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# κ-Opioid Receptor Activation of a Dendrotoxin-Sensitive Potassium Channel Mediates Presynaptic Inhibition of Mossy Fiber Neurotransmitter Release

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**SUMMARY**

Activation of κ-opioid receptors on mossy fiber terminals in the hippocampus inhibits excitatory amino acid release. The mechanism of presynaptic inhibition at the mossy fiber synapse was investigated through whole-cell voltage-clamp of CA3 pyramidal cells. The application of a κ-opioid agonist, U69593, reduced the amplitude of the excitatory postsynaptic current response, and this effect was reversed with a κ receptor antagonist. Presynaptic potassium channels were blocked by bath application of channel toxins, and the effect of κ receptor activation was tested. The inhibition caused by U69593 was blocked by low doses of 4-aminopyridine (30 μM) and the selective peptide toxins dendrotoxin and mast cell degranulating peptide. The inhibition was not blocked by low doses of tetraethylammonium chloride (1 mM), barium, or glibenclamide. Thus, we conclude that presynaptic κ-opioid receptors are coupled to a Shaker-type voltage-dependent potassium channel that is sensitive to dendrotoxin and mast cell degranulating peptide. An increase in presynaptic potassium conductance would enhance the rate of repolarization after action potential invasion, thereby limiting calcium influx and neurotransmitter release. This is the first physiological demonstration of the involvement of a dendrotoxin-sensitive potassium current in presynaptic inhibition mediated by a G protein-coupled receptor.

Dynorphin peptides are endogenous ligands for κ-opioid receptors, which are important regulators of excitatory transmission in areas such as the hippocampus, striatum, brainstem, cerebellum, and spinal cord (1). In the hippocampus, κ-opioid receptors are localized on presynaptic terminals, and their activation inhibits excitatory amino acid release (2, 3). The mechanism by which this presynaptic inhibition is accomplished in the hippocampus has not yet been elucidated; however, several mechanisms have been proposed. For example, receptor activation may inhibit the opening of voltage-dependent calcium channels or activate specific potassium channels. Presynaptic inhibition also may occur via mechanisms downstream of voltage-dependent ion channel regulation (4).

Although in other cell types, such as dorsal root ganglion neurons, κ receptors inhibit calcium influx through N-type calcium channels (5), we reported previously that neither N-type nor L-type calcium channel antagonists block the presynaptic effects of κ receptors in the hippocampus (6). The possibility that κ receptors inhibit transmitter release by increasing presynaptic potassium efflux is supported by studies reporting that κ receptors can couple to somatic potassium channels in several systems (7–10).

In this study, we used selective potassium channel blockers to determine the pharmacological profile of the presynaptic ion conductance mediating the κ-opioid effect. By recording evoked EPSCs in guinea pig CA3 pyramidal cells with the use of the whole-cell voltage-clamp technique, we determined that the presynaptic inhibition caused by κ-opioid activation is likely mediated by a voltage-dependent potassium channel that is sensitive to 4-AP, DTx, and MCDP. These data have been presented in preliminary form (11).

## Materials and Methods

Hippocampal slices were prepared as described previously (6). Briefly, transverse hippocampal slices (500 μm) were made from male Hartley guinea pigs (Simonsen Labs, Gilroy, CA) with a Campden Vibroslicer. Slices were then submerged in the recording chamber, warmed to 34°, and continuously perfused with oxygenated Krebs-bicarbonate buffer containing 120 mM NaCl, 3.5 mM KCl, 1.3

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**ABBREVIATIONS:** EPSC, excitatory postsynaptic current; nBNI, norbinaltorphimine; 4-AP, 4-aminopyridine; TEA, tetraethylammonium chloride; DTx, dendrotoxin; MCDP, mast cell degranulating peptide; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GIRK, G protein-activated inwardly rectifying potassium; $K_{ATP}$, ATP-gated potassium current; $V_h$, holding potential.

mM $MgCl_2$, 2.5 mM $CaCl_2$, 1.25 mM $NaH_2PO_4$, 25.6 mM $NaHCO_3$, and 10 mM) glucose. EPSCs were evoked with a bipolar stimulating electrode (0.1 mm contact diameter; Kopf) placed in the dentate granule cell layer to stimulate mossy fibers (Fig. 1A). Stimuli were delivered as 0.3-msec square waves, 10–90 μA, at ~0.2 Hz. Responses were recorded in the whole-cell mode with glass microelectrodes (3–5 MΩ) filled with an intracellular solution that blocked postsynaptic potassium currents and maximized recording stability over long periods of time (90–120 min; modified from Ref. 12). This solution contained 120 mM CsF, 10 mM CsCl, 10 mM EGTA, 10 mM HEPES, and 5 mM Mg-ATP, pH adjusted to 7.2–7.3 with CsOH (osmolarity, 260–270 mOsM). Cells were held at −70 mV, except in a few experiments, as noted.

