Dynorphin Selectively Augments the M-Current in Hippocampal CA1 Neurons by an Opiate Receptor Mechanism

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INTRODUCTION

Although the rodent hippocampus contains opioid peptides and opiate receptors, the functional role of these elements is still under investigation. Nonetheless, after two decades of research, several concepts have emerged (see Siggins et al. 1986); these include 1) the reported lack of direct effect of the opioids on pyramidal neurons (Nicoll et al. 1980; Siggins 1990; Siggins and Ziegglänsberger 1981; Ziegglänsberger et al. 1979), 2) the well-known disinhibitory effect of δ and μ opioids mediated by inhibitory opiate receptors on interneurons (Lupica and Dunwiddie 1991; Madison et al. 1987; Ziegglänsberger et al. 1979), and 3) the presence of presynaptic κ opiate receptors that inhibit transmitter release (Castillo et al. 1996; Salin et al. 1995; Wagner et al. 1992; Weisskopf et al. 1993) and may be involved in depressing some forms of long-term potentiation (LTP) (Wagner et al. 1993; Weisskopf et al. 1993). However, subsequent intracellular studies in our laboratory (Moore et al. 1994) showed that opioids could act directly on rat CA3 hippocampal pyramidal neurons (HPNs): agonists selective for κ opiate receptors enhanced the voltage-dependent $I_M$, whereas δ (but not μ) agonists reduced $I_M$. In contrast, Salin et al. (1995) reported that CA3 HPNs in several species did not show such direct postsynaptic effects of opioids.

Autoradiographic studies have revealed κ receptor binding in the rat hippocampal pyramidal cell layer (Tempel and Zukin 1987; Zukin et al. 1988). In an immunohistochemical study, Arvidsson et al. (1995b) showed that κ receptors are located on both postsynaptic elements and guinea pig. In contrast, Drake et al. (1996) reported that κ receptors are located presynaptically in the guinea pig hippocampal formation. Electrophysiological studies have suggested that dynorphin may activate μ but not κ receptors in CA1 neurons (Chavkin et al. 1985a; Neumaier et al. 1988). Similarly, results of previous electrophysiological and binding studies suggested that opioid receptors (mostly μ and δ) were located primarily on elements presynaptic to CA1 and CA3 HPNs (Unnerstall et al. 1983; Ziegglänsberger et al. 1979). However, the recent ultrastructural studies of Commons and Milner (1997), using antisera raised against the cloned δ opiate receptor, have revealed postsynaptic δ opiate receptors on dendrites and cell bodies of CA1 and CA3 HPNs. Thus these anatomic findings seemed to corroborate our previous positive findings in CA3 HPNs that δ agonists postsynaptically reduced the $I_M$ K+ conductance (Moore et al. 1994). Therefore, reasoning that previous studies may have overlooked voltage-dependent postsynaptic effects of the opioids in CA1 HPNs, we reevaluated the action of various opioids on these principal neurons. We found that CA1 HPNs showed no response to application of several δ- and μ-selective agonists, yet low concentrations of dynorphin A more selective for κ receptors augmented the voltage-sensitive $I_M$.

METHODS

Slice preparation

We used standard intracellular current- and voltage-clamp recording techniques in the rat hippocampal slice, prepared as described previously (Madamba et al. 1996). In brief, male Sprague-Dawley rats (100–170 g) were anesthetized with 3% halothane, decapitated, and...
their brains rapidly removed and placed in ice-cold artificial cerebral spinal fluid (ACSF) gassed with 95% O₂-5% CO₂. We cut transverse hippocampal slices 350 µm thick on a McIlwain-type brain slicer, incubated them in an interface configuration for ~30 min, and then completely submerged and continuously superfused the slices with gassed ACSF of the following composition (in mM): 130 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 1.5 MgSO₄·7H₂O, 2.0 CaCl₂, 24 NaHCO₃, and 10 glucose. Other ions and drugs were added to the ACSF in known concentrations. Slices were superfused with warm (31°C), gassed ACSF, at a continuous, constant flow rate of 2–4 ml/min. We used sharp glass micropipettes filled with 3 M KCl (tip resistance, 67 ± 2 MΩ; mean ± SE) to penetrate HPNs. Methods of superfusion, voltage-clamp recording, cell identification, drug administration, and data analysis were as described previously (Madamba et al. 1996; Moore et al. 1994; Schweitzer et al. 1993).

