

OPIOID PEPTIDES SELECTIVE FOR MU- AND DELTA-OPIATE RECEPTORS REDUCE CALCIUM-DEPENDENT ACTION POTENTIAL DURATION BY INCREASING POTASSIUM CONDUCTANCE

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We suggest that both mu- and delta-opiate receptors on dorsal root ganglion neuron somata are coupled to voltage- and/or calcium-dependent potassium channels since opioid peptide decreases of calcium-dependent action potential duration were: (1) not associated with a change of resting membrane potential or conductance; (2) accompanied by an increase in action potential after-hyperpolarization, and (3) blocked by intracellular injection of the potassium channel blocker cesium [18]. In contrast, norepinephrine [4] and cadmium [9], which have been reported to act on voltage-dependent calcium rather than potassium channels, shortened action potential duration and decreased after-hyperpolarization amplitude, an action not blocked by intracellular iontophoresis of cesium.

Opiates decrease the duration of somatic calcium-dependent action potentials of mouse [19, 20] and chick [14] dorsal root ganglion (DRG) neurons grown in primary dissociated cell culture. This opiate action is antagonized by naloxone. It is likely, therefore, that opiate receptors on the somata of these DRG neurons are functionally similar to opiate receptors on primary afferent terminals [5, 6, 10] where a decrease in calcium entry would correlate with a decrease of neurotransmitter release [7, 12, 14]. We have recently reported that opioid peptides selective for both mu- and delta-opiate receptors decrease the duration of DRG neuron somatic calcium-dependent action potentials [20]. At present, the identity of the ion channels coupled to mu- and delta-opiate receptors is uncertain. It has been suggested that opiates decrease a voltage-dependent calcium conductance [14] or produce a membrane hyperpolarization by increasing a potassium conductance [15, 18, 21]. We now suggest that in mouse DRG neurons in cell culture both mu- and delta-

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receptors are coupled to voltage- and/or calcium-dependent potassium channels.

Preparation of mouse spinal cord and DRG neuron co-cultures and electrophysiological techniques were as previously described [16]. Recording medium was a Tris-HCl-buffered (pH 7.2–7.4) balanced saline (320 mOsm) containing (in mM): NaCl, 137.0; KCl, 5.3; MgCl₂, 0.8; CaCl₂, 5.0; Tris-base, 13.0; glucose, 5.6, and tetraethylammonium-chloride (TEA), 5.0. TEA, a potassium channel blocker, augmented the calcium component of action potentials. DRG neurons were impaled with either 4 M potassium acetate (KAc)- or 4 M cesium acetate (CsAc)-filled micropipettes (20–40 MΩ) and somatic calcium-dependent action potentials were evoked by brief (100–500 μsec) depolarizing current pulses at a frequency of 4 per min. Morphiceptin (Peninsula), leucine-enkephalin (Calbiochem), norepinephrine (Sigma), or cadmium (Sigma) were applied to single neurons by pressure ejection (0.5–2.0 psi with 1 psi = 6.76 kPa) from micropipettes with tip diameters of 2–5 μm. Application was for 1 sec delivered 4 sec prior to evoking an action potential.

During recording with KAc-filled micropipettes, action potentials were evoked from resting membrane potential (–45 to –70 mV) and generally had durations between 7 and 20 msec. Following determination of the effects of the opioid peptides, norepinephrine or cadmium on calcium-dependent action potential duration, the KAc-filled recording micropipette was gently withdrawn from the neuron. The neuron was then reimpaled with a recording micropipette containing CsAc and action potentials were evoked. Initially following neuronal impalement with a CsAc-filled micropipette, resting membrane potential, action potential duration and action potential after-hyperpolarization corresponded to those observed during KAc recordings. Over the next 1–15 min, membrane potential declined to 0 to –20 mV, action potential duration increased to 100–1500 msec, and the after-hyperpolarization was abolished, consistent with substantial blockade of potassium conductance by intracellular cesium. Holding current was applied to maintain baseline membrane potential at about –60 mV. The action of the opioid peptides, norepinephrine and cadmium was then reassessed on the prolonged calcium-dependent action potentials obtained following intracellular cesium injection. A more detailed description of methods used can be found in Werz and Macdonald [21].

