMPA receptor activation recovered with a time constant of 1.4 s compared with 5.5 s for kainate receptors when the results were fitted with a first order exponential function. Given our relatively slow drug delivery (20–50 ms), we may draw only qualitative conclusions about recovery time constants obtained in our experimental settings. Nevertheless, there is a clear separation in recovery kinetics between AMPA and kainate receptors.

Selective activation of AMPA and kainate receptors causes action potential firing

We have compared the ability of kainate and glutamate to drive firing of action potentials following selective activation of AMPA and kainate receptors. Under current clamp, application of 100 μM kainate to cultured DRG neurons caused a strong depolarization that led to action potential firing (Fig. 4). In Fig. 4A, kainate was applied to a DRG neuron that expressed both kainate and AMPA receptors. It caused firing of action potentials that was sustained for the first few seconds of kainate application. When kainate was applied in the presence of SYM 2081(Fig. 4Ab), action potential firing remained strong for a similar duration, consistent with the minimal desensitization of AMPA receptors when activated by kainate. Glutamate at 5 mM evoked an action potential and a sustained steady-state depolarization in this neuron (Fig. 4Ac). Even though both kainate and AMPA receptors were expressed by this neuron, the sustained steady-state component in Fig. 4Ac was due mainly to AMPA receptor activation because kainate receptors on DRG neurons desensitized rapidly and almost completely with glutamate activation (Figs. 3Ba, 4Bc).

In a DRG neuron expressing only kainate receptors, kainate caused a burst of action potential firing at the beginning of drug application (Fig. 4Ba), suggesting that kainate activation is strongest during the earliest period of agonist application. This is consistent with the larger initial peak current and stronger desensitization mediated by kainate receptors compared with AMPA receptors (Figs. 1A, 1B, 2A, 2B). Evidence that only kainate receptors were expressed by this neuron is shown in Fig. 4Bb where kainate applied in the presence of 3 μM SYM 2081 did not cause any depolarization or action potential firing. Glutamate caused a strongly desensitizing depolarization that did not evoke any action potential firing (Fig. 4Bc and 4E; n=7). Glutamate was not always able to induce action potential firing even in neurons expressing both AMPA and kainate receptors (n=3/11), and these were included in the calculation of average number of spikes. It is important to note that the responses to agonist application will be influenced by the intrinsic excitability of the neurons, the kinetics of the drug application system as well as the kinetics of the receptors in response to different agonists.

To compare the action potential firing pattern due to activation of kainate or AMPA receptors, we pharmacologically isolated each receptor type. The kainate receptor-mediated action potential firing pattern was assessed from recordings performed in the presence of 10 μM GYKI 53655 (n=3/11) or recordings that showed complete block by SYM 2081 (n=8/11). AMPA receptor-mediated firing pattern was measured from the recordings performed in the presence of SYM 2081 (n=9). The number of action potentials occurring in 1 s intervals for the first 5 s of kainate application was counted, normalized to the total number of action potentials occurring in 5 s, averaged across cells, and plotted in Fig. 4C. This figure suggests that AMPA receptors may contribute to action potential firing more in the later phase of activation, and that kainate receptors contribute more in the early phase of activation during prolonged exposure to agonist.

The effect of glutamate on action potential firing and membrane potential is summarized in Fig. 4D and 4E. When glutamate was applied to neurons that expressed both AMPA and kainate receptors, it elicited an average of 1.45±0.46 action potentials (n=11), whereas glutamate did not elicit any action potentials in neurons that expressed only kainate receptors (Fig. 4D; n=7). Glutamate also induced a substantial and significantly different depolarization at the end of a 5 s application of glutamate in the neurons that expressed both AMPA and kainate receptors compared with kainate receptor only neurons (Fig. 4E). These data show that AMPA receptor activation by glutamate induces action potential firing and that the effect of glutamate mediated by AMPA receptors is much more prolonged than its effect on kainate receptors.