Electrophysiological methods

Current and voltage were recorded with an Axoclamp-2A preamplifier (Axon Instruments) and filtered at 0.3 kHz. Continuous current and voltage records were stored on polygraph paper. We digitized data by D/A sampling and acquisition software (pClamp; Axon Instruments) and stored them on computer disk for data analysis via Clampfit software (Axon Instruments). Tetrodotoxin (TTX; 1 µM) was added to block Na⁺-dependent action potentials and inhibit synaptic transmission. In discontinuous single-electrode voltage-clamp mode, the switching frequency was 3–4 kHz; we continuously monitored electrode settling time and input capacitance neutralization at the headstage on a separate oscilloscope (Finkel and Redman 1985). Current-voltage (I-V) relationships were generated from a holding potential of −60 ± 0.3 mV with hyperpolarizing and depolarizing steps of 1.5 s duration. Problems (e.g., space clamping) associated with voltage clamp in neurons with extended processes are discussed elsewhere (Finkel and Redman 1985; Halliwell and Adams 1982; Johnston and Brown 1983). These problems are less acute when studying relative changes following drug application (see Madison et al. 1987), especially in the presence of agents such as TTX and Cd²⁺ that block large Na⁺ and Ca²⁺ conductances.

M-current analysis

In CA1 HPNs, IₖM is seen with holding potentials around ~45 mV and hyperpolarizing steps of 5–25 mV and 700–1,000 ms durations (Halliwell and Adams 1982; Moore et al. 1994); under these conditions it appears as a slow inward “relaxation” following the instantaneous (ohmic) inward current drop (see Fig. 1). We measured IₖM amplitude with software (Clampfit, Axon Instruments) that fitted two exponential curves to the IₖM relaxation and used the difference between the instantaneous peak current at command onset and the steady-state current just before command offset to quantify the amplitude of IₖM (Fig. 1). Tail (off-command) currents were not analyzed because of possible contamination with other currents (e.g., Iₙ or Iₐ). Usually, an initial current (capacitative) artifact of 5–20 ms duration was present at command onset (Halliwell and Adams 1982). Therefore we fitted the current relaxation via Clampfit (Chebyshev method) to the peak of the initial current that usually fell within 5–20 ms of step onset after complete settling of the capacity transient (see Fig. 1).

Drug administration

Drugs and peptides were made from a stock solution and added to the ACSF in known concentrations immediately before administration to the slice chamber. The usual drug protocol followed for agonist testing was to record currents during superfusion of ACSF alone (“control”), followed by switching to ACSF with drug and repeating these current measures after 4–10 min of drug superfusion, followed by switching again to ACSF alone for 10–30 min with subsequent current measures (“washout”). The cell was depolarized to −40 to −49 mV for IₖM analysis at each of the three periods but was held near resting membrane potential (RMP) between these periods to avoid the instabilities that might develop with prolonged depolarization (see Halliwell and Adams 1982). For tests of the opioid antagonists, the usual protocol involved first applying the opioid agonist, followed by washout with ACSF alone, followed by adding the antagonist to the superfusate (to test possible effects on baseline properties), followed by addition of the opioid agonist together with the antagonist. At the end of some recordings, we added 30 µM carbachol (CCh) or 2 mM Ba²⁺ to the superfusate to verify IₖM relaxation.

We obtained dynorphin A, DAMGO, DPDPE, deltorphin, D-T-dyn, and DADLE from Peninsula Laboratories (Belmont, CA). U69,593, and DADLE from National (Natick, MA). We obtained naloxone from Sigma (St. Louis, MO) and TTX from Calbiochem-Novabiochem (San Diego, CA).

Quantification and statistics

All measures are reported as means ± SE. I-V relationships were plotted by Origin 4.0 software (Microcal Software) using B-spline lines. We determined statistical significance by two-way ANOVA for repeated measures and the Newman-Keuls post hoc test when appropriate.

Results

We recorded from a total of 57 CA1 HPNs. These neurons had an average RMP of ~69 ± 0.3 mV (n = 51) and average spike amplitude of 105 ± 1 mV (n = 44). Stable recordings could be maintained for up to 4 h, suggesting a relative lack of injury by the electrode penetration. We studied IₖM using voltage clamp at a mean holding potential of −43 ± 0.2 mV. Several lines of evidence suggest, as reported previously (Halliwell and Adams 1982; Moore et al. 1994), that the current relaxations we recorded represented IₖM; 1) the relaxations were suppressed by the muscarinic agonist carbachol (30 µM) or by 2 mM Ba²⁺, and 2) the magnitude, kinetics, and voltage dependence of the relaxations were equivalent to those of IₖM previously reported (Halliwell and Adams 1982; Moore et al. 1988a; Schweitzer et al. 1993).