During intracellular recording with KAc-filled micropipettes, the opioid peptides (Fig. 1A₁), norepinephrine (Fig. 1B₁) and cadmium (Fig. 1C₁) decreased the duration of somatic calcium-dependent action potentials. As previously reported, the opioid peptides affected only a portion of DRG neurons. Of 90 DRG neurons tested, only 13 responded to 5–10 μM leucine-enkephalin or 5–10 μM morphiceptin with a decrease of somatic calcium-dependent action potential duration of at least 25%. Norepinephrine at 100 μM decreased calcium-dependent action potential duration in 6 of 6 neurons by 11–35%, with a mean reduction of 20%. Cadmium at 100–500 μM abolished the calcium-dependent component in 11 of 11 neurons leaving an action potential of 2 msec duration which was sodium-dependent.

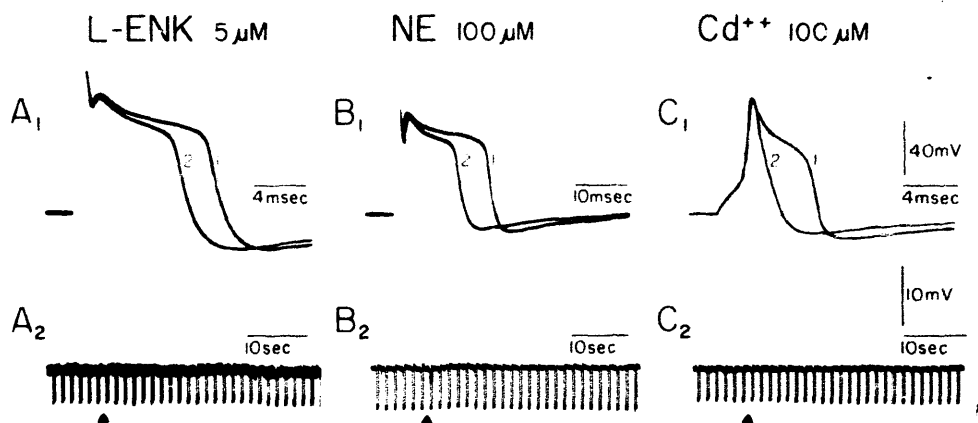


Fig. 1. Leucine-enkephalin (L-ENK) (A₁, A₂), norepinephrine (NE) (B₁, B₂), and cadmium (Cd⁺⁺) (C₁, C₂) decreased DRG neuron somatic calcium-dependent action potential duration without affecting resting membrane potential or conductance. Intracellular recordings from 3 different neurons (A, B and C) obtained using KAc-filled micropipettes. A₁, B₁ and C₁: action potentials were evoked from neurons bathed in medium containing 5 mM TEA and therefore had durations of 5–20 msec. Approximately 2 msec was dependent upon sodium with the remaining broad plateau dependent upon calcium. Data in this and subsequent figures are superimposed action potentials evoked prior to (1) and subsequent to (2) L-ENK, NE, or Cd⁺⁺ application. A₂, B₂ and C₂: depolarizing stimuli to evoke action potentials were discontinued. L-ENK (A₂), NE (B₂) and Cd⁺⁺ (C₂) applied for 1 sec at triangle did not affect membrane potential or the voltage responses to injection of small, hyperpolarizing constant current pulses.

Decreases of DRG neuron somatic calcium-dependent action potentials by the opioid peptides (Fig. 1A₂), norepinephrine (Fig. 1B₂) and cadmium (Fig. 1C₂) were not associated with a change of resting membrane potential or conductance.