Evoked responses were considered to be mediated primarily by mossy fibers if they met criteria described previously (13) [i.e., if they could be elicited by low-intensity stimulation of the granule cells (≤40 μA), appeared at a characteristically short and constant latency, and had a smooth rising phase]. At various times during each experiment, current-voltage relationships were measured with 20-mV steps from −120 mV to +20 mV. Current-voltage curves were generated from the steady state currents and used to monitor the stability of the recording. Experiments were excluded from analysis if the capacitance was <3 pF, the series resistance was >20 MΩ, or the series resistance changed by ≥20% during opioid application.

Cells were allowed to stabilize for ~15 min after establishing the whole-cell configuration. A potassium channel blocker was then bath applied, and EPSCs were measured after ~40 min. Perfusion with the potassium channel blocker continued for the duration of the experiment. The selective κ agonist U69593 (1 μM) was bath applied next, and responses were measured after 15–20 min. Whenever possible, the selective κ antagonist nBNI (100 nM) was then bath applied, and responses were again measured after 15–20 min. Responses were digitized, stored, and analyzed with pClamp 6.0. Each trace that is shown is the average of three consecutive responses, and each amplitude value is the average of six responses at a given stimulus intensity. EPSCs were recorded at two or more stimulus intensities. The effects of U69593 and nBNI were calculated as the percent change from the pre-U69593 EPSC amplitude. The percent change values for two stimulus intensities were averaged to determine the overall U69593 and nBNI effects for each experiment.

Stock solutions of U69593 were dissolved in 50% ethanol; the final concentration of ethanol applied to the slices was ≤0.001%. Glibenclamide was dissolved in dimethylsulfoxide, and the final concentration of dimethylsulfoxide applied to the slices was ≤0.1%. All other drugs were dissolved in water. U69593, nBNI, and glibenclamide were obtained from Research Biochemicals (Natick, MA); 4-AP, TEA, and $BaCl_2$ were from Sigma Chemical Co. (St. Louis, MO); DTx was from Biomol (Plymouth Meeting, PA); and MCDP was from Bachem (King of Prussia, PA).

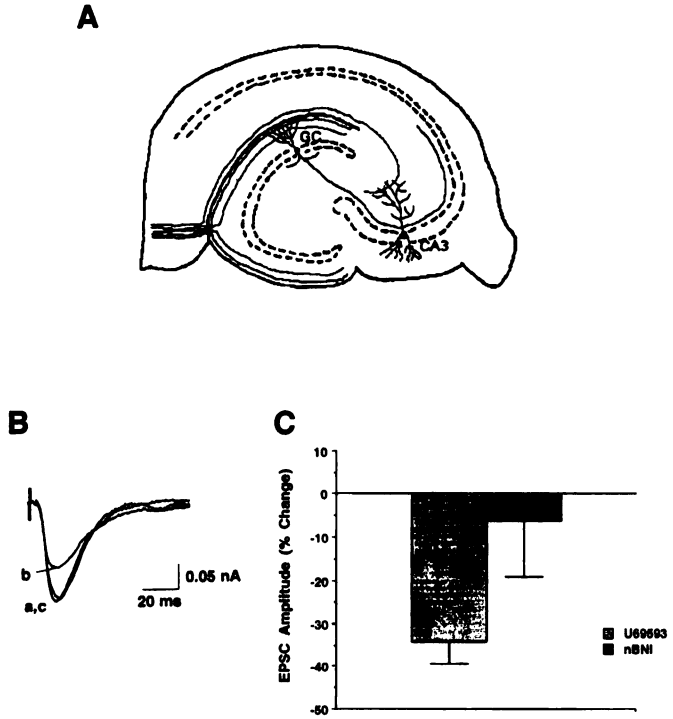


**Fig. 1.** A, Transverse hippocampal slice. *Dashed lines*, stratum granulosum and stratum pyramidale. A granule cell (*GC*) is shown with its mossy fiber axon making a synapse with the proximal dendrite of a CA3 pyramidal cell. A stimulating electrode was placed in the granule cell layer, and the recording electrode was placed in the pyramidal layer. B, Superimposed EPSCs recorded after the application of no drug (*a*), U69593 (*b*), and nBNI plus U69593 (*c*). Each trace is the average of three consecutive responses, and stimulus artifacts are truncated. $V_h$ = −50 mV; stimulus = 80 μA. C, Group data showing that the EPSC amplitude was reduced by U69593 and that this effect was reversed by nBNI (six experiments). Values are mean ± standard error.

## Results

Mossy fiber EPSCs were evoked through stimulation of dentate granule cells. The κ-opioid agonist U69593 (1 μM) consistently caused an inhibition of the EPSC amplitude (six of six experiments; Fig. 1, B and C). Mean inhibition was 34.2 ± 5.3%, and the inhibition was reversed in five of six experiments by subsequent treatment with the κ antagonist nBNI (100 nM) to 5.4 ± 11.1% (six experiments) below the pre-U69593 amplitude. U69593 did not alter the pyramidal cell holding current or membrane resistance (data not shown), indicating that the agonist was not acting directly on the pyramidal cell. This observation further supports previous observations that κ receptors in the guinea pig CA3 region are presynaptic (3).

