Activation of Kainate Receptors on Rat Sensory Neurons Evokes Action Potential Firing and May Modulate Transmitter Release

C. JUSTIN LEE,[a] H. S. ENGELMAN, AND A. B. MACDERMOTT

Department of Physiology and Cellular Biophysics and
Center for Neurobiology and Behavior, Columbia University,
630 West 168th Street, New York, New York 10032, USA

Evidence for functional presynaptic kainate receptors in the cortex and spinal cord has been slowly accumulating over the last 20 years. Recently, glutamate release from CA1 hippocampal pyramidal neurons was shown to be transiently enhanced, then depressed by selective activation of presynaptic kainate receptors.[1] GABA release is also depressed by activation of presynaptic kainate receptors.[2,3] *In vivo* expression of kainate receptors by nociceptive primary afferents was suggested by recordings from isolated rat dorsal-root preparations[4,5] and acutely dissociated dorsal root ganglia (DRG) neurons.[6] However, the role of kainate receptors in the regulation of synaptic transmission in the superficial dorsal horn where nociceptive primary afferents terminate remains uncertain. Using microisland cocultures of DRG and dorsal horn neurons and acutely prepared spinal cord slices, we have begun to investigate the possible presence of presynaptic kainate receptors and their function in regulating synaptic transmission onto dorsal horn neurons. We demonstrate here that pharmacologically defined kainate receptors are expressed by DRG neurons grown in coculture with dorsal horn neurons, that kainate receptor activation of DRG neurons elicits a burst of action potential firing, and that application of kainate to the spinal cord slice increases the frequency of spontaneous postsynaptic currents (sPSCs) recorded from dorsal horn neurons.

DRG and dorsal horn neurons were harvested from E16 embryos, dissociated, and plated on top of previously prepared astrocytes growing on collagen-coated microislands. Cultures were grown for at least a week before recording. DRG neuron recordings were made using perforated patch technique with 25 μg/mL gramicidin and (mM) 75 K_2SO_4, 10 KCl, 0.1 $CaCl_2$, 10 HEPES, pH 7.2. Spinal cord slices were acutely prepared from postnatal day 7–10 rats. Slices were 400 μm thick and lamina II was visually identified. Whole-cell patch recordings were made using (mM) 140 Cs-methanesulfonate, 10 HEPES, 10 NaCl, 5 EGTA, 0.5 $CaCl_2$, pH 7.2.

Application of 100 μM kainate evoked an inward current from DRG neurons in coculture voltage clamped at −60 mV. As shown in FIGURE 1A, the inward current had both transient and steady-state components, as originally described for kainate currents in DRG neurons by Huettner.[6] CNQX (250 μM), a non-NMDA antagonist, mostly blocked the kainate-evoked current. It was completely blocked by applying 3 μM SYM 2081, a selec-

[a]Address for correspondence: C. Justin Lee, Department of Physiology, Columbia University, 630 West 168th Street, New York, New York 10032. Phone: 212/305-3817; fax: 212/305-3723; e-mail: cjl2@columbia.edu

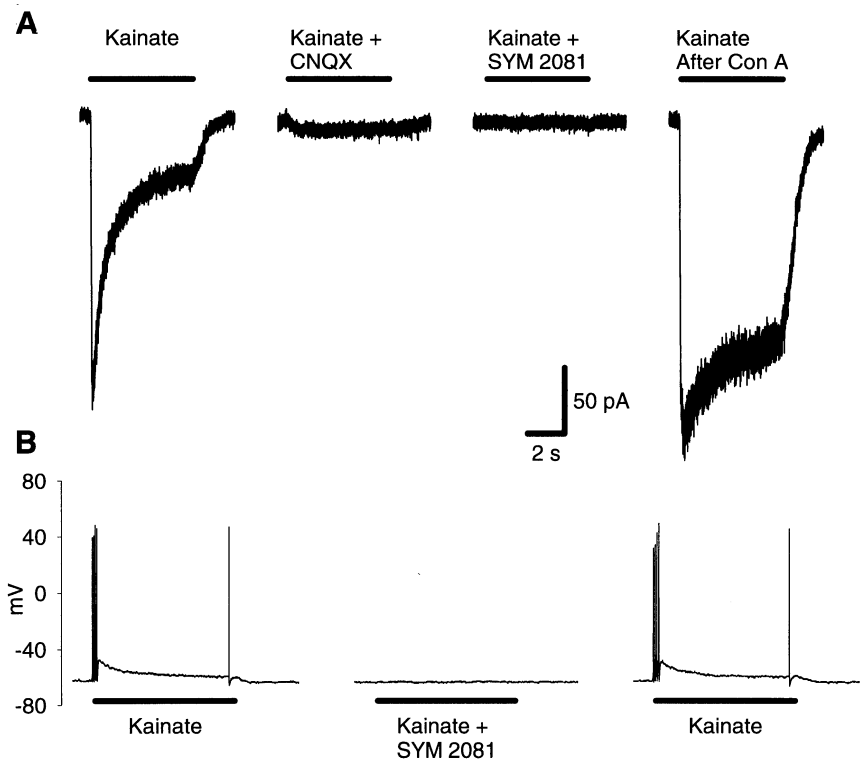

FIGURE 1. Kainate evokes whole-cell currents **(A)** and action potential firing **(B)** in cultured DRG neurons by activating kainate receptors. **(A)** 5-s application of 100 μM kainate evoked a desensitizing current, which could be mostly blocked by 250 μM CNQX, completely blocked by 3 μM SYM 2081, and enhanced by 5-min pretreatment with 300 μg/ml Con A. Holding potential: −60 mV. **(B)** Under current clamp, a 5-s application of 100 μM kainate elicited a burst of action potentials at the onset of the application, which is completely and reversibly blocked by 3 μM SYM 2081.