Selective activation of AMPA and kainate receptors depresses evoked postsynaptic responses

We have previously provided direct evidence for expression of functional AMPA and kainate receptors at or near central terminals of nociceptive DRG neurons in acutely prepared spinal cord slice (Lee et al., 2002). To investigate whether AMPA and kainate receptors expressed on the DRG terminals influence glutamate release in a more isolated system, we used microisland configurations of DRG and dorsal horn neurons grown in co-culture. The microisland co-culture system is useful because we can easily identify DRG and dorsal horn neurons and, by using paired

cell recording, be certain that the synapse under study is monosynaptic.

First we examined the effect of selective AMPA and kainate receptor activation on evoked EPSCs. We chose microislands with only one dorsal horn neuron and one or more DRG neuron (example shown in Fig. 5A). Under these conditions, synaptic contributions from other dorsal horn neurons are minimized, even though contributions from autaptic connections cannot be ruled out. A synaptic pair of DRG and dorsal horn neurons was obtained by patching both neurons simultaneously under perforated-whole-cell configuration. The DRG neuron was patched under current-clamp and a brief current injection was used to elicit action potentials. Simultaneously, a dorsal horn neuron was patched under voltage-clamp control to detect postsynaptic currents. Selective activation of AMPA receptors was achieved by applying kainate in the presence of 3 μM SYM 2081. Kainate was used at low concentrations, between 0.5 and 10 μM, which rarely evoked action potentials in the absence of current steps in DRG neurons. The effect on synaptic transmission was monitored by assessing changes in AMPA receptor-mediated EPSC amplitudes. Kainate receptors were selectively activated by applying either kainate in the presence of 100 μM GYKI 53655 to monitor changes in postsynaptic responses mediated by NMDA receptors, or a GluR5 selective agonist, ATPA (Clarke et al., 1997; Hoo et al., 1999; Kerchner et al., 2001) to monitor changes in postsynaptic responses mediated by AMPA receptors.

Selective activation of AMPA receptors on a DRG neuron by kainate caused a decrease in the amplitude of evoked EPSCs (Fig. 5A). In this example, recordings were made from three neurons simultaneously; one dorsal horn neuron (DH) and two DRG neurons (DRG1 and DRG2) located nearby. Upon stimulation, both DRG neurons evoked EPSCs in the dorsal horn neuron under study. Five micromolar kainate applied in the presence of SYM 2081 caused a significant depression of EPSC amplitude from DRG1 (27% depression) but not from DRG2 (7% depression). DRG1 was later shown to express AMPA receptors by direct response to 100 μM kainate in the presence of SYM 2081, whereas DRG2 did not (not shown). This suggests that the main effect of kainate is due to activation of presynaptic AMPA receptors. Only a small portion of the EPSC amplitude depression is due to postsynaptic kainate action as indicated by the minimal depression of EPSC amplitude from the DRG neuron not expressing AMPA receptors. This is also consistent with a kainate-induced decrease in mEPSC amplitude observed in the presence of TTX (see Fig. 6C). Overall, however, the observed depression of EPSC amplitude under these conditions was significantly greater when the DRG neurons expressed AMPA receptors (34.5±6.0%, $n=6$, $P<0.05$) than when they did not (11.2±1.6%, $n=3$; Fig. 5C). The presence of a small but significant depression in the absence of AMPA receptors in DRG neuron (Fig. 5C) suggests activation of postsynaptic AMPA receptors by low concentrations of kainate.

The impact of selective activation of presynaptic kainate receptors on glutamate release was similarly tested in microisland co-cultures. Some of these experiments (e.g. Fig. 5B) were performed by applying kainate in the presence of GYKI 53655 to block AMPA receptors. Therefore we recorded synaptic activity mediated by NMDA receptors. To optimize the NMDA receptor mediated responses, Mg^{2+} was eliminated from the bath solution to relieve the Mg^{2+} block of NMDA receptors. The NMDA receptor-mediated responses were assessed by recording either postsynaptic potentials or currents. Because we used microislands with multiple dorsal horns neurons for these recordings, we added bicuculline and strychnine to eliminate any contribution of $GABA_A$ and glycine receptors. Furthermore, we included 0.5 μM TTX to block any evoked release of transmitters from other dorsal horn neurons. Under these conditions, we could still evoke glutamate release from the DRG terminals due to the presence of TTX resistant Na^+ channels as described in our previous study (Gu and MacDermott, 1997).