We tested seven different opioid agonists: 1) DPDPE, a δ₁-selective opioid peptide; 2) deltorphin, a δ₁-selective opioid; 3) DAMGO, a μ-selective opioid; 4) DADLE, a broadly ef-
fective opioid peptide capable of activating several receptor subtypes but especially μ and δ; 5) dynorphin A (1–17), an opioid relatively selective for κ receptors at low concentrations but less selective at higher concentrations; 6) U50,488h, and 7) U69,593, opioids selective for the κ opiate receptor subtype. We also tested D-T-dyn to rule out possible nonopioid subtype.

For any given CA1 neuron, the effects measured were usually opioid-induced currents at rest, changes in \( I_M \) amplitudes, and changes in \( V \)-relationships. For the δ agonists, we also examined \( Ca^{2+} \)-dependent spikes.

**Resting and steady-state currents**

On average, superfusion of DPDPE, deltorphin, DAMGO, or DAMLE (1–8 μM concentrations) induced little current measured near RMP and had no effect on \( V \)-curves (Table 1; Fig. 2C). At more depolarized membrane potentials (near −45 mV), these opioid peptides only induced small, statistically insignificant inward currents (mean −32 ± 49, −15 ± 22, and −18 ± 57 pA, respectively; DADLE effects were not determined; see Fig. 3 and Table 1). In contrast, 0.5 μM dynorphin had a marked effect near −45 mV: it induced a mean outward current of 178 ± 13 pA, but only 3 ± 11 pA near RMP. Similarly 1 μM dynorphin induced 214 pA near −45 mV and only 12 pA near RMP (Table 1). Figure 4A shows the effect of dynorphin on the steady-state current values of a CA1 neuron held at −59 mV and subjected to hyperpolarizing and depolarizing voltage steps. Superfusion of 0.5 μM dynorphin induced an outward current at depolarized membrane potentials that reversed around −90 mV (Fig. 4A) but induced little current near RMP (−69 mV for this cell). A lower concentration of dynorphin (0.1 μM) had little effect (4 pA) near −45 mV in two cells. Although on average 4–8 μM U50,488h had no significant effect on mean \( I_M \) amplitudes (see \( M \)-currents), it evoked a mean outward current of 98 ± 22 pA near −45 mV in three cells. In contrast, 1 μM U69,593 induced little current across the voltage range tested. Similarly, 1 μM D-T-dyn had no effect on steady state currents (Table 1).

**\( Ca^{2+} \)-dependent spikes**

Because of the relative lack of effect of δ agonists on resting currents, and because of the clear presence of δ receptors on CA1 HPNs (Commons and Milner 1997), we also explored a possible effect of δ agonists on \( Ca^{2+} \)-dependent spikes. Because of difficulties in adequately recording \( Ca^{2+} \) currents in slice preparations, these experiments were performed in current-clamp mode in the presence of 1 μM TTX to block \( Na^+ \) spikes. \( Ca^{2+} \)-dependent spikes were elicited by depolarizing current steps (current protocol in bottom left of each panel). A: superfusion of 1 μM DPDPE for 10 min had little effect on voltage responses to current steps. Subsequent \( Ca^{2+} \) (100 μM) superfusion for 7 min almost totally blocked the spikes, suggesting that they were \( Ca^{2+} \)-dependent. Resting membrane potential (RMP) was −68 mV. B: superfusion of 1 μM deltorphin for 10 min onto another neuron had no effect on the \( Ca^{2+} \)-dependent spikes evoked in the presence of 1 μM TTX. RMP was −68 mV (arrow). C: \( V-I \) relationships of the steady-state potentials from the neuron shown in B.