tive kainate receptor desensitizing agonist, for 1 minute before and during kainate application. These data indicate that the kainate-induced current was mediated exclusively by kainate receptors. To further confirm receptor identity, 300 μg /mL of Concanavalin A (ConA) was added to the bath for 5 min before kainate application, and the subsequent kainate-induced current was greatly enhanced (FIG. 1A). ConA has been shown to selectively block desensitization of kainate receptors.[7] Thus these data provide strong evidence for the expression of kainate receptors by DRG neurons grown in coculture with dorsal horn neurons. Under current clamp conditions, a 5-s application of 100-μM kainate to a DRG neuron elicited a burst of action potential firing, an effect that was reversibly blocked by SYM 2081 (FIG. 1B). These data suggest that activation of DRG neurons through kainate receptors is potentially able to drive action potential-evoked transmitter release from DRG central and peripheral terminals.

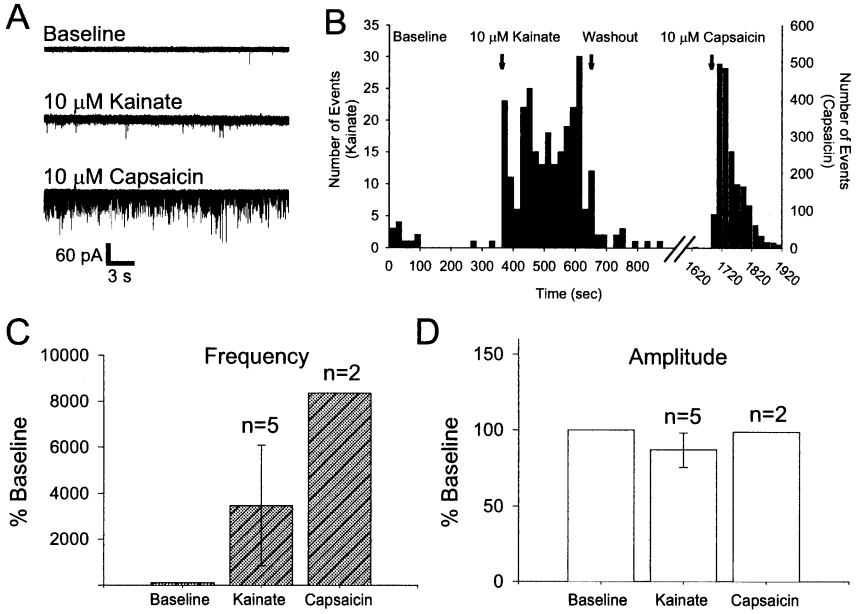

FIGURE 2. Kainate and capsaicin induce an increase in the frequency of spontaneous dorsal horn neuron PSCs in the presence of TTX. **(A)** Representative traces are shown from recordings in normal bath, 10 μM kainate, and 10 μM capsaicin from a single dorsal horn neuron held at −70 mV. Recordings are from a transverse spinal cord slice taken from a P8 rat. **(B)** For the same neuron as in (**A**), the number of PSCs is plotted versus time (*bars* represent the number of events over 20-s intervals). Note difference in scale for kainate and capsaicin. **(C)** Average PSC frequency was calculated over 4–5 min under baseline conditions. Changes in frequency in the presence of kainate and capsaicin determined over 4–5 min were calculated as a percent of baseline. The average percent of baseline was then computed for all cells tested and plotted (for kainate, $n = 5$, for capsaicin, $n = 2$). **(D)** PSC amplitudes were measured over the same periods as for frequency and expressed as percent of baseline.

We have begun to investigate the effect of kainate on spontaneous release of transmitter onto lamina II neurons in the postnatal rat dorsal horn. This region of the spinal cord receives inputs from cutaneous nociceptive primary afferents that express Ca^{2+}-permeable capsaicin receptors on their central terminals.[8] Capsaicin was used to confirm that the dorsal horn neurons under study with kainate received input from nociceptors by recording both the kainate- and capsaicin-induced change in frequency of spontaneous PSCs from dorsal horn neurons. Bath application of 10 μM kainate to a lamina II neuron in the presence of 0.5 μM TTX increased the sPSC frequency 17-fold, suggesting the presence of presynaptic receptors sensitive to kainate (FIG. 2A and 2B). After washout of kainate, sPSC frequency decreased back to baseline levels. Subsequent bath application of 10 μM capsaicin increased sPSC frequency 163-fold. This change in sPSC frequency in response to capsaicin indicates that this neuron received innervation from primary afferents. sPSC frequency was expressed as a percentage of baseline and averaged across cells (FIG. 2C).

Both kainate and capsaicin caused dramatic and significant, though widely variable, increases in sPSC frequency with no significant change in amplitude (FIG. 2D).

In these studies, kainate receptors were pharmacologically identified on cultured DRG neurons. Activation of kainate receptors elicited a burst of action potential firing, raising the possibility that if kainate receptors are expressed presynaptically on DRG terminals, they could initiate action potential firing and transmitter release when activated by glutamate. The kainate-induced increase in frequency of sPSCs recorded from lamina II neurons in spinal cord slices supports our hypothesis of a role for presynaptic kainate-sensitive receptors in the regulation of synaptic transmission in the nociceptive pathway.

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