As shown in Fig. 5B, 0.5 μM kainate application in the presence of GYKI 53655 decreased the amplitude of NMDA receptor-mediated postsynaptic potentials by 28%, indicating that kainate receptor activation decreases release of glutamate from DRG terminals. We sometimes observed kainate receptor-mediated response in the dorsal horn neurons, manifested as a change in the holding current during kainate application. Such synaptic pairs were not included in the analysis. The results from this series of experiments are summarized in Fig. 5D for five DRG and dorsal horn synaptic pairs. In summary kainate significantly depressed the amplitude of synaptic responses by an average of 20±4% ($n=5$, $P<0.05$) compared with the baseline. In a separate series of experiments, we recorded AMPA receptor-mediated EPSCs from DH neurons while activating presynaptic kainate receptors by applying 5 μM ATPA. ATPA also significantly depressed the amplitude of EPSCs (30.7+3.0%, $n=4$, $P<0.005$) compared with the baseline, consistent with the results of kainate-induced depression of NMDA receptor-mediated postsynaptic responses.

These data are consistent with the recent reports on presynaptic inhibition of glutamate release by activation of AMPA and kainate receptors expressed at the central terminals of nociceptors (Lee et al., 2002; Kerchner et al., 2001). Our results here provide more direct support for the inhibitory action of presynaptic AMPA and kainate receptors on glutamate release because they were performed on an isolated system in which a synaptic pair of DRG and dorsal horn neurons was confined to a microisland. Experiments in the two previous studies were performed on slice preparations or mass culture systems in which many secondary effects may have complicated the interpretation of results.

Activation of AMPA and kainate receptors causes an increase in the frequency of spontaneous EPSCs

We examined the impact of AMPA and kainate receptor activation on spontaneously occurring release of transmitter from DRG nerve terminals. For this set of experiments, we specifically selected microislands with only one dorsal horn and one or more DRG neuron to minimize the contribution of glutamate release from dorsal horn terminals.