4-AP is a relatively nonselective potassium channel blocker, although certain voltage-dependent potassium channels are particularly sensitive to 4-AP, with $IC_{50}$ values in the micromolar range (14). Pretreatment of the hippocampal slice with 30–100 μM 4-AP produced a dramatic facilitation of the evoked EPSC, increasing both the amplitude and the duration of the response. The EPSC amplitude was increased ~10-fold with 30 μM 4-AP; an even greater facilitation was observed with 100 μM 4-AP. Because most postsynaptic potassium channels were blocked by the cesium-filled patch pipette, it is likely that the facilitation produced by 4-AP was presynaptic. Facilitation of transmitter release is a well-established effect of 4-AP (15). In the presence of 4-AP, U69593 did not affect the evoked EPSC. In 30 μM 4-AP, treatment with U69593 decreased the EPSC amplitude by only 7.0 ± 2.9% (five experiments; Fig. 2). In 100 μM 4-AP, the EPSC amplitude increased 5.6 ± 6.1% (three experiments; Fig. 2B) after U69593 application.

TEA is another agent that can be used to block a wide variety of potassium channels (14). At a dose of 10 mM, TEA enhanced the evoked EPSC amplitude by ~10-fold, which is similar to the effect of 4-AP. In 10 mM TEA, the EPSC amplitude was not affected by U69593 (−4.7 ± 11.6%, four experiments; Fig. 2B). The application of 1 mM TEA had a smaller facilitatory effect on the EPSC, usually increasing the amplitude by <2-fold. In the presence of 1 mM TEA, U69593 reversibly inhibited the EPSC amplitude in four of seven experiments. The average inhibition was 20.3 ± 8.0%

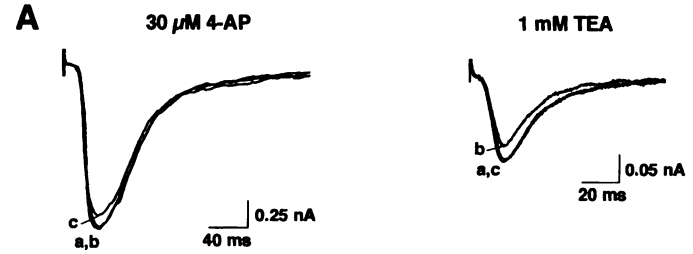


**Fig. 3.** *Left*, superimposed EPSCs recorded before the application of cadmium (*a*), in 20 μM cadmium (*b*), and after washout (*c*). $V_h$ = −70 mV; stimulus = 50 μA. *Right*, superimposed EPSCs recorded after the application of 30 μM 4-AP (*a*), 20 μM cadmium (*b*), and after washout of cadmium (*c*). $V_h$ = −50 mV; stimulus = 30 μA.

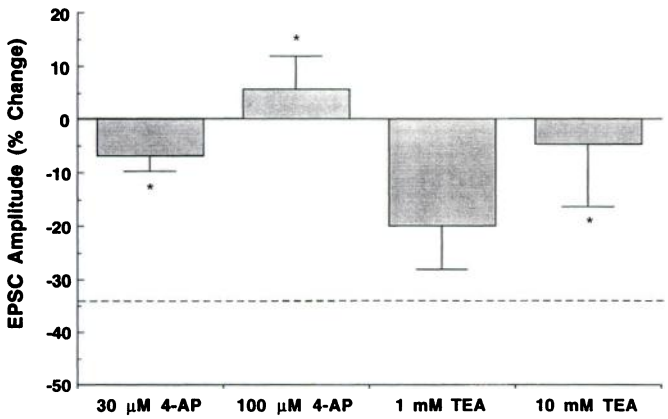


**Fig. 2.** A, *Left*, superimposed EPSCs recorded after the application of 30 μM 4-AP (*a*), U69593 (*b*), and nBNI plus U69593 (*c*). $V_h$ = −50 mV; stimulus = 25 μA. *Right*, superimposed EPSCs recorded sequentially after the application of 1 mM TEA (*a*), U69593 (*b*), and nBNI plus U69593 (*c*). $V_h$ = −70 mV; stimulus = 40 μA. B, Group data showing the effect of U69593 in the presence of various potassium channel inhibitors. The effect of U69593 was significantly reduced in the presence of 100 μM 4-AP (three experiments), 30 μM 4-AP (five experiments), and 10 mM TEA (four experiments) compared with the U69593 effect in the absence of inhibitors (*, $p < 0.05$, one-way analysis of variance with least squared differences post-hoc test). *Dashed line*, inhibitory effect of U69593 in the absence of any potassium channel inhibitors (see Fig. 1C). Values are mean ± standard error.

(seven experiments), was reversed by nBNI (to +29.7 ± 37.7%), and was not statistically different from the control effect of U69593 (Fig. 2).

