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μ-Opioid Peptides Inhibit Thalamic Neurons

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Opioidergic inhibition of neurons in the centrolateral nucleus of the thalamus was investigated using an *in vitro* thalamic slice preparation from young rats. The μ-opioid receptor agonist D-Ala^2,*N*-Me-Phe^4,glycinol^5-enkephalin (DAMGO) evoked a hyperpolarization and decrease in input resistance that was reversible, concentration-dependent, and persisted in the presence of tetrodotoxin. Application of the specific μ-receptor antagonist Cys^2,Tyr^3,Orn^5,Pen^7-amide blocked this response. The respective δ- and κ-opioid receptor agonists, (D-Pen^2,D-Pen^5)-enkephalin and ($\pm$)-*trans*-U-50488 methanesulfonate had no effect. Voltage-clamp experiments showed that DAMGO activated an inwardly rectifying potassium conductance (GK_{IR}) characterized by rectification at hyperpolarized potentials that increased in elevated extracellular potassium concentrations, a complete block by Ba^{2+} (1 mM), and a voltage-dependent block by Cs^+. The extent of μ-opioid inhibition in other thalamic nuclei was then investigated. Widespread inhibition similar to that seen in the centrolateral nucleus was observed in a number of sensory, motor, intralaminar, and midline nuclei. Our results suggest that the net action of opioids would depend on their source: exogenous (systemically administered) opiates inhibiting the entire thalamus and favoring the shift of cell firing from tonic to bursting mode; and endogenously released opioids acting on specific thalamic nuclei, their release depending on the origin of the presynaptic input.

Key words: μ-opioids; inhibition; thalamus; intralaminar nuclei; centrolateral nucleus; inwardly rectifying potassium conductance; cesium

The classical pain pathway from peripheral nociceptors to the thalamus is conveyed principally by neurons of the spinothalamic tract. These neurons are functionally characterized as nociceptive specific or nonspecific (wide dynamic range), can receive convergent somatic and visceral inputs, and project to two groups of thalamic nuclei: the ventrobasal and posterior complex and the centrolateral nucleus (CLN) (Applebaum et al., 1979; Giesler et al., 1981; Brüggemann et al., 1994; Craig et al., 1994). Neurons of the ventrobasal nucleus and posterior complex process and transmit the discriminative aspects of pain, and their response properties reflect the stimulus characteristics (Peschanski et al., 1980; Simone et al., 1993; Apkarian and Shi, 1994). The response properties of centrolateral neurons and their spinothalamic afferents (large, overlapping receptive fields and slower conduction velocities) indicate that the CLN is poorly suited to this task. It has therefore been suggested that the CLN processes signals associated with the affective properties of pain (Giesler et al., 1981; Nakahama et al., 1981; Peschanski et al., 1981; Jones, 1985).

Several lines of evidence suggest that the activity of thalamic neurons, specifically those associated with the transmission of pain signals, are modulated by opiates and opioids. First, anatomical studies have demonstrated the presence of the three opioid peptide precursors proenkephalin, prodynorphin, and proopiomelanocortin as well as opioid ligand binding sites and receptor mRNA transcripts throughout the thalamus (Fallon and Leslie, 1986; Merchenthaler et al., 1986; Mansour et al., 1987, 1994; Nahin, 1988; Loughlin et al., 1995). Furthermore, opiates and opioids modulate thalamic nociceptive cell activity. In the ventrobasal complex, the neuronal response to a noxious stimulus is reduced by morphine and μ-receptor agonists injected intravenously, whereas spontaneous cell firing and responses to innocuous stimuli are unaffected (Shigenaga and Inoki, 1976; Benoist et al. 1986). Intravenous morphine similarly reduces nociceptive responses of medial thalamic (including CLN) cells (Nakahama et al. 1981), but when locally injected, it depresses only the affective response to painful stimuli, whereas the motor response (tail flick) remains intact (Yeung et al., 1978, Carr and Bak, 1988).

These results prompted us to systematically analyze the role of opioid peptides in the thalamus. In the present study, we have examined the postsynaptic effects of opioids, focusing our attention primarily on the CLN. We subsequently analyzed the extent of opioidergic inhibition in the thalamus to determine whether it is restricted to thalamic nuclei implicated in the processing of pain. Using current- and voltage-clamp techniques in thalamic slices of young rats, we found that CLN neurons were inhibited by opioid peptides, an effect that was mediated by an increase in potassium conductance. Surprisingly, the result could be extended to many other sensory, motor, association, and intralaminar nuclei.

MATERIALS AND METHODS

Preparation of thalamic slices. Coronal thalamic slices were prepared from young Wistar and Sprague Dawley rats (12–23 d) of both sexes. Rats were anesthetized with a ketamine and xylazine solution (100 and 16 mg/kg, i.p., respectively) before decapitation. The skull was opened, and the brain was excised and placed in cold (4°C) oxygenated physiological solution containing (in mM): NaCl 126, KCl 2.7, NaH_2P0_4 1.25, $MgCl_2$ 1, $CaCl_2$ 2, $NaHC0_3$ 26.0, glucose 10, and pyruvic acid 5. A block of thalamic tissue was prepared by making the following cuts: two trans-

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versal (one rostral to the optic chiasm and one caudal to the mamillary body), two parasaggital (lateral to the reticular nucleus), and one horizontal (dorsal to the third ventricle). We cut 400 μM coronal slices using a vibratome (Campden Instruments, Sileby, UK), and stored them submerged in oxygenated solution in a small vial for 1 hr. For recording, slices were transferred to a chamber perfused (2 ml/min) with heated physiological solution (30–35°C).