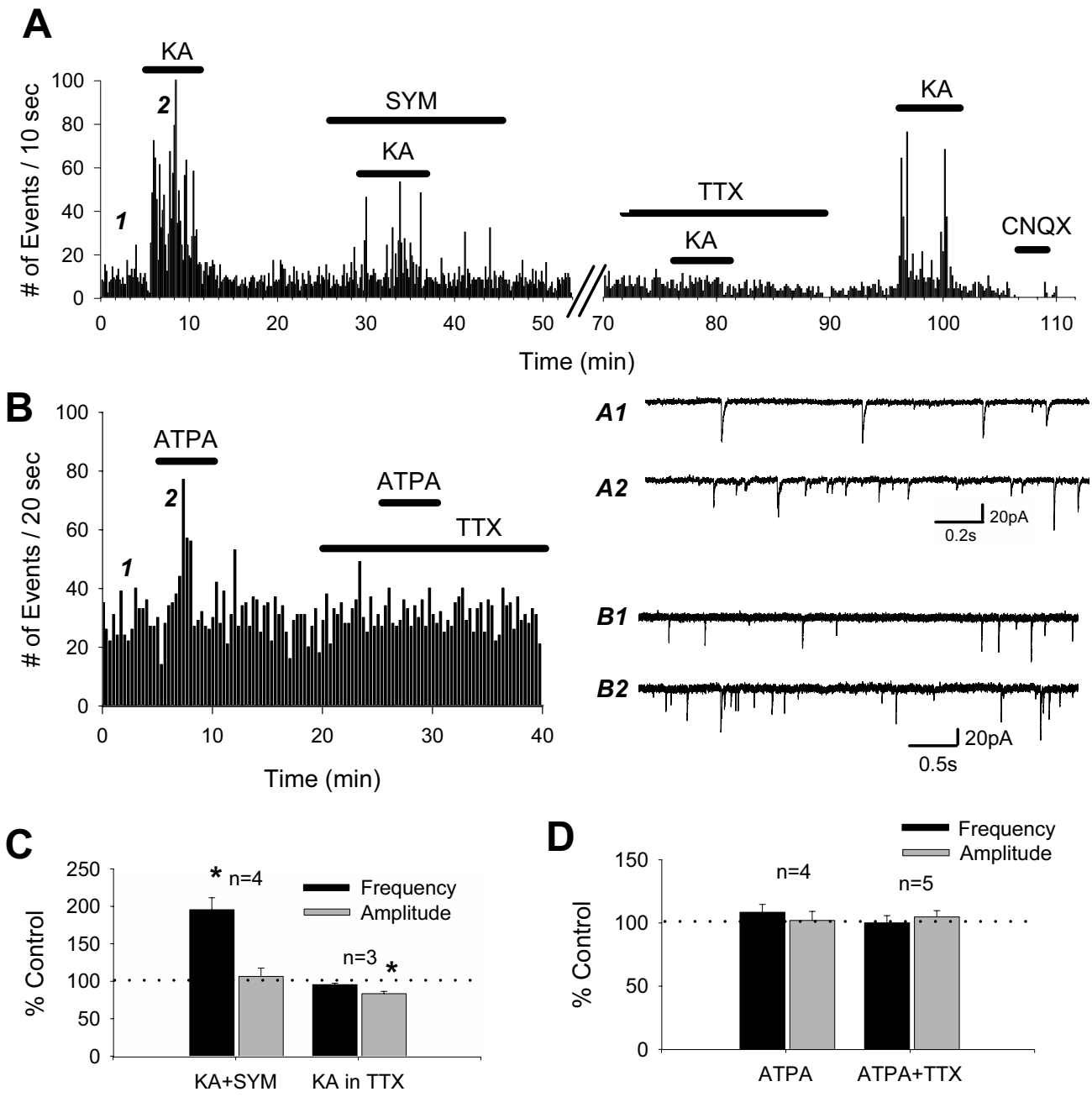

Fig. 6. Activation of AMPA receptors increases frequency of spontaneous EPSCs and this effect is TTX sensitive. (A) A microisland with one dorsal horn and one or more DRG neurons is selected and the dorsal horn is patched. The spontaneous EPSCs are recorded and the frequency of activity is monitored and plotted as the number of events per 10 s. Drugs are applied at times indicated by horizontal bars. Selective activation of presynaptic AMPA receptor is achieved by applying kainate in the presence SYM 2081. (B) Selective activation of presynaptic kainate receptor is achieved by applying 3 μM ATPA. Raw traces are shown for time point A1 (before kainate) and A2 (during kainate), and B1 (before ATPA) and B2 (during ATPA). (C) Data are pooled to generate this summary bar graph. The changes from control baseline values of frequency and amplitudes are expressed as percent control and averaged across number of cells indicated above each set of bars. Plot shows an AMPA receptor mediated decrease in the frequency but not amplitude. It also shows that there is a decrease in amplitude of EPSCs in TTX. (D) Data for ATPA applications are summarized. ATPA was tested at 3 and 5 μM.

Spontaneous EPSCs (sEPSCs) were recorded from individual dorsal horn neurons. As shown in Fig. 6A, application of 5 μM kainate caused an immediate increase in the frequency of sEPSCs. The average amplitudes of control sEPSCs ranged from 20 to 50pA. The kainate-induced increase in frequency persisted in the presence 3 μM SYM 2081, indicating that AMPA receptor activation was driving a portion of this increase. However, when kainate was applied in the presence of 0.5 μM TTX, the increase in frequency was blocked. The sEPSCs were completely

blocked by 50 μM CNQX, indicating that the sEPSCs were mediated by post-synaptic AMPA receptors and that the released transmitter was glutamate.