**TABLE 1. Summary of opioid-induced currents in CA1 HPNs at two different membrane potentials**

<table>
<thead>
<tr>
<th>Opioid Tested</th>
<th>Near −45 mV</th>
<th>Near RMP</th>
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<tbody>
<tr>
<td>DPDPE 1–5 μM</td>
<td>−32 ± 49 (4)</td>
<td>−10 ± 9 (8)</td>
</tr>
<tr>
<td>Deltorphin II 1 μM</td>
<td>−11 ± 22 (5)</td>
<td>−2 ± 11 (5)</td>
</tr>
<tr>
<td>DAMGO 1–2 μM</td>
<td>−18 (2)</td>
<td>3 ± 11 (5)</td>
</tr>
<tr>
<td>DADLE 4–8 μM</td>
<td>ND</td>
<td>−2 (2)</td>
</tr>
<tr>
<td>Dynorphin 0.1 μM</td>
<td>−4 (2)</td>
<td>−18 (2)</td>
</tr>
<tr>
<td>Dynorphin 0.5 μM</td>
<td>178 ± 13 (6)</td>
<td>3 ± 14 (6)</td>
</tr>
<tr>
<td>Dynorphin 1.0 μM</td>
<td>214 (2)</td>
<td>12 (2)</td>
</tr>
<tr>
<td>U50,488h 4 μM</td>
<td>98 ± 22 (3)</td>
<td>4 ± 17 (6)</td>
</tr>
<tr>
<td>U69,593 1 μM</td>
<td>−9 ± 28 (6)</td>
<td>−1 ± 15 (6)</td>
</tr>
<tr>
<td>D-T-Dyn 1 μM</td>
<td>16 ± 50 (3)</td>
<td>−11 ± 24 (3)</td>
</tr>
</tbody>
</table>

Values are means ± SE in pA; numbers in parentheses refer to number of cells measured near −45 mV or near resting membrane potential (RMP). Positive numbers represent outward (hyperpolarizing) current, and negative numbers represent inward (depolarizing) current. HPNs, hippocampal pyramidal neurons; ND, not determined.

FIG. 2. δ Receptor agonists (DPDPE and deltorphin) have little effect on \( Ca^{2+} \)-dependent spikes or voltage-current (\( V-I \)) curves in CA1 neurons: superimposed voltage records in the presence of 1 μM TTX to block \( Na^+ \) spikes. \( Ca^{2+} \)-dependent spikes were elicited by depolarizing current steps (current protocol in bottom left of each panel). A: superfusion of 1 μM DPDPE for 10 min had little effect on voltage responses to current steps. Subsequent \( Ca^{2+} \) (100 μM) superfusion for 7 min almost totally blocked the spikes, suggesting that they were \( Ca^{2+} \)-dependent. Resting membrane potential (RMP) was −68 mV. B: superfusion of 1 μM deltorphin for 10 min onto another neuron had no effect on the \( Ca^{2+} \)-dependent spikes evoked in the presence of 1 μM TTX. RMP was −68 mV (arrow). C: \( V-I \) relationships of the steady-state potentials from the neuron shown in B.

None of the μ- or δ-selective opiate agonists altered \( I_M \) relaxation amplitudes in CA1 neurons (Fig. 3). Thus, averaging data from seven neurons, superfusion of 1–5 μM DPDPE did not significantly (\( P > 0.1 \)) alter \( I_M \) amplitudes when compared...
with control values (Fig. 3C, left panel). Likewise, the selective δ2-receptor agonist deltorphin (1 μM) did not significantly change $I_M$ amplitudes in five cells (Fig. 3, B and C, middle panel; $P > 0.9$). The μ-selective agonist DAMGO (1–2 μM; $n = 5$) also did not significantly ($P > 0.5$) change $I_M$ amplitudes (Fig. 3C, right panel). In two cells, 4–8 μM DADLE also had little effect on $I_M$ amplitudes (data not shown).

Interestingly, in the same cells where 1 μM deltorphin had no effect on $I_M$ amplitude, subsequent superfusion of 0.5 μM dynorphin clearly augmented $I_M$ (data not shown). Dynorphin A (0.5 μM) markedly induced an outward holding current concomitant with an increased $I_M$ amplitude, with recovery on washout (Fig. 4B). In the same cell after 23 min washout of the first exposure, a second application of 0.5 μM dynorphin again increased $I_M$ amplitudes, suggesting a lack of tachyphylaxis over this period. In data pooled from eight cells, superfusion of 0.5 μM dynorphin significantly $[F(2,14) = 79.11, P < 0.001; \text{Newman-Keuls}, P < 0.01]$ increased mean $I_M$ relaxation amplitudes to 160% of control with recovery on washout (Fig. 5B1). Similarly, in three cells 1 μM dynorphin increased $I_M$ amplitudes to 169% of control (averaged values taken from 10 to 20 mV hyperpolarizing steps). In contrast, a lower concentration of dynorphin (0.1 μM) had no effect on $I_M$ ($n = 2$; data not shown). Thus the maximally effective concentration for dynorphin was 0.5 μM, and the apparent $EC_{50}$ for this relatively steep dose-response relationship must fall between 0.1 and 0.5 μM. Figure 5A shows a representative recording: superfusion of 0.5 μM dynorphin A enhanced $I_M$ amplitudes, elicited an inward steady-state current and totally blocked the inward current relaxations, verifying the identity of the relaxations as due to $I_M$. On average, 1 μM nBNI ($n = 3$) or 4 μM naloxone ($n = 1$) totally prevented the augmenting effect of 0.5 μM dynorphin on $I_M$ (Fig. 5B2).