Electrophysiological recordings and cell staining. Whole-cell current-clamp and voltage-clamp recordings of membrane properties were obtained using borosilicate pipettes (2–5 MΩ resistance) filled with (in mM): $MgCl_2$ 2, HEPES 10, K-gluconate 140, KCl 10, ATP-Mg 2, GTP-Tris 0.2 and neurobiotin (2 mg/ml). For recordings using tetraethylammonium (TEA^+) or Cs^+ as the main permeant ion, TEA-acetate or Cs-gluconate was substituted for K-gluconate. Thalamic cells were patched under visual control, using an infrared-sensitive video camera (Hamamatsu, Hamamatsu-City, Japan), and recordings were performed using an Axoclamp 200A amplifier (Axon Instruments, Foster City, CA) and a microcomputer equipped with pCLAMP software (Axon Instruments). Data were digitized (Neurocorder DR-890; Neuro Data Instruments Corp., Delaware Water Gap, PA) and stored on videotape. Potential values were corrected for the junction potential (11 mV) during analysis. In voltage-clamp recordings, whole-cell capacitance and series resistance were compensated and monitored throughout the experiment; recording was continued only if the series resistance did not exceed 20 MΩ. Drugs, TTX, and potassium channel blockers were applied by bath perfusion. At the end of the experiment, slices were fixed in a phosphate buffer solution (0.1 M, pH 7.4) containing 4% paraformaldehyde and 0.1% glutaraldehyde. Within 2–3 d, slices were incubated in an avidin–biotin complex solution (Vectastain ABC kit; Vector Laboratories, Burlingame CA) and further processed to reveal neurobiotin-labeled cells (Horikawa and Armstrong, 1988). To determine the location of the recorded cells within the thalamus, thin sections (60 μM) were cut from each slice and mounted on gelatin-coated slides. The sections were counterstained with cresyl violet to aid in defining the thalamic nuclei. The location of each labeled cell was determined using the atlas *The Rat Brain in Sterotaxic Coordinates*, second edition (Paxinos and Watson, 1986). Camera lucida reconstructions were made for each neurobiotin-injected cell.

Chemicals. The opioid peptide agonists D-Ala^2,*N*-Me-Phe^4,$glycinol^5$-enkephalin (DAMGO), (D-Pen^2,D-Pen^5)-enkephalin (DPDPE), and the μ-opioid receptor antagonist Cys^2, Tyr^3, Orn^5, Pen^7-amide (CTOP) were purchased from Bachem (Basel, Switzerland); the κ-opioid receptor agonist, (±)-*trans*-U-50488 methanesulfonate, was purchased from Research Biochemicals International (Natick, MA); TTX was purchased from Latoxan (Rosnans, France); and the neurobiotin and avidin–biotin complex was purchased from Vector Laboratories (Burlingame, CA).

RESULTS

A total of 221 thalamic neurons were recorded: 155 were located in the CLN, and 66 were located throughout most of the remaining thalamic nuclei. Most of the cells (197 of 221) were inhibited by opioids, whereas the remaining cells showed no response. Our first analysis (pharmacological and electrophysiological) was aimed primarily at the CLN.

Membrane properties of centrolateral neurons

Centrolateral (CL) neurons studied in detail ($n = 28$) had a mean ± SEM resting potential of −72.5 ± 5.9 mV. The input resistance of these cells ranged from 100 to 300 MΩ, and values generally decreased in older animals. Firing properties of a typical CL neuron in our system, held at −60 and −82 mV, are shown in Figure 1*A*. The firing pattern of CL neurons, and indeed all of the thalamic neurons that were recorded, presented two distinctive characteristics: membrane rectification at hyperpolarized potentials and a powerful low-threshold calcium spike. The inward rectification at hyperpolarized potentials was both immediate and time-dependent, although the time-dependent rectification (produced by the activation of the hyperpolarization-activated cation current, I_h) was relatively weak. The immediate rectification was most likely attributable to the activation of an inwardly rectifying potassium current, IK_{IR} (see below). Large hyperpolarizing pulses applied at resting potential were followed by the activation of a low-threshold calcium spike, crowned by three to six action potentials (Fig. 1*A*, *left*). This low-threshold spike pattern was also seen when cells were depolarized from more negative potentials (Fig. 1*A*, *right*) and is characteristic of the burst firing mode of thalamic relay neurons, which switch to tonic (action potential) firing mode at depolarized potentials (Jahnsen and Llinás, 1984).