To activate presynaptic kainate receptors expressed by DRG neurons selectively, we have utilized a GluR5 selective agonist, ATPA. In one example, ATPA at 3 μM caused a slight increase in the frequency of sEPSCs, while the amplitude was not significantly changed (Fig. 6B). This increase in frequency by ATPA was no longer present when TTX was included in the bath (Fig. 6B). With other cell pairs, however, ATPA at 5 μM was not able to elevate sEPSC frequency (Fig. 6D).

The effect of AMPA and kainate receptor activation on sEPSCs is summarized in Fig. 6C and 6D, respectively. The average changes in frequency and amplitude of sEPSCs are plotted in bar graphs as percent of control. Kainate (1–5 μM) in the presence SYM 2081 caused about two-fold increase in the frequency of sEPSCs (195±61%, $n=4$) while not significantly affecting the amplitude (106±11%, $n=4$). In the presence of TTX, kainate no longer induced an increase in the frequency of sEPSCs but instead caused a slight reduction in the frequency (94±3% of control) and a significant reduction in amplitude (85±5%, $n=3$). In contrast, ATPA (3 or 5 μM) on average caused a small but not significant increase in frequency (108±6%, $n=4$) and amplitude (101±7, $n=4$) of sEPSCs. In the presence of TTX, ATPA produced no increase in frequency (100±6%, $n=5$) or amplitude (104±5%, $n=5$). These data suggest that low concentrations of kainate activate presynaptically localized AMPA receptors causing an increase in frequency of spontaneous release of glutamate from the DRG terminals.

DISCUSSION

Functional AMPA and kainate receptor expression by cultured DRG neurons

Essentially all embryonic DRG neurons in culture express kainate receptors and over half of those neurons express AMPA receptors. In previous experiments, we demonstrated functional AMPA and kainate receptor expression near central terminals of sensory neuron on different subpopulations of DRG neurons (Lee et al., 2001, 2002). Nevertheless, we found less than 1% of DRG cell bodies acutely dissociated from postnatal day 3–9 rats express functional AMPA receptors. The reason for more frequent detection of functional AMPA receptors in cultured embryonic DRG neurons reported here compared with the acutely dissociated DRG preparation may be because DRG neurons extend their neurites in culture and form extensive synaptic connections (Gu and MacDermott, 1997). Thus, functional AMPA receptors may be expressed preferentially on distal neurites of DRG neurons in culture where they will be functionally detectable. The acutely dissociated postnatal neurons have their neurites removed. Recent immunocytochemical studies indicate that AMPA receptor subunits are widely distributed in DRG neurons (Lu et al., 2002; Chambille and Rampin, 2002), consistent with the frequent detection of functional AMPA receptors in culture. However, it is also possible that AMPA receptor expression, along with other cellular markers, becomes more promiscuous under culture conditions.

Similarities and differences in functional properties of AMPA and kainate receptors on DRG neurons

Glutamate is an endogenous ligand for AMPA and kainate receptors. The consequences of glutamate activation of DRG neurons through AMPA and kainate receptors depend on the kinetic properties of these receptors as well as the time course of elevated glutamate concentrations in the vicinity of the receptors. Glutamate causes rapid and nearly complete desensitization of kainate receptors (Huettner, 1990; Wilding and Huettner, 1997), whereas AMPA receptor activation by glutamate has been shown to cause a variable degree of steady-state current (Patneau and Mayer, 1990; Sommer et al., 1990). Even at saturating concentrations, however, glutamate activation of AMPA receptors on DRG neurons was associated with a substantial steady-state current while glutamate activation of kainate receptors on DRG neurons produces almost fully desensitizing current. Thus, in our studies, AMPA receptors more potently induced action potential firing in the presence of elevated glutamate concentrations. At the same time, sustained depolarization mediated by AMPA receptors in the continued presence of glutamate might dampen action potential propagation to the central terminals of the primary afferents depending on the degree of depolarization and cellular location of the receptors (Engelman and MacDermott, 2004).