These data suggest that the κ-opioid receptor may act via a potassium conductance that is highly sensitive to 4-AP and only moderately sensitive to TEA. However, because 4-AP caused such a large increase in transmitter release, it was not certain that a κ receptor-mediated inhibition of calcium channels could have been detected if it had occurred. To test this possibility, slices were treated with a known calcium channel blocker, cadmium, in the presence and absence of 4-AP. Like U69593, cadmium inhibits transmitter release from the presynaptic terminal (16, 17). $CdCl_2$ (20 μM) decreased mossy fiber-evoked EPSCs by approximately the same amount as 1 μM U69593 (34.3 ± 14.0%, three experiments; Fig. 3). In the presence of 30 μM 4-AP, the inhibitory effect of $CdCl_2$ was not diminished (38.4 ± 7.0%, three experiments; Fig. 3). This result is in agreement with previous results (16), and it indicates that a partial reduction in presynaptic calcium influx could still be detected during potassium channel blockade. Therefore, the results suggest that the κ-opioid effect was mediated via the activation of a 4-AP-sensitive potassium conductance rather than via inhibition of a calcium conductance. Further characterization of the underlying potassium conductance was carried out with more-selective potassium channel inhibitors.

A strong candidate is the GIRK channel previously shown to functionally couple to the κ-opioid receptor (10). In contrast to voltage-gated potassium channels, inward rectifying potassium currents would inhibit transmitter release by stabilizing the membrane potential to prevent firing (18). Block of GIRK channels with 300 μM $BaCl_2$ resulted in a variable and often dramatic increase in both the amplitude and duration of the EPSC. In the presence of $BaCl_2$ the EPSC amplitude was inhibited by U69593 in four of four experiments (Fig. 4). Even at stimulus intensities that evoked EPSCs of >1 nA, the effect of U69593 was equal to or greater than its effect in control buffer (two of two experiments; data not shown). The average inhibition was 45.0 ± 18.5% (four experiments; Fig. 4), and the effect was reversed by nBNI to 7.3 ± 14.2% below the pre-U69593 amplitude. Thus, the κ-opioid effect at the mossy fiber/CA3 synapse was not mediated by GIRK channels. Furthermore, the inhibition seen in $BaCl_2$ suggests that increased excitability alone is not sufficient to explain the observation that the U69593 effect was blocked by low doses of 4-AP.

$K_{ATP}$ is thought to be related to the GIRK channels (18). This current was considered as a possible mediator of the κ-opioid effect because a selective antagonist of the $K_{ATP}$ current, glibenclamide, was shown to inhibit the effect of morphine in the hippocampus (19). We found that 3 μM glibenclamide had little effect on the amplitude of the mossy fiber EPSC. Furthermore, in the presence of glibenclamide, U69593 still decreased the EPSC amplitude in three of three experiments. The average inhibition was 35.1 ± 14.6% (three experiments; Fig. 4), and this was reversed to −0.5 ± 4.7% with subsequent application of nBNI. From these results, it is concluded that the glibenclamide-sensitive current ($K_{ATP}$) does not mediate the κ-opioid effect in the CA3 region.

Voltage-dependent potassium channels of the Shaker family are characteristically sensitive to the peptide toxin, DTx (20). These channels, particularly those with fast inactivation kinetics (also called A currents), are localized presynaptically (21) and participate in the repolarization of the action potential and neurotransmitter release (14). Application of 200 nM DTx greatly increased the amplitude and duration of the evoked EPSC. As was observed with 4-AP, the increase in the EPSC amplitude was ~10-fold. Furthermore, U69593 did not affect the EPSC in any experiment in the presence of DTx. The amplitude of the response was increased 2.9 ± 8.3%