Opiate action differed from that of norepinephrine and the calcium channel blocker cadmium in two ways. Firstly, the peak amplitude of action potential after-hyperpolarization was increased 1–7 mV (mean 2.4 mV) by opioid peptides (9 of 12 neurons) (Fig. 2A) but decreased 1–2.5 mV (mean 1.7 mV) by norepinephrine (6 of 6 neurons) (Fig. 2B) and 1–10 mV (mean 3.3 mV) by cadmium (9 of 9 neurons) (Fig. 2C). The opioid peptides did not affect the amplitude of the after-hyperpolarization in 2 neurons and decreased it slightly in one. Secondly, in DRG neurons that had responded to the opioid peptides during recording with KAc-filled micropipettes, the response was blocked by intracellular cesium (Fig. 3A, B) (10 of 10 neurons). Opioid peptide responses were blocked by intracellular cesium in neurons that responded to both morphiceptin and leucine-enkephalin (Fig. 3A) (3 of 3), presumably mu-receptor-mediated responses, and in neurons that responded to leucine-enkephalin but not morphiceptin (3 of 3) (Fig. 3B), presumably delta-receptor-mediated responses. In contrast, DRG neurons (n = 6) that responded to norepinephrine with a decrease of action potential duration of 11–35% (mean reduction 20%) during recording with KAc-filled micropipettes responded with a reduction of 25–66% (mean reduction 37%) during subsequent recording with CsAc-filled micropipettes (Fig. 3C). Cadmium abolished the calcium-dependent

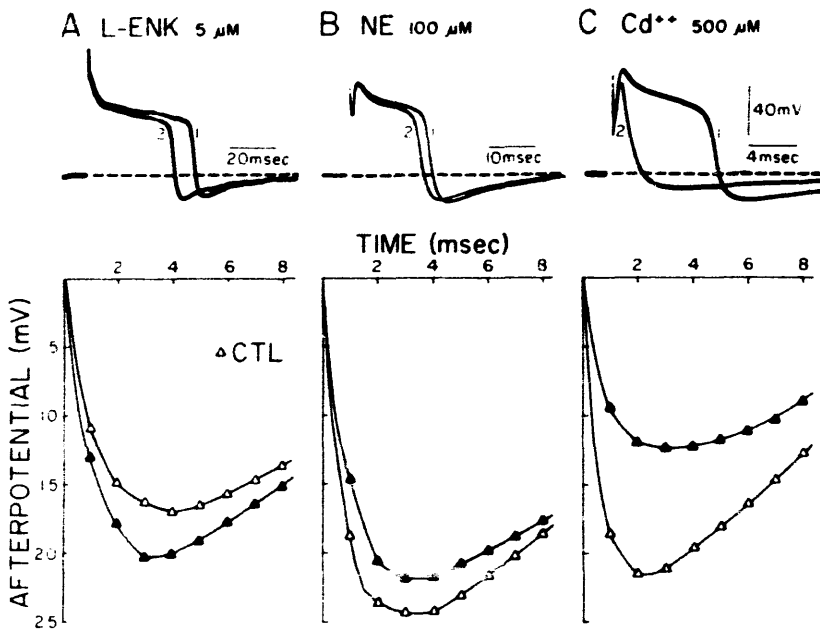


Fig. 2. Opioid peptides increased while norepinephrine and cadmium decreased action potential after-hyperpolarization. Upper panel: effect of L-ENK (A), NE (B), and Cd^{++} (C) on action potential duration and after-hyperpolarization. Broken line indicates resting membrane potential. Lower panel: after-hyperpolarization amplitudes of action potentials shown in upper panel are plotted as a function of time. Measurement of after-hyperpolarizations was begun where the repolarizing limb of the action potential intersected resting membrane potential. In each case, control is designated by open triangles and opioid peptide, norepinephrine, or cadmium treated by closed triangles.

component of action potentials during recording with either KAc- or CsAc-filled recording micropipettes ($n = 5$) (Fig. 3C).

The opioid peptides leucine-enkephalin and morphiceptin, norepinephrine and cadmium decreased the duration of the calcium component of DRG neuron somatic calcium-dependent action potentials without affecting resting membrane potential or conductance, suggestive of an action to decrease voltage-dependent calcium conductance or to increase voltage- and/or calcium-dependent potassium conductance. Norepinephrine and cadmium appear to act by the first mechanism, a decrease of voltage-dependent calcium conductance, as shown by decreased calcium-dependent action potential duration under conditions of substantial potassium channel blockade and by the reduced amplitude of action potential after-hyperpolarizations. A decrease of calcium entry would be expected to produce less activation of calcium-activated potassium conductance. This interpretation is supported by voltage clamp studies demonstrating that cadmium abolished the early calcium inward current evoked by depolarizing voltage steps and also reduced the magnitude of the late outward current [14]. Our results that norepinephrine decreased a voltage-dependent calcium conductance are consistent with the findings of Dunlap and Fischbach [5].