Pharmacological characterization of opioidergic inhibition of centrolateral neurons

We first compared the action of DAMGO, DPDPE, and U50488, respective agonists selective for the μ-, δ-, and κ-opioid receptor types. Current-clamp experiments ($n = 11$) showed that cells in the CLN hyperpolarize in response to DAMGO (2.5 μM, applied for 30 sec). The hyperpolarization was reversible and was accompanied by a decrease in input resistance, as reflected by the decrease in voltage deflection in response to constant current pulses (Fig. 1B_1). Application of DPDPE and U50488 (also 2.5 μM for 30 sec) had no effect ($n = 11$). The response to DAMGO persisted in TTX (1 μM; $n = 25$) and in a low-calcium, high-magnesium solution (0.2 and 5 mM, respectively; $n = 3$, data not shown), suggesting that the effect was direct (Fig. 1B_1, *bottom*). An expansion of the voltage trace in Figure 1B_1 shows that the cell shifts from tonic to burst firing mode (characterized by a low-threshold calcium spike) on activation of μ-opioid receptors (Fig. 1B_2). This effect was mimicked by the μ-receptor agonist morphine (0.1 mM; $n = 4$) (data not shown) and blocked by the selective μ-receptor antagonist CTOP (1 μM; $n = 3$) (Fig. 1*C*).

All further experiments were performed in the presence of 1 μM TTX. The response to DAMGO was concentration-dependent, first appearing at a concentration of 2.5×10^{-8} M. Both the size of the hyperpolarization and the change in input resistance increased with DAMGO concentration (Fig. 2*A*, *left*). An average dose–response curve (from four cells, held at −60 mV) was constructed using the ratio of the voltage deflection in response to a constant current pulse in control to the same voltage deflection at the peak of the DAMGO effect ($\Delta V_{CONTROL}/\Delta V_{DAMGO}$); the membrane potential was manually clamped at resting potential (Fig. 2*A*, *right*). A 23% decrease in input resistance was measured at the EC_{50} (2.5×10^{-7} M).

Ionic basis of μ-opioidergic inhibition

The mean reversal potential of the effect, determined in current-clamp experiments (in TTX, $n = 15$) was −98.0 ± 6.3 mV, suggesting the involvement of a potassium conductance. The reversal potential was determined either by taking the intersection of the current–voltage curves in control and at the peak of the DAMGO response (Fig. 2*B*, *right*) or by applying DAMGO while holding the cell at various membrane potentials (Fig. 2*B*, *left*). Similar values were obtained by both methods.

The involvement of potassium was further investigated in voltage-clamp experiments in which the extracellular potassium concentration was increased from 2.7 to 5.4 and 10.8 mM ($n = 10$, 6, and 3; Fig. 3*B*). The current activated by DAMGO, I_{DAMGO}, was obtained by subtracting voltage-clamp current traces obtained in control from those at the peak of the effect (as in Fig. 3*A*, *bottom*). A plot of the voltage dependency of I_{DAMGO} was then obtained by plotting the early peak current amplitude, taking care to avoid contamination by the more slowly activating I_h. The reversal potential of I_{DAMGO}, extrapolated from current–voltage