Considering the differences in depolarization kinetics and firing patterns, the contribution of AMPA and kainate receptors to generation of action potential firing patterns and dorsal root reflexes is expected to be different. Recently, the dorsal root reflex was shown to be mediated not only by GABA and $GABA_A$ receptors but also by glutamate and non-NMDA receptors (Russo et al., 2000; Lee et al., 2002). Based on the response of DRG neurons to glutamate shown here, AMPA receptors would be expected to contribute much more to the generation of dorsal root reflexes and primary afferent depolarizations than kainate receptors, especially in the prolonged presence of glutamate.

AMPA receptors expressed by central neurons display a more rapid recovery from desensitization (Patneau and Mayer, 1991; Trussell and Fischbach, 1989) than kainate receptors on DRG neurons (Huettner, 1990; Wong and Mayer, 1993; Swanson and Heinemann, 1998). Similarly, we have shown a substantial difference in the time constants of recovery from desensitization between AMPA and kainate receptors both expressed on DRG neurons when glutamate was used as the agonist. A consequence of the more rapid recovery time for AMPA receptors is that it may further enhance the robust effect of glutamate on action potential firing and steady-state depolarization mediated by AMPA receptors. When transient elevations of glutamate concentration occur at central terminals of the DRG neurons (Russo et al., 2000; Lee et al., 2002), the

faster recovery time constant for AMPA receptors is expected to make them more readily available for repeated activation than is possible for kainate receptors. These differences between AMPA and kainate receptors in their response to glutamate suggest they are likely to play different roles in sensory transmission.

Despite the differences in functional properties of receptor activation and recovery from desensitization, AMPA and kainate receptors both cause depression of glutamate release from DRG terminals when activated by exogenously applied kainate. Activation of presynaptic AMPA and kainate receptors similarly inhibit the evoked release of glutamate in acute spinal cord slice preparations (Lee et al., 2002; Kerchner et al., 2001). Our data for AMPA receptor-mediated inhibition of transmitter release is consistent with the recent report by Satake et al. (2000), who showed that glutamate released from climbing fibers activates presynaptic AMPA receptors to depress GABA release from neighboring interneuron terminals onto Purkinje cells in the cerebellum. The authors eliminated possible involvement by G-protein-coupled receptors or intracellular second messengers. Similarly, our data suggest that the inhibition of glutamate release by kainate displayed fast onset and fast recovery making the involvement of metabotropic mechanism unlikely. In addition, the involvement of $GABA_B$ receptor is not likely because we selected microislands with only one dorsal horn neuron (only possible source of GABA) before patching, although autaptic release of GABA is still possible. In our previous reports, we observed that presynaptic inhibition persisted in the presence of a cocktail containing blockers for GABAA, GABAB, glycine, P2X, A1, and group I, II, III metabotropic glutamate receptors (Lee et al., 2002).

Interestingly, we have observed that activation of AMPA receptors by kainate causes a TTX-sensitive increase in the frequency of sEPSCs without a change in amplitude. The TTX sensitivity of the frequency increase suggests synaptic involvement of TTX-sensitive voltage gated Na^+ channels on the presynaptic DRG neuron. Activation of presynaptic AMPA receptors may depolarize the membrane sufficiently to recruit voltage-gated Na^+ channels. This could result in frank action potential generation or more simply further depolarize the terminals, allowing Ca^{2+} entry through voltage-gated Ca^{2+} channels and enhancing spontaneous glutamate release. Prolonged exposure to kainate may inactivate TTX sensitive Na^+ channels as well as voltage-gated Ca^{2+} channels. This could explain how evoked release is inhibited under prolonged exposure to kainate. Consistent with this idea, kainate activation of presynaptic kainate receptors on mossy fibers terminals decreases action potential-induced Ca^{2+} elevation at these terminals (Kamiya and Ozawa, 2000).