In contrast, opioid peptide effects on action potential duration were frequently associated with an increase in amplitude of after-hyperpolarization and were block-

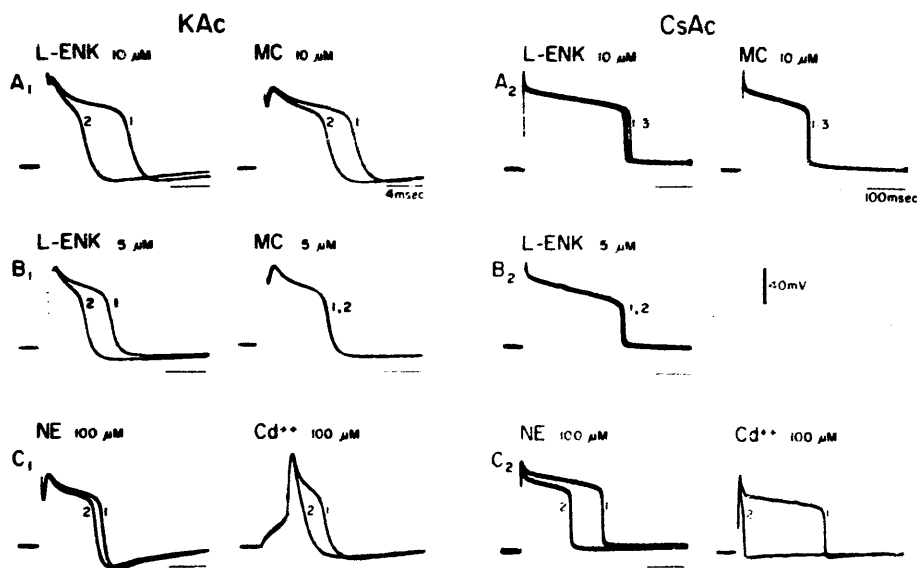


Fig. 3. Intracellular injection of cesium blocked neuronal responses to L-ENK and morphiceptin (MC) but not to NE or Cd^{++} . Opioid peptides, NE and cadmium were tested on somatic calcium-dependent action potentials during recording with a KAc-filled micropipette and again following reimpalment of the neuron with a CsAc-filled micropipette. A and B: a DRG neuron that responded well to L-ENK and MC (A_1), presumably a neuron with mu-receptors [20], and a neuron that responded to L-ENK but not MC (B_1), presumably a neuron with predominantly delta-receptors, during recording with KAc-filled micropipettes. Subsequent reimpalment of the neurons with a CsAc-filled micropipette and intracellular injection of cesium blocked the response to the opioid peptides in both cases (A_2 , B_2). Two neurons that responded to NE and Cd^{++} during recording with KAc-filled micropipettes (C_1) continued to show large decreases of action potential duration during subsequent recording with CsAc-filled micropipettes (C_2). Calibrations represent 4 msec (A_1 , B_1 , C_1) or 100 msec (A_2 , B_2 and C_2).

ed by intracellular cesium, suggesting that opiates act by increasing a potassium conductance. Our conclusion is in conflict with the finding of Mudge et al. [14] who suggested that enkephalins decrease a voltage-dependent calcium conductance. However, opiates have been reported to act on potassium conductances in several systems: a potassium conductance present at resting membrane potential [15, 22] as well as a potassium conductance activated by calcium [18]. We have not observed an opiate action to increase a potassium conductance at resting membrane potential, consistent with an action on one of several neuronal voltage- or calcium-dependent potassium conductances [1, 2, 11]. Further work will be necessary to define which potassium conductance is acted upon by opiates in primary sensory neurons and to determine if mu- and delta-receptors are coupled to the same or different potassium channels.

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