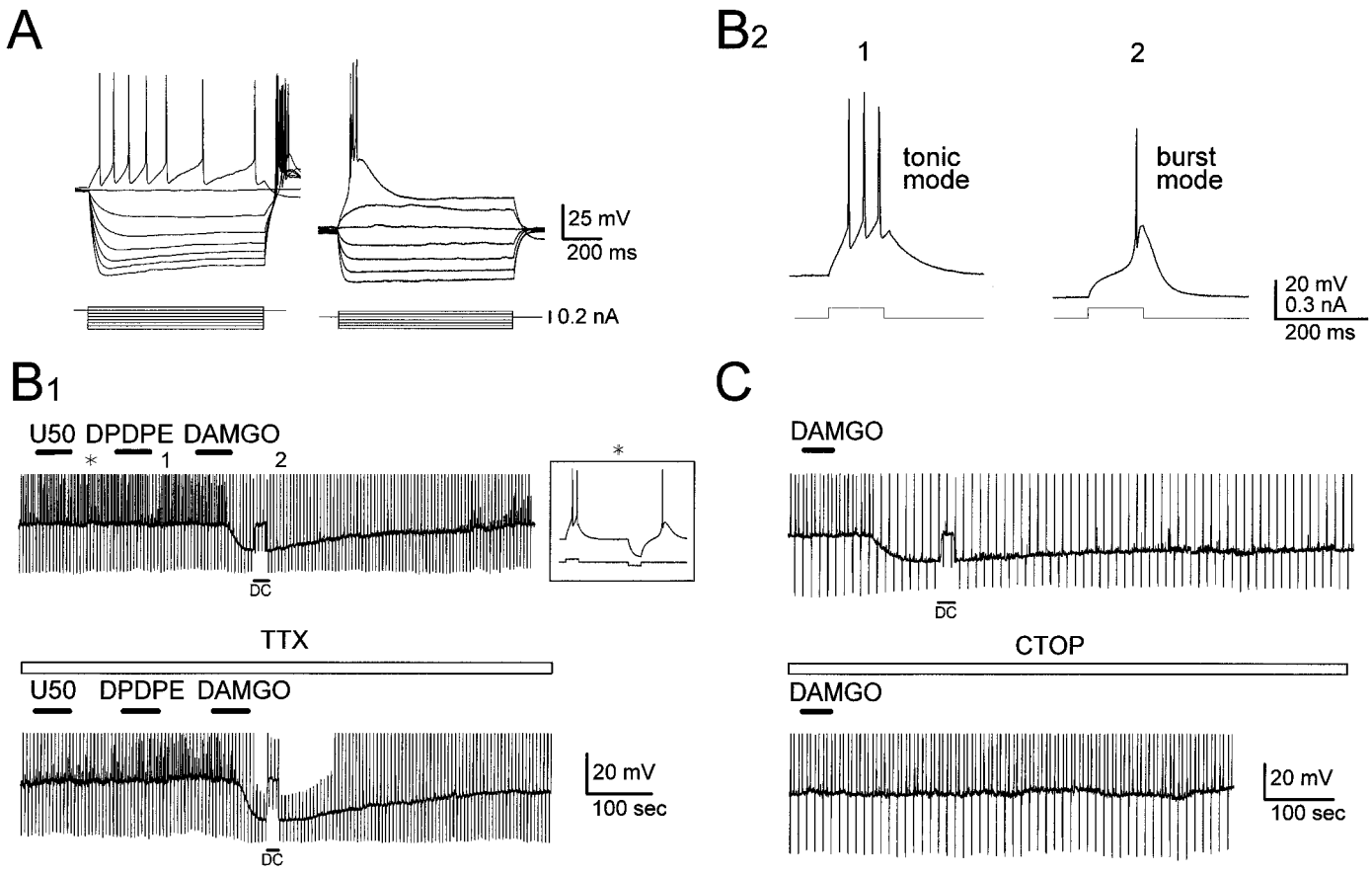

Figure 1. μ-Opioid receptor agonists inhibit centrolateral neurons. *A*, Firing properties of a typical centrolateral neuron, held at −60 (*left*) and −82 mV (*right*). B_1, Current-clamp experiment (same cell as in *A*) in which application of DAMGO (2.5 μM) provoked a hyperpolarization and decrease in input resistance. The respective δ- and κ-opioid receptor agonists, DPDPE and U50488 (2.5 μM), had no effect. *Inset*, Membrane potential response to the current protocol, a series of alternating pulses of ±0.1 nA. At the peak of the response to DAMGO, the cell was manually clamped at resting potential. Note the decrease in membrane voltage deflection in response to current pulses. The effect is direct (it remains in the presence of TTX, 1 μM) and reversible. B_2, Expansion of the voltage trace in B_1, at points *1* and *2*, shows that the cell shifts from tonic to burst firing mode (characterized by a low-threshold calcium spike) on application of DAMGO. *C*, The response to DAMGO is blocked by CTOP (1 μM), an antagonist selective for the μ-opioid receptor.

curves, shifted with $[K^+]_{EXT}$ (−92.3 ± 1.3, −76.2 ± 2.6, and −60.0 ± 4.1 mV, respectively; the expected shift in E_K at 30°C resulting from a twofold increase in extracellular potassium of +18 mV), demonstrating that I_{DAMGO} is indeed carried by potassium (Fig. 3*B*). The slope of the best-fit line connecting the points was 53.1.

Characterization of I_{DAMGO}

Because μ-opioids have been shown to activate an inwardly rectifying potassium conductance (GK_{IR}) in other CNS structures, we examined the voltage dependency of I_{DAMGO}, its dependence on extracellular K^+ concentration, and its sensitivity to the K^+ channel blockers Ba^{2+} and Cs^+. In voltage-clamp recordings (with extracellular TTX, 1 μM, and TEA, 10 mM), I_{DAMGO} rectified at hyperpolarized potentials, and its voltage dependency varied with extracellular potassium concentration (2.7, 5.4, and 10.8 mM; $n = 16$). I_{DAMGO} is plotted for one cell at all three concentrations (Fig. 3*A*, *top*), with the voltage-clamp pulses shown below (Fig. 3*A*, *bottom*). Inward rectification was modest in 2.7 mM K^+_{EXT} and robust in 5.4 and 10.8 mM K^+_{EXT}. It should be noted that the large values of IK_{IR} obtained in 10.8 mM external potassium may induce some error in voltage estimation attributable to series resistance (10 MΩ). In several cells ($n = 7$, not shown), I_{DAMGO} showed no voltage dependence. It is likely that in at least some cells a leak current is also increased, as with adenosine activation of a K^+ conductance, which shows no obvious voltage dependency (Pape, 1992).

We then tested the sensitivity of I_{DAMGO} to the potassium channel blockers cesium and barium. Figure 3*C* illustrates two different experiments in which the outward current is blocked partially by Cs^+ (2 mM; *top*) and completely by Ba^{2+} (1 mM; *bottom*). The mean decrease in I_{DAMGO} in the presence of barium (1 mM) and cesium (2 mM) was 95.9 ± 2.8% ($n = 6$) and 66.5 ± 20.3% ($n = 6$), respectively. Analysis of voltage-clamp ramp and pulse protocol data indicated that I_{DAMGO} was blocked across the complete voltage range by 1 mM Ba^{2+}. The block by Cs^+, however, appeared to be voltage-dependent and was studied further. Figure 3*D* shows an experiment in which I_{DAMGO} was blocked by cesium in a concentration- and voltage-dependent manner, the block increasing at hyperpolarized potentials. Both the inward-rectifying properties and the block of I_{DAMGO} by cesium and barium are consistent with μ-opioid activation of GK_{IR}.