If the AMPA receptors expressed near glutamate release sites are Ca^{2+} permeable, it is expected that their activation will elevate local Ca^{2+} concentration and increase the quantal release of glutamate. In fact, activation of presynaptic AMPA receptors causes a TTX-insensitive increase in frequency of miniature IPSCs by cerebellar stellate cells (Bureau and Mulle, 1998) and by dorsal horn interneurons (Engelman, Anderson and MacDermott, unpublished observation). The presence of GluR2 subunit renders AMPA receptor Ca^{2+} impermeable (reviewed in Seeburg and Hartner, 2003). The Ca^{2+} permeability of AMPA receptors expressed by DRG neurons is not known but variable expression of the GluR2 subunit among DRG neurons (Lee et al., 2002) suggests Ca^{2+} permeability is variable. The lack of increase in frequency of sEPSCs in the presence TTX suggests that presynaptic AMPA receptors are Ca^{2+}-impermeable under our culture conditions or not near transmitter release sites.

The role of AMPA and kainate receptors at the peripheral and central terminals of sensory neurons

Accumulating data suggest that AMPA as well as kainate receptors participate in sensory transduction on the peripheral terminals of nociceptors. In behavioral tests, injection of non-NMDA receptor agonists into peripheral tissue was shown to cause hyperalgesia that was blocked by non-NMDA receptor antagonists (Carlton et al., 1995; Jackson et al., 1995). Peripheral injection of kainate receptor selective antagonist, SYM 2081 was shown to attenuate the allodynia and hyperalgesia in a freeze-injury model (Ta et al., 2000). Furthermore, glutamate is released peripherally during formalin-induced inflammation (Omote et al., 1998) and during low and high intensity sciatic nerve stimulation (deGroot et al., 2000). Taken together, these reports suggest that peripheral fibers of nociceptive DRG neurons express functional AMPA and kainate receptors and that glutamate is released from either the peripheral terminals or surrounding tissue to activate them.

Ault and Hildebrand (1993) demonstrated that kainate and glutamate applied to the exposed skin of rat tails cause spinal reflexes in the rat spinal cord–tail preparation. Selective antagonists able to distinguish AMPA and kainate receptors were not available so the identity of the receptors mediating the response was not clear. It was assumed that kainate receptors mediated the peripheral excitation because only functional kainate receptors were known to be present at the soma of DRG neurons (Huettner, 1990). However, it is possible that AMPA receptors were partly responsible for the generation of spinal reflexes at peripheral nerve endings. This hypothesis is supported by the fact that AMPA and quisqualate also induced partial spinal reflexes compared with kainate or glutamate (Ault and Hildebrand, 1993). In addition, glutamate-evoked responses were similar in duration and magnitude to those evoked by kainate. We would expect much smaller duration and magnitude if the responses were solely mediated by kainate receptors because of kainate receptor's rapid and nearly complete desensitization to glutamate. In our experiments, AMPA receptors induced more robust and prolonged action potential firing than kainate receptors in DRG neurons. Future studies testing selective agonists and antagonists in spinal cord–tail preparation will determine the involvement of AMPA receptors in the peripheral sensory processing at the peripheral terminals.

Presynaptic AMPA and kainate receptors expressed on central terminals of sensory neurons in the spinal cord

consistently play an inhibitory role in nociceptive processing. These receptors might function as autoreceptors for glutamate released by the primary afferents. Alternatively, they may receive excitatory synaptic inputs from dorsal horn interneurons. It is plausible that selective activation of presynaptic AMPA and kainate receptors might be a potential target for analgesic agents. In addition, the differences in current kinetics and action potential firing pattern suggest that AMPA and kainate receptors would contribute very differently to the generation of dorsal root reflexes, spinal activities that have been implicated in the generation of neurogenic inflammation (Willis, 1999).

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