The blockade by nBNI of the dynorphin-induced $I_M$ augmentation indicated that this effect was mediated by κ receptors. We therefore tested the selective κ agonist U50,488h. Superfusion of 4 μM U50,488h clearly can increase $I_M$ amplitudes in some individual CA1 HPNs (Fig. 6, A and B), with recovery on washout. In addition, in the same cell a 0.5 μM dynorphin-induced $I_M$ increase was blocked by 4 μM naloxone. However, when averaged across all six cells studied, superfusion of 4–8 μM U50,488h only slightly increased $I_M$ amplitudes. This effect did not reach statistical significance ($n = 6; P > 0.1$; Fig. 6C). Similarly, in five cells 1 μM U69,593 had no significant ($P > 0.5$) effect on $I_M$ amplitude.

**DISCUSSION**

In this study we found that, in the presence of TTX, superfusion of opiates selective for δ1, δ2, and μ receptors did not alter $I_M$ or other postsynaptic properties in rat CA1 HPNs. By contrast, dynorphin A significantly enhanced $I_M$ amplitudes, and this effect was blocked by naloxone and the selective κ receptor antagonist, nBNI. However, U69,593 and U50,488h did not significantly alter $I_M$ amplitudes, although some individual cells showed a clear $I_M$ augmentation with U50,488h. Both U50,488h and dynorphin A induced an outward steady-state current near −45 mV where $I_M$ persists. These findings suggest that a pharmaco-
reported postsynaptic localization of μ receptors in CA1 neurons (Arvidsson et al. 1995b). It may be argued that we have not examined several other voltage-sensitive currents, such as $I_A$, $I_D$, $I_T$, and delayed rectifier currents. These are inactivating currents that may not influence resting properties. Modulatory effects of the opioids on other postsynaptic transmitter receptors, such as those for glutamate (see Deisz et al. 1988; Martin et al. 1997), could also be a site of action. Thus postsynaptic δ receptors could play a role in phosphorylation and desensitization of receptors, leading to neuroadapative processes as demonstrated for the μ receptor (Zhang et al. 1996).

Nevertheless, our results indicating a lack of clear postsynaptic effect of δ- or μ-selective agonists in CA1 HPNs are in agreement with previous studies supporting the disinhibition hypotheses (Nicoll et al. 1980; Siggins and Gruol 1986; Siggins and Zieglgänsberger 1981; Zieglgänsberger et al. 1979). These opiate receptor agonists have been shown to reduce GABAergic transmission in hippocampus (Lupica and Dunwiddie 1991; Lupica et al. 1992), and an intracellular study of hippocampal interneurons showed that they were directly hyperpolarized by enkephalins (Madison and Nicoll 1988).

**Post synaptic effects of dynorphin**

In contrast to the lack of effects of μ and δ agonists on CA1 HPNs, we found that dynorphin A significantly enhanced $I_M$ amplitudes, with recovery on washout. Dynorphin A also induced

![Image](http://jn.physiology.org/)

**FIG. 4.** Dynorphin A elicits an outward current and augments $I_M$ in CA1 HPNs. A: control-subtracted $I-V$ curve; superfusion of 0.5 μM dynorphin A induced an outward current of ~180 pA at depolarized membrane potentials but little current near RMP (~69 mV for this cell; $V_h = -59$ mV). B: in a different neuron, superfusion of 0.5 μM dynorphin A for 5 min elicited an outward holding current (compare to dashed line) and increased the amplitude of $I_M$ relaxations and overall conductance (note larger spacing between current steps). On washout, $I_M$ relaxations, overall conductance and the outward current all returned to control levels. A 2nd dynorphin application (0.5 μM; 5 min) 23 min later again induced an outward current with an increase of $I_M$ and overall conductance. RMP = -67 mV; $V_h = -45$ mV.

logically functional κ receptor subtype exists in rat CA1 neurons and that opioids may have more complex actions than the simple disinhibitory or presynaptic effects that have received the most attention in hippocampus.