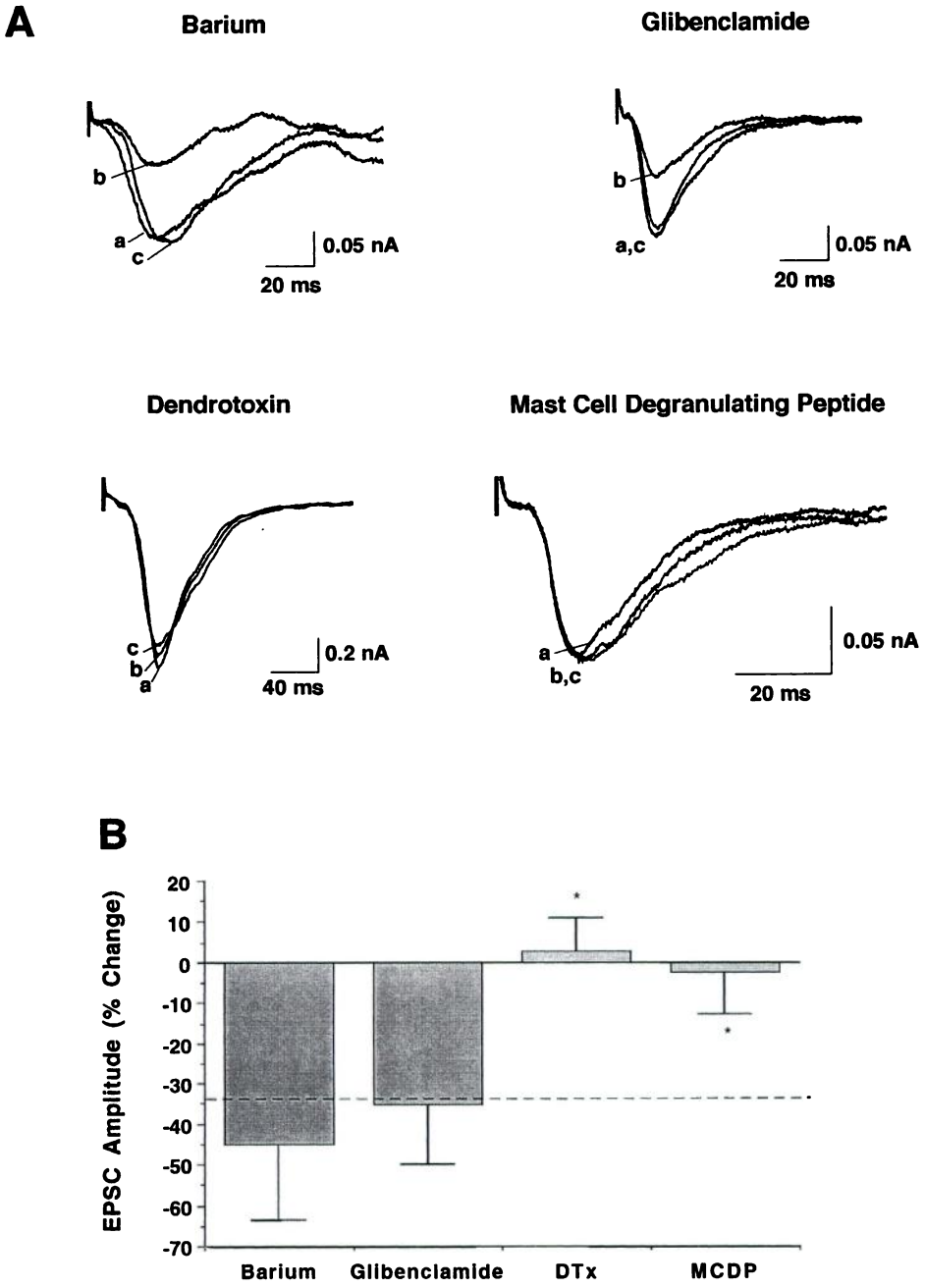


**Fig. 4.** A, Superimposed EPSCs recorded after the application of a potassium channel blocker (*a*), U69593 (*b*), and nBNI plus U69593 (*c*). *Top left*, GIRK channels were blocked with 300 μM $BaCl_2$. $V_h$ = −70 mV; stimulus = 20 μA. *Top right*, ATP-gated potassium conductance was blocked with 3 μM glibenclamide. $V_h$ = −70 mV; stimulus = 50 μA. *Bottom left*, voltage-dependent potassium channels were blocked with 200 nM DTx. $V_h$ = −70 mV; stimulus = 25 μA. *Bottom right*, voltage-dependent potassium channels were blocked with 300 nM MCDP. $V_h$ = −70 mV; stimulus = 40 μA. B, Group data showing the effect of U69593 in the presence of various potassium channel inhibitors. The effect of U69593 was significantly reduced in the presence of DTx (five experiments) and MCDP (four experiments) compared with the U69593 effect in the absence of inhibitors (*, $p < 0.05$; one-way analysis of variance with least squared differences post-hoc test). *Dashed line*, inhibitory effect of U69593 in the absence of any potassium channel inhibitors (see Fig. 1C). Values are mean ± standard error.

(five experiments; Fig. 4). This result suggests that κ-opioid receptors may inhibit transmitter release by increasing a presynaptic DTx-sensitive potassium conductance.

DTx-sensitive potassium channels also are, in many cases, sensitive to another peptide toxin, MCDP (20). Treatment with 300 nM MCDP typically enhanced the amplitude of the evoked EPSC by <3-fold. This increase is smaller than that observed with 4-AP and DTx, suggesting that MCDP may act on a smaller subset of voltage-dependent channels than the other two blockers. Even with this relatively moderate degree of enhanced transmission, the U69593 effect was completely abolished in the presence of MCDP. That is, the EPSC amplitude was reduced by only 2.4 ± 10.3% (four experiments; Fig. 4) during U69593 application compared with the pre-U69593 response. Taken together, our results suggest that κ-opioid receptors in mossy fiber terminals are functionally coupled to a voltage-dependent potassium channel that is sensitive to low doses of 4-AP as well as to the peptide toxins DTx and MCDP.

## Discussion

We used whole-cell voltage-clamp recording of mossy fiber-evoked EPSCs to characterize the mechanism by which κ-opioid receptors inhibit excitatory neurotransmission. Previous work from Weisskopf *et al.* (3) demonstrated that κ-opioid receptors are localized on mossy fiber terminals and not on CA3 pyramidal cell somata or collaterals in the guinea pig. Thus, as in other regions of the CNS, these κ receptors mediate presynaptic inhibition of transmitter release. In contrast to results obtained in other cell types (5), our data suggest that κ-opioid receptors on mossy fiber terminals in

the hippocampus do not inhibit calcium channel activity but rather increase the conductance of a specific class of potassium channels.

