Kainate Acts at Presynaptic Receptors to Increase GABA Release From Hypothalamic Neurons

QING-SONG LIU, PETER R. PATRYLO, XIAO-BING GAO, AND ANTHONY N. VAN DEN POL
Department of Neurosurgery, Yale University Medical School, New Haven, Connecticut 06520

Liu, Qing-Song, Peter R. Patrylo, Xiao-Bing Gao, and Anthony N. van den Pol. Kainate acts at presynaptic receptors to increase GABA release from hypothalamic neurons. J. Neurophysiol. 82: 1059–1062, 1999. Recent reports suggest that kainate acting at presynaptic receptors reduces the release of the inhibitory transmitter GABA from hippocampal neurons. In contrast, in the hypothalamus in the presence of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-d-aspartate (NMDA) receptor antagonists [1-(4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466) and D,L-2-amino-5-phosphonopentanoic acid (AP5)], kainate increased GABA release. In the presence of tetrodotoxin, the frequency, but not the amplitude, of GABA-mediated miniature inhibitory postsynaptic currents (IPSCs) was enhanced by kainate, consistent with a presynaptic site of action. Postsynaptic activation of kainate receptors on cell bodies/dendrites was also found. In contrast to the hippocampus where kainate increases excitability by reducing GABA release, in the hypothalamus where a much higher number of GABAergic cells exist, kainate-mediated activation of transmitter release from inhibitory neurons may reduce the level of neuronal activity in the postsynaptic cell.

INTRODUCTION

Kainate receptors are expressed widely throughout the brain (Herb et al. 1992; Hollmann and Heineman 1994), including the hypothalamus (van den Pol et al. 1994), the focus of the present experiments. Although ionotropic glutamate receptors are generally considered as postsynaptic receptors on the cell body or dendrites of neurons, recent evidence suggests that kainate can activate a presynaptic receptor and that activation of this receptor inhibits transmitter release from glutamatergic (Chittajalul et al. 1996) and GABAergic neurons (Clarke et al. 1997; Rodriguez-Moreno et al. 1997) in the hippocampus.

In the present study, we used whole cell recordings of hypothalamic neurons in culture and in brain slices to examine kainate responses on hypothalamic neurons. In contrast to previous reports based on neurons from other regions of the brain, we find that kainate evokes an increase in the release of the inhibitory transmitter GABA by activating receptors that appear to be on the axon terminal.

METHODS

Slice recording

Hypothalamic slices (400 μm thick) containing the arcuate and ventromedial nuclei were prepared and maintained from postnatal day 7–8. Methylenedioxymethylamphetamine (MDMA) was used as a excitatory neurotransmitter. The data were analyzed using the Clampfit 9.0 program.

RESULTS

Kainate increases GABA activity in hypothalamic slices

In the presence of 1-(4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466) (100 μM) and AP5 (50 μM), kainate (1 μM) increased the mean frequency of spontaneous IPSPs from 4.6 ± 0.8 events/s (mean ± SE; range, 1.4–8.4 events/s) to 5.9 ± 1.1 events/s (range, 1.9–11 events/s; n = 9 neurons; P < 0.04; paired t-tailed t-test; Fig. 1). When we used a minimum change criterion of 20% in IPSP frequency, we found that kainate reversibly increased the frequency of GABA-mediated PSPs in five of nine neurons to 157 ± 11.1% of baseline activity (P < 0.03; paired t-tailed t-test). In three of three neurons the experiment was repeated with the same protocol and the same results were obtained.
same response. In the remaining four neurons, a nonsignificant change in the frequency of spontaneous IPSPs was observed with kainate (97 ± 6% of baseline; P = 0.4). Nonresponding cells were distributed evenly in both the arcuate and ventromedial nucleus.

Glutamate and GABA actions in cultured hypothalamic neurons

After 2–3 wk in culture, virtually all hypothalamic neurons showed spontaneous GABA-mediated synaptic currents. At a holding potential of −70 mV, excitatory postsynaptic currents (EPSCs) were inward currents that were blocked by the non-NMDA receptor antagonist CNQX (10 μM, n = 7), and IPSCs were barely visible (Fig. 2A). At a holding potential of 0 mV, IPSCs were outward currents that were blocked by the GABA_A receptor antagonist bicuculline (10 μM, n = 7), whereas EPSCs were barely visible (Fig. 2B). At a holding potential of −70 mV, EPSCs were completely and reversibly blocked by the selective AMPA receptor antagonist GYKI 52466 (100 μM, n = 7; Fig. 2A), indicating that AMPA receptors are a primary mediator of spontaneous glutamate activity in the hypothalamus. Inhibitory PSCs reversed at −75 mV, typical of GABA-mediated events under these conditions (Fig. 2C).