**Absence of voltage-dependent effects of δ and μ agonists on HPNs**

The selective δ₁ and δ₂ receptor agonists (DPDPE and deltorphin) did not alter Ca²⁺-dependent spikes in CA1 HPNs, suggesting a lack of effect on voltage-dependent Ca²⁺ currents. However, there is the caveat that such current-clamp studies offer a relatively insensitive index of drug effects on Ca²⁺ currents, and that reciprocal opioid effects on the different subtypes of Ca²⁺ currents could result in false-negative results. Unfortunately, voltage-clamping Ca²⁺ currents in CA1 slice neurons with extended processes is difficult due to space-clamp confounds combined with the positive step potentials needed (around 0 mV) and the typically large Ca²⁺ current amplitudes. However, M-currents have smaller current amplitudes and are easier to record at more negative potentials (near ~45 mV).

In voltage-clamp recordings, opioid peptides selective for δ₁, δ₂, and μ receptors had little effect on the voltage-dependent $I_M$. On average, these peptides had no effect near RMP or near ~45 mV (see Table 1). In view of the recent observation of δ receptors on CA1 HPNs (Commons and Milner 1997) and our finding of $I_{hi}$ depression by δ agonists in CA3 (Moore et al. 1994), the lack of postsynaptic effect of δ receptor agonists on $I_M$ in CA1 HPNs was unexpected. Similarly, the μ agonist DAMGO had no clear effect on $I_M$ or Ca²⁺ spikes, despite the
There are reports of dynorphin-like immunoreactive fibers located in the hippocampal CA1 region (Chavkin et al. 1985b; McGinty et al. 1983), and several κ receptor subtypes (e.g., κ₁, κ₂, or epsilon) are located in the rat brain (Nock et al. 1988, 1990, 1993; Zukin et al. 1988), where the κ₁ receptor subtype predominates. Dynorphin has a higher potency than U50,488h for the κ₂ subtype (Zukin et al. 1988). The lower affinity of the κ₂ receptor for U50,488h may explain the lack of significant I₂M enhancement by U50,488h. Furthermore, U69,593 is specific for κ₁ receptor and is inactive at the κ₂ receptor, suggesting that a κ₁ receptor is not involved. However, our finding of nBNI blockade of dynorphin enhancement of I₂M might suggest κ₁ receptor mediation. Although it has been suggested that nBNI is a selective κ₁ antagonist (Nock et al. 1990), it may also interact with the κ₂ receptor subtype (see Wollemann et al. 1993 for review). Previously, we reported that U50,488h significantly enhanced I₂M amplitudes in CA3 HPNs (Moore et al. 1994), with nBNI blockade of this effect. It is possible that some new, uncharacterized κ receptor subtype or splice variant is responsible for the dynorphin effect on I₂M in CA1 HPNs (see Clark et al. 1989; and our accompanying paper Madamba et al. 1999). Furthermore, the exact localization of κ receptors in the rat CA1 region is presently unknown.

Our finding of large I₂M increases induced by dynorphin in rat CA1 HPNs is highly consistent with the significant dynorphin-induced I₂M increases we reported previously for rat CA3 HPNs (Moore et al. 1994). In contrast to our CA3 findings of decreased I₂M by higher dynorphin concentrations (>0.5 μM), CA1 neurons showed only augmentation of I₂M at all dynorphin concentrations tested above 0.1 μM. However, Salin et al. (1995) found no postsynaptic dynorphin effects in CA3 HPNs of several species, including rat. The inability to replicate our I₂M data may arise from several differences in our experimental conditions, such as 1) possible contamination of the apparent I₂M relaxation with the Q-current relaxation that is not altered by opioids in CA3 neurons (Moore et al. 1994); 2) the method for quantifying the I₂M relaxations; 3) bath temperature; 4) dynorphin concentrations; and 5) the presence or absence of Ca²⁺ channel antagonists.