In the current study, we used potassium channel blockers, which prolong the depolarization of the presynaptic terminal, thereby allowing increased calcium influx and increased transmitter release. It was important to demonstrate that this increase in calcium influx was not merely overwhelming the otherwise moderate effect of the κ-opioid agonist. First, we demonstrated that a partial reduction in calcium influx produced by a low dose (20 μM) of cadmium was still evident in the presence of 4-AP. Therefore, if κ receptors did inhibit presynaptic calcium channels, the effect of U69593 should have been seen during 4-AP treatment. We further showed that the degree of enhancement of base-line EPSC amplitudes did not correlate with the blockade of the κ-opioid effect. That is, when the base-line EPSC amplitude was enhanced by treatment with barium, U69593 was still effective in reducing the response; conversely, the effect of U69593 was not seen in the presence of MCDP, which only moderately increased base-line transmission. Together with our previous findings that the inhibitory effect of U69593 was not altered by prior treatment of the slice with L-type and N-type calcium channel antagonists (6), these results indicate that κ-opioid receptors on mossy fiber terminals do not couple to voltage-dependent calcium channels.

Our data demonstrate that κ-opioid receptors inhibit neurotransmitter release by modulating presynaptic potassium channels. Potassium channels localized on presynaptic terminals control the rate of repolarization after an action potential invasion. Shorter action potentials allow less calcium entry into the terminal and, therefore, less transmitter release. Several types of potassium currents are thought to participate in action potential repolarization, including inactivating voltage-dependent currents, noninactivating voltage-dependent currents, and the large conductance, $Ca^{2+}$-dependent potassium current (14). These currents can be differentiated by pharmacological, as well as electrophysiological, characteristics.

The combination of sensitivity to low doses of 4-AP, as well as DTx and MCDP, is characteristic of the Shaker (Kv1) voltage-dependent potassium currents (20, 23). Shaker potassium channels are heteromeric combinations of α and β subunits, and different combinations exhibit different electrophysiological and pharmacological properties (24–26). It is likely that the channels coupled to κ receptors contain either Kv1.1, Kv1.2, or Kv1.6 α subunits, which confer sensitivity to DTx (27). It is also possible that the opioid-sensitive channel contains Kv1.4 and/or the β1 subunit because in the rat hippocampus, these two subunits are enriched in the mossy fiber terminal zone (21, 28, 29). Channels that include the Kv1.4 α subunit or the β1 subunit exhibit rapidly inactivating A currents (26).

A number of other G protein-coupled receptors have been reported to act via voltage-dependent potassium currents, including muscarinic acetylcholine receptors (16, 30), $\alpha_1$-adrenergic receptors (31), and dopamine receptors (32). In addition, we previously found that μ-opioid receptors in interneurons from the rat subiculum can potentiate at least two distinct potassium currents: one is an inward rectifier and the other is a voltage-dependent outward current (33). This study is the first to physiologically show that presynaptic inhibition of neurotransmission caused by the activation of a G protein-coupled receptor can be mediated by an increase in a DTx-sensitive potassium conductance.

The signal transduction pathway coupling the κ receptor to the potassium channel remains unknown, but many examples exist of modulation of potassium channel activity via a change in phosphorylation state (23, 34, 35). Indeed, μ-opioid receptor activation of a voltage-dependent potassium current was blocked by stimulation of cAMP-dependent protein kinase (36). Arachidonic acid increases a potassium conductance that is sensitive to both DTx and MCDP (37), so this second messenger must also be considered. The single-channel basis for modulation of whole-cell potassium current remains to be determined. In the case of $\gamma$-aminobutyric $acid_B$ receptor activation, potentiation of an A current is achieved by shifting the voltage dependence of inactivation to more positive potentials, which results in a greater proportion of available channels and thus a larger whole-cell current (38).

κ opioids inhibit long term potentiation in the hippocampus (2, 3, 39). Interestingly, potassium channel toxins, including MCDP, induce long term potentiation (40). The functional association of the κ receptor with a MCDP-sensitive potassium channel suggests that a common mechanism may account for both observations. For example, presynaptic calcium concentrations are likely to be important for induction of long term potentiation; increased potassium conductance by κ receptor activation would limit presynaptic calcium influx, and the potassium channel toxin would have the opposite effect. Furthermore, κ-opioid agonists inhibit seizures in some animal models of epilepsy (41), whereas blockade of voltage-dependent potassium channels with MCDP induces epileptiform activity that is believed to originate in the hippocampus (42). It is likely, therefore, that stimulation of the MCDP-sensitive potassium channel by κ opioids accounts for the anticonvulsant actions of these drugs.