In the course of experiments, it was also noted that both cadmium and nickel (0.5–1 mM) reversibly blocked the activation

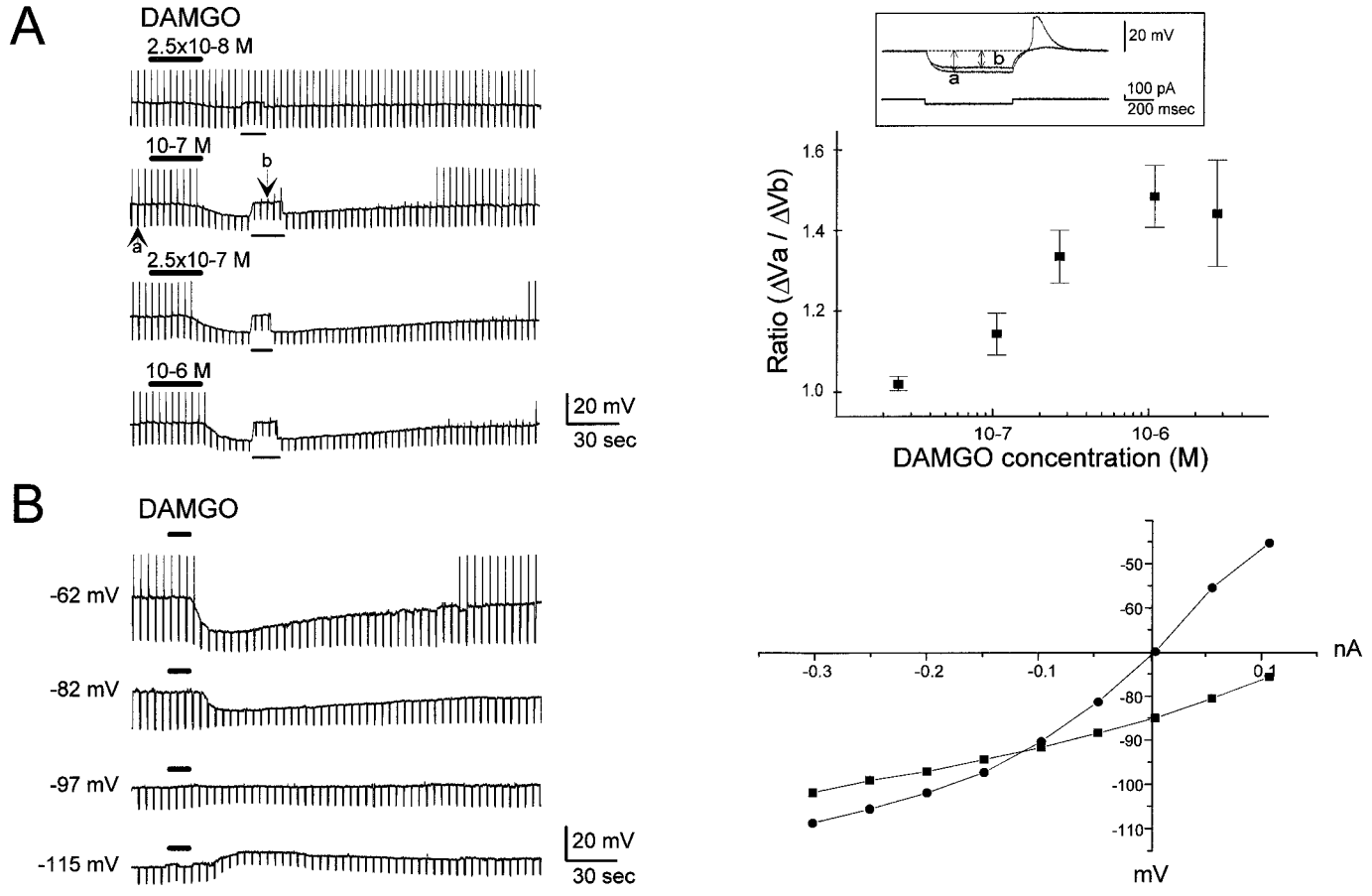

Figure 2. The response to DAMGO is concentration-dependent and reverses near E_K. *A*, *left*, Effect of increasing concentrations of DAMGO (V_m = −60 mV). Hyperpolarizing pulses (−0.1 nA) were applied every 4 sec. *Inset* (*right*, *top*), Expanded trace (see *A*, *left*) of the membrane potential response to the current pulse in control (*a*) and in DAMGO (*b*). Below is a dose–response curve from four centrolateral neurons. The decrease in input resistance is measured as $\Delta V_a/\Delta V_b$ (see *inset*). Error bars indicate SEM. The EC_{50} was at $\sim 2.5 \times 10^{-7}$ M. *B*, *left*, The reversal potential of the current activated by DAMGO was determined by studying the effect at various membrane potentials. The effect reverses at approximately −97 mV. A similar value (−93 mV) was obtained at the intersection of current–voltage plots taken in control (*circles*) and at the peak of the response to DAMGO (*squares*, *right*).