Kainate increases spontaneous IPSCs

In the presence of GYKI 52466 (100 μM), kainate (10 μM, 3 min) reversibly increased the spontaneous IPSC frequency to 273 ± 41% of baseline (baseline = 100%; P < 0.01, paired t-test) in 9 of 10 neurons (Fig. 2, D and E). This effect had a rapid onset and often desensitized to a stable value within 1–2 min. This stable value was still higher than the baseline fre-
DISCUSSION

In hippocampal neurons, activation of kainate receptors caused a decrease in transmitter release from axon terminals (Lerma 1997). In contrast, our data show that kainate presynaptically causes an increase in GABA release from cultured hypothalamic neurons. We do not view our data as contradicting earlier reports; rather, our data appear to indicate that the actions of kainate may be strongly dependent on neuronal type; hippocampal neurons show a decrease, whereas hypothalamic neurons show a substantial increase in transmitter release in response to presynaptic actions of kainate. These appear to be the first data showing that a kainate receptor generally exerts an enhancing effect on transmitter release at a presynaptic site. This may be of significant and fundamental importance related to how glutamate may regulate general brain activity. GABA is found in a large number of hypothalamic neurons (Tappaz et al. 1982), is present in at least half of all presynaptic boutons (Decavel and van den Pol 1990), and acts as the primary transmitter mediating inhibition in the hypothalamus (Kim and Dudek 1992; Randle et al. 1986; Tasker and Dudek 1993) including the arcuate nucleus (Belousov and van den Pol 1997). That kainate can enhance GABA release in hypothalamic neurons would have the ultimate result of increasing inhibition by greater activation of GABA receptors on the postsynaptic cell. The absence of a kainate-mediated decrease in GABA release in hypothalamic neurons is interesting given that many presynaptic neuromodulator receptors (e.g., neuropeptide Y, GABA$_B$, mGluRs) act to depress hypothalamic transmitter release through different mechanisms (Chen and van den Pol 1996, 1998; Obrietan and van den Pol 1998).

Neurons of the hypothalamic arcuate nucleus are involved in secretion of pituitary tropins, and release of these tropins is facilitated by bursting patterns of action potentials. On a speculative note, the enhanced GABA inhibition evoked by kainate may hyperpolarize the postsynaptic neuron, and a negative membrane potential has been suggested as a mechanism to modulate the burst-responsiveness of arcuate neurons to synaptic input (MacMillan and Bourque 1993); whether these bursting cells receive axonal input responsive to kainate remains to be determined. A large number of neuroactive substances are found in different neurons of the arcuate nucleus, many colocalized with GABA (Meister and Hokfelt 1988). Although kainate receptor mRNAs have been found in hypothalamic neurons with in situ hybridization (van den Pol et al. 1994), the transmitter phenotype of the neurons that express kainate receptors and what presynaptic effect kainate has in these circuits remains to be determined. Because kainate receptors may be on presynaptic axon terminals or the postsynaptic somato-dendritic area of hypothalamic neurons, or both, the cellular location of the receptors in specific circuits would be critical for determining the action of activated kainate receptors.

Previous reports found a decrease in transmitter release with kainate activation of presynaptic receptors in hippocampal neurons in slice and culture (Clarke et al. 1997; Rodriguez-Moreno et al. 1997), whereas we find an increase in GABA release with kainate activation of hypothalamic neurons. This raises the question as to what might be the mechanism for this difference. A recent paper (Rodriguez-Moreno and Lerma 1998) suggested that kainate inhibited transmitter release by a presynaptic mechanism was based on activation of a G protein by an ionotropic receptor; kainate activated protein kinase C (PKC), which lead to phosphorylation of a protein involved in transmitter exocytosis and thus inhibited release. Although in that case an increase in PKC was suggested to mediate a
decrease in transmitter release, other reports have shown that a PKC increase can lead to an increase in GABA (Capogna et al. 1995) and glutamate release (Malenka et al. 1986). In fact, when Rodriguez-Moreno and Lerma (1998) first treated their hippocampal neurons with phorbol esters, kainate application caused an increase in GABA release. The increase in GABA release in hypothalamic neurons could be due to either a different set of proteins being phosphorylated by PKC activation, or if the idea that there are two different pools of PKC (Rodriguez-Moreno and Lerma 1998) has credence, then hypothalamic neurons may have a greater pool of the PKC that enhances neurotransmission. We recently described a parallel mechanism whereby strong activation of protein kinase A would lead to an inhibition of GABA activity in hypothalamic neurons, but a small increase could enhance GABA activity (Obrietan and van den Pol 1997). Alternatively, kainate may activate a different subset of kainate receptors in hypothalamic neurons that are coupled differently to G proteins in these cells.

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Address for reprint requests: A. N. van den Pol, Dept. of Neurosurgery, Yale University Medical School, 333 Cedar St., New Haven, CT 06520.

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