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**References**

1. Akil, H., S. J. Watson, E. Young, M. E. Lewis, H. Khachaturian, and J. M. Walker. Endogenous opioids: biology and function. *Annu. Rev. Neurosci.* **7**:233–255 (1984).
2. Wagner, J. J., G. W. Terman, and C. Chavkin. Endogenous dynorphins inhibit excitatory neurotransmission and block LTP induction in the hippocampus. *Nature (Lond.)* **363**:451–454 (1993).
3. Weisskopf, M. G., R. A. Zalutsky, and R. A. Nicoll. The opioid peptide dynorphin mediates heterosynaptic depression of hippocampal mossy fibre synapses and modulates long-term potentiation. *Nature (Lond.)* **362**:423–427 (1993).
4. Cohen, G. A., V. A. Doze, and D. V. Madison. Opioid inhibition of GABA release from presynaptic terminals of rat hippocampal interneurons. *Neuron* **9**:325–335 (1992).
5. Gross, R. A., and R. L. Macdonald. Dynorphin A selectively reduces a large transient (N-type) calcium current of mouse dorsal root ganglion neurons in cell culture. *Proc. Natl. Acad. Sci. USA* **84**:5469–5473 (1987).
6. Simmons, M. L., G. W. Terman, S. M. Gibbs, and C. Chavkin. L-type calcium channels mediate dynorphin neuropeptide release from dendrites but not axons of hippocampal granule cells. *Neuron* **14**:1265–1272 (1995).
7. Grudt, T. J., and J. T. Williams. κ-Opioid receptors also increase potassium conductance. *Proc. Natl. Acad. Sci. USA* **90**:11429–11432 (1993).
8. Moore, S. D., S. G. Madamba, P. Schweitzer, and G. R. Siggins. Voltage-dependent effect of opioid peptides on hippocampal CA3 pyramidal neurons *in vitro*. *J. Neurosci.* **14**:809–820 (1994).
9. Baraban, S. C., E. W. Lothman, A. Lee, and P. G. Guyenet. *Kappa* opioid receptor-mediated suppression of voltage-activated potassium current in a catecholaminergic neuronal cell line. *J. Pharmacol. Exp. Ther.* **273**:927–933 (1995).
10. Henry, D. J., D. Grandy, H. A. Lester, N. Davidson, N. F. Lim, and C.