Past publications (e.g., North et al. 1987; Piguet and North 1993; Williams and North 1984; Williams et al. 1988) on neuronal opiate effects had postulated, in addition to the well-known presynaptic inhibitory effects of opioids on transmitter release, agonists specific for μ and δ receptors exerted their inhibitory effects by opening K⁺ channels, whereas those specific for κ receptors did not, but rather inhibited Ca²⁺ channel function (Gross and MacDonald 1987). However, several studies (Deisz et al. 1988; Martin et al. 1997; Moore et al. 1988b; Siggins and Ziegglansberger 1981; Sutor and Ziegglansberger 1984; Yuan et al. 1992), including the present investigation, found that many central neurons showed no direct inhibitory effects of μ or δ agonists attributable to K⁺ channel activation. By contrast, the Moore et al. (1994) studies and those of others (Grudt and Williams 1993; Henry et al. 1995; Ikeda et al. 1995; Ma et al. 1995; Simmons and Chavkin 1996), have shown that κ agonists clearly can activate K⁺ channels in several neuron types and in Xenopus oocytes coexpressing κ-opioid receptors and K⁺ channels. As the M-current is carried through K⁺ channels, the present study, and the accompanying paper on nociceptin, adds further evidence that κ agonists open K⁺ channels.
Physiological role of hippocampal dynorphin

As for the function of endogenous opioids in CA1 hippocampus, regulation of the M-current could play an important role in events that involve prolonged depolarizations, such as those triggered during theta burst activity. Dynorphin- or nociceptin-induced augmentation of \( I_M \) would be predicted to counter prolonged depolarizations and reduce bursting activity (Halliwell and Adams 1982). Considerable evidence suggests that opiates play some as yet undefined role in epileptiform activity. The epileptogenic action of \( \mu \) agonists in CA1 and CA3 neurons may arise from inactivation of inhibitory interneurons in hippocampus (Nicoll et al. 1980; Siggins and Groul 1986; Siggins and Zieglgänsberger 1981; Zieglgänsberger et al. 1979). By contrast, several reports suggest that dynorphins dampen epileptiform activity (Jones 1991; Tortella and Holaday 1986), consistent with an inhibitory action via \( K^+ \) channel/\( I_K \) augmentation. Changes of dynorphin levels and metabolism in hippocampus following evoked seizure activity (Gall 1988; Hong et al. 1988) also may indicate a role for dynorphin in seizures.

Dynorphin also could be involved in some form of synaptic plasticity. Several studies have shown that dynorphin depresses LTP, a cellular model for learning and memory, in guinea pig hippocampus (Terman et al. 1994; Wagner et al. 1993; Weisskopf et al. 1996). However, the locus of this effect is thought to be presynaptic, in part because dynorphin was assumed to have no postsynaptic action. By contrast, \( \delta \)- and/or \( \mu \)-selective agonists enhanced LTP in rat hippocampus (Derrick et al. 1992; Gramham et al. 1991; Xie and Lewis 1995). DAMGO facilitation of mossy fiber LTP may be partially due to inhibition of GABA\(_B\) receptor activation (Jin and Chavkin 1999). Although the role of \( I_M \) in LTP processing is still unknown, it seems logical that augmenting \( I_M \) should prevent the postsynaptic depolarization needed for the expression of many forms of LTP. Interestingly, a novel form of long-term depression (LTD) found in the rat CA1 hippocampus is blocked by naloxone (Francesconi et al. 1997), suggesting that endogenous opioids could also play a role in this form of synaptic plasticity. Considering the \( I_K\)-augmenting (i.e., inhibitory) effect of dynorphin in this region, dynorphin could be implicated in mediating this form of LTD. Dynorphin could also be involved in other phenomena such as paired-pulse inhibition or short-term potentiation. Based on its potent hippocampal actions, it is reasonable to suggest that endogenous dynorphin could play a role in memory function, an idea supported by data showing naloxone-sensitive impairment of spatial memory by dynorphin microinjection into the hippocampus (McDaniel et al. 1990). Furthermore, dynorphin B impairs spatial learning in rats via \( \kappa \) receptors (Sandin et al. 1998). Additional studies will be required to determine whether the M-current is involved in these seizure-, plasticity-, or memory-related phenomena and whether endogenous dynorphin in hippocampus might have similar effects. As implied in the data of our accompanying paper (Madamba et al. 1999), similar considerations apply to the possible role of nociceptin in hippocampus.

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DYNORPHIN INCREASES M-CURRENTS IN CA1 HIPPOCAMPUS