of I_{DAMGO} (data not shown). The block by Ni^{2+} was studied further ($n = 3$). In voltage-clamp experiments with 7 mM external potassium, 10 mM TEA, and 1 μM TTX, it was noted that on application of nickel (1 mM), IK_{IR} was substantially blocked and was no longer activated on application of a large hyperpolarizing voltage step. Application of DAMGO no longer had an effect on IK_{IR}, and at −60 mV, no outward current was seen. It is unlikely that the block of I_{DAMGO} by nickel is mediated by calcium, because responses to DAMGO were recorded when 10 mM BAPTA was added to the intracellular solution ($n = 4$).

Opioid modulation of I_h?

Finally, we investigated the effect of DAMGO on the hyperpolarization-activated cation current, I_h. When the potassium response was blocked by adding 1 mM Ba^{2+} to the bath, hyperpolarizing pulses across the activation range of I_h showed no change in amplitude after the application of DAMGO ($n = 3$).

Distribution of μ-opioidergic inhibition in the thalamus

Thalamic cells located in the principal relay, midline, and intralaminar nuclei were tested in the presence of TTX for their sensitivity to DAMGO. Cells located in the nucleus reticularis were investigated in another paper (Brunton and Charpak, 1997). Current-clamp experiments were conducted as shown in Figure 1 (DAMGO, 2.5 μM, applied for 10–30 sec). The location of cells injected with neurobiotin was identified in thin cresyl violet-stained sections. Of a total of 66 cells recorded throughout the thalamus, 59 were sensitive to μ-opioids. Thirty-four cells were successfully marked and located. Of those marked, all but three cells showed a positive response (a hyperpolarization) to DAMGO. Of these, one each was recorded in the anterior dorsal, laterodorsal, and ventromedial nuclei. Positive responses were recorded from labeled cells located in the following nuclei: paraventricular, anterior and posterior (PVA, PVP) ($n = 1$ and 1, respectively); mediodorsal, central (MDC) ($n = 3$); mediodorsal, lateral (MDL) ($n = 1$); anterior, ventral, dorsal, and medial (AV, AD, and AM) ($n = 1$, 1, and 1, respectively); paracentral, ($n = 3$); centromedial (CM) ($n = 3$); paracentral–ventrolateral border ($n = 3$); laterodorsal, dorsomedial and ventrolateral (LDDM, LDVL) ($n = 2$, 1, and 1, respectively, on the LDDM–LDVL border); reuniens (Re) ($n = 1$); ventrolateral (VL) ($n = 4$); ventromedial (VM) ($n = 1$); ventroposterior, medial and lateral (VPM and VPL) ($n = 2$); and dorsal lateral geniculate (DLG) ($n = 1$). A schematic representation of μ-opioid-sensitive nuclei is shown in Figure 4, along with camera lucida (300×) reconstructions of selected neurobiotin-injected cells (for clarity only one cell is shown for each nucleus). Coronal thalamic slices are shown

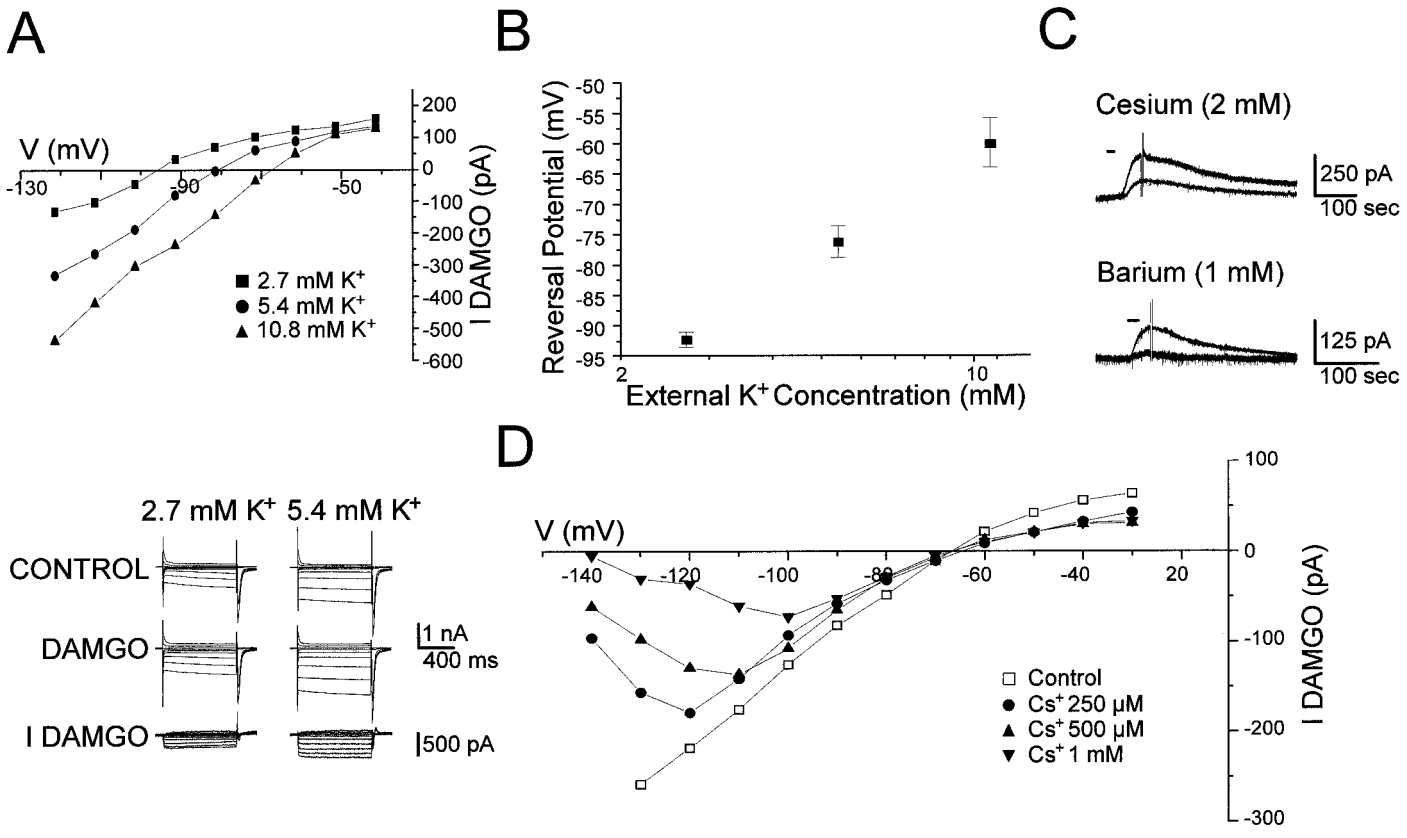

Figure 3. I_{DAMGO} demonstrates properties of an inwardly rectifying potassium current. *A*, *top*, Voltage-clamp data showing that I_{DAMGO} rectifies and that its rectification increases with extracellular potassium concentration (2.7, 5.4, and 10.8 mM; *squares*, *circles*, and *triangles*, respectively). I_{DAMGO} was obtained by subtracting the current traces in response to voltage steps in control from those at the peak of the response (*bottom*). *B*, The reversal potential of I_{DAMGO} shifts with increasing extracellular potassium concentration. Data were taken from 10, 6, and 3 cells, respectively. *C*, Steady-state current traces ($V_h = -60$ mV) show that the outward current activated by DAMGO is blocked partially by cesium (2 mM) and completely by barium (1 mM). *D*, Voltage-clamp data showing that the cesium block of I_{DAMGO} ($[Cs^+]_{EXT} = 0$ μM, *open squares*; 250 μM, *circles*; 500 μM, *upright triangles*; 1 mM, *inverse triangles*) is both concentration- and voltage-dependent. The extracellular potassium concentration for this experiment was 7 mM.

in anterior–posterior order (Fig. 4, *middle*), with *dots* marking the relative position of the cells within each nucleus. Detailed analysis of morphology was not undertaken, but cell bodies were ovoid or polygonal, 15–30 μM in diameter, with 5–10 primary dendrites branching close to the cell body, producing dendritic fields that had either radial, tufted, or asymmetric patterns (Sawyer et al., 1989; Tömböl et al., 1990).

DISCUSSION

This study examines opioidergic inhibition in the centrolateral nucleus of the thalamus. Activation of μ-opioid receptors results in an increase of an inwardly rectifying potassium conductance on the postsynaptic membrane, hyperpolarizing the cell and shifting its firing pattern from tonic to burst firing. Widespread μ-opioidergic inhibition was then demonstrated in many other relay, intralaminar, and midline nuclei.

Membrane properties of centrolateral neurons

Firing properties of CLN neurons were similar to those reported elsewhere (Jahnsen and Llinas, 1984), displaying two modes of firing: tonic and bursting, generating either trains of action potentials or low-threshold calcium spikes, as well as voltage- and time-dependent rectification at hyperpolarized potentials. Some important differences were noted. I_h was much weaker in our preparation than that reported in the adult guinea pig (McCormick and Pape, 1990), most closely resembling the I_h reported in the auditory thalamus of the young rat (Tennigkeit et al., 1996). It was similarly uncovered when leak and hyperpolarization-activated K^+ currents were blocked by extracellular barium. The difference from I_h in the adult rat and guinea pig is likely attributable to developmental changes, because the cells recorded were in good condition (had high input resistances), and developmental changes in I_h have been reported (Ramoa and McCormick, 1994; Pirchio et al., 1997). Rectification at hyperpolarized potentials was immediate, indicative of the activation of GK_{IR} (Hille, 1992; Tennigkeit, 1996). Indeed, this rectification increased when the extracellular potassium concentration was increased. Cells with exceptionally high intraburst frequencies, as in the large-cell region of the CLN in the adult cat (Steriade et al., 1993), were not observed in our preparation.

Characterization of opioid inhibition

Similar inhibition by opioids has been shown in a number of CNS structures, its mechanism being the modulation of a potassium conductance through the activation of G-protein-coupled receptors (Yoshimura and North, 1983; North et al., 1987; Williams et al., 1988). Inhibition through activation of the same potassium conductance has been observed with other neurotransmitters, such as serotonin, adenosine, acetylcholine, noradrenaline, and GABA (Andrade et al., 1986; Christie and North, 1988; North, 1989; Pape, 1992). Calcium channels are another common target

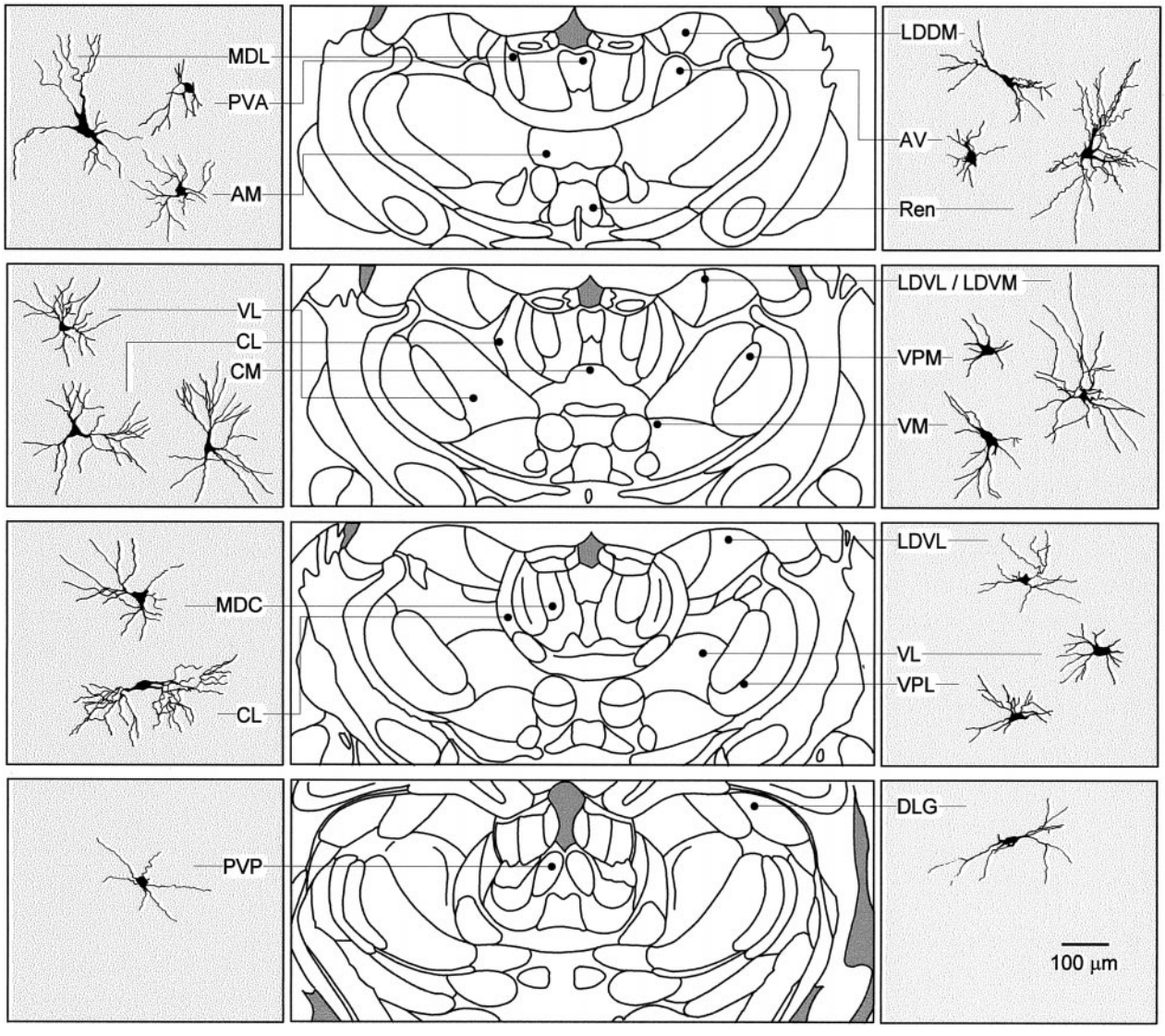

Figure 4. μ-Opioids inhibit cells in many thalamic nuclei. Camera lucida drawings of neurobiotin-labeled μ-opioid-sensitive cells and their corresponding locations are indicated. Coronal thalamic slices are shown in anterior to posterior order. *AM*, Anteromedial nucleus; *AV*, anteroventral nucleus; *CL*, centrolateral nucleus; *CM*, centromedial nucleus; *DLG*, dorsal lateral geniculate nucleus; *LDDM*, laterodorsal nucleus, dorsomedial; *LDVL*, laterodorsal nucleus, ventrolateral; *LDVM*, laterodorsal nucleus, ventromedial; *MDC*, mediodorsal nucleus, central; *MDL*, mediodorsal nucleus, lateral; *PVA*, paraventricular nucleus, anterior; *PVP*, paraventricular nucleus, posterior; *