Chavkin. Kappa-opioid receptors couple to inwardly rectifying potassium channels when coexpressed by *Xenopus* oocytes. *Mol. Pharmacol.* **47**:551–557 (1995).
11. Simmons, M. L., and C. Chavkin. Kappa opioid receptors in the guinea pig hippocampus are functionally coupled to a 4-AP-sensitive potassium channel. *Soc. Neurosci. Abstr.* **21**:1362 (1995).
12. Langdon, R. B., J. W. Johnson, and G. Barrioneuvo. Asynchrony of mossy fibre inputs and excitatory postsynaptic currents in rat hippocampus. *J. Physiol.* **472**:157–176 (1993).
13. Claiborne, B. J., Z. Xiang, and T. H. Brown. Hippocampal circuitry complicates analysis of long-term potentiation in mossy fiber synapses. *Hippocampus* **3**:112–122 (1993).
14. Storm, J. F. Potassium currents in hippocampal pyramidal cells, in *Progress in Brain Research* (J. Storm-Mathisen, J. Zimmer, and O. P. Ottersen, eds.). Elsevier Science Publishers, Oxford, 161–187 (1990).
15. Buckle, P. J., and H. L. Haas. Enhancement of synaptic transmission by 4-aminopyridine in hippocampal slices of the rat. *J. Physiol.* **326**:109–122 (1982).
16. Fredholm, B. B. Differential sensitivity to blockade by 4-aminopyridine of presynaptic receptors regulating [$^3$H]acetylcholine release from rat hippocampus. *J. Neurochem.* **54**:1386–1390 (1990).
17. Lupica, C. R., W. R. Proctor, and T. V. Dunwiddie. Presynaptic inhibition of excitatory synaptic transmission by adenosine in rat hippocampus: analysis of unitary EPSP variance measured by whole-cell recording. *J. Neurosci.* **12**:3753–3764 (1992).
18. Doupnik, C. A., N. Davidson, and H. A. Lester. The inward rectifier potassium channel family. *Curr. Opin. Neurobiol.* **5**:268–277 (1995).
19. Uchida, Y., K. Saito, T. Ohnishi, S. Maeda, T. Matsuya, and R. Inoki. Antagonism by glibenclamide of the effect of morphine in hippocampal preparations. *Neurosci. Lett.* **162**:114–116 (1993).
20. Dreyer, F. Peptide toxins and potassium channels, in *Reviews of Physiology, Biochemistry and Pharmacology* (M. P. Blaustein, O. Creutzfeldt, H. Grunicke, E. Habermann, H. Neurath, B. Sakmann, M. Schweiger, U. Trendelenburg, K. J. Ullrich, and E. M. Wright, eds.). Springer-Verlag, Berlin, 93–136 (1990).
21. Sheng, M., Y. J. Liao, Y. N. Jan, and L. Y. Jan. Presynaptic A-current based on heteromultimeric $K^+$ channels detected *in vivo*. *Nature (Lond.)* **365**:72–75 (1993).
22. Twitchell, W. A., and S. G. Rane. Opioid peptide modulation of $Ca^{2+}$-dependent $K^+$ and voltage-activated $Ca^{2+}$ currents in bovine adrenal chromaffin cells. *Neuron* **10**:701–709 (1993).
23. Rehm, H., C. Cochet, E. Chambaz, B. L. Tempel, W. Trautwein, D. Pelzer, and M. Lazdunski. Dendrotoxin-binding brain membrane protein displays a $K^+$ channel activity that is stimulated by both cAMP-dependent and endogenous phosphorylations. *Biochemistry* **28**:6455–6460 (1989).
24. Ruppersberg, J. P., K. H. Schroter, B. Sakmann, M. Stocker, S. Sewing, and O. Pongs. Heteromultimeric channels formed by rat brain potassium-channel proteins. *Nature (Lond.)* **345**:535–537 (1990).
25. Po, S., S. Roberds, D. J. Snyders, M. M. Tamkun, and P. B. Bennett. Heteromultimeric assembly of human potassium channels: molecular basis of a transient outward current? *Circ. Res.* **72**:1326–1336 (1993).
26. Rettig, J., S. H. Heinemann, F. Wunder, C. Lorra, D. N. Parcej, J. O. Dolly, and O. Pongs. Inactivation properties of voltage-gated $K^+$ channels altered by presence of β-subunit. *Nature (Lond.)* **369**:289–294 (1994).
27. Pongs, O. Molecular biology of voltage-dependent potassium channels. *Physiol. Rev.* **4**(suppl.):S69–S88 (1992).
28. Rhodes, K. J., S. A. Keilbaugh, N. X. Barrezueta, K. L. Lopez, and J. S. Trimmer. Association and colocalization of $K^+$ channel α- and β-subunit polypeptides in rat brain. *J. Neurosci.* **15**:5360–5371 (1995).
29. Veh, R. W., R. Lichtinghagen, S. Sewing, F. Wunder, I. M. Grumbach, and O. Pongs. Immunohistochemical localization of five members of the Kv1 channel subunits: contrasting subcellular locations and neuron-specific co-localizations in rat brain. *Eur. J. Neurosci.* **7**:2189–2205 (1995).
30. Zoltay, G., and J. R. Cooper. Dendrotoxin blocks a class of potassium channels that are opened by inhibitory presynaptic modulators in rat cortical synaptosomes and slices. *Cell. Mol. Neurobiol.* **13**:59–68 (1993).
31. Aghajanian, G. K. Modulation of a transient outward current in serotonergic neurones by α1-adrenoceptors. *Nature (Lond.)* **315**:501–503 (1985).
32. Liu, L., R.-Y. Shen, G. Kapatos, and L. A. Chiodo. Dopamine neuron membrane physiology: characterization of the transient outward current ($I_A$) and demonstration of a common signal transduction pathway for $I_A$ and $I_K$. *Synapse* **17**:230–240 (1994).
33. Wimpey, T. L., and C. Chavkin. Opioids activate both an inward rectifier and a novel voltage-gated potassium conductance in the hippocampal formation. *Neuron* **6**:281–289 (1991).
34. Chung, S., and L. K. Kaczmarek. Modulation of the inactivation of voltage-dependent potassium channels by cAMP. *J. Neurosci.* **15**:3927–3935 (1995).
35. Prevarskaya, N. B., R. N. Skryma, P. Vacher, N. Daniel, J. Djiane, and B. Dufy. Role of tyrosine phosphorylation in potassium channel activation. *J. Biol. Chem.* **270**:24292–24299 (1995).
36. Wimpey, T. L., and C. Chavkin: 8-Bromo-cAMP blocks opioid activation of a voltage-gated potassium current in isolated hippocampal neurons. *Neurosci. Lett.* **137**:137–140 (1992).
37. Zoltay, G., and J. R. Cooper. Presynaptic modulation by eicosanoids in cortical synaptosomes. *Neurochem. Res.* **19**:1211–1215 (1994).
38. Saint, D. A., T. Thomas, and P. W. Gage. $GABA_B$ agonists modulate a transient potassium current in cultured mammalian hippocampal neurons. *Neurosci. Lett.* **118**:9–13 (1990).
39. Terman, G. W., J. J. Wagner, and C. Chavkin. *Kappa* opioids inhibit induction of long-term potentiation in the dentate gyrus of the guinea pig hippocampus. *J. Neurosci.* **14**:4740–4747 (1994).
40. Cherubini, E., Y. Ben-Ari, M. Gho, J. N. Bidard, and M. Lazdunski. Long-term potentiation of synaptic transmission in the hippocampus induced by a bee venom peptide. *Nature (Lond.)* **328**:70–73 (1987).
41. Tortella, F. C., L. Robles, and J. W. Holaday. U50,488, a highly selective *kappa* opioid: anticonvulsant profile in rats. *J. Pharmacol. Exp. Ther.* **237**:49–53 (1986).
42. Bidard, J.-N., C. Mourre, G. Gandolfo, H. Schweitz, C. Widmann, C. Gottesmann, and M. Lazdunski. Analogies and differences in the mode of action and properties of binding sites (localization and mutual interactions) of two $K^+$ channel toxins, MCD peptide and dendrotoxin I. *Brain Res.* **495**:45–57 (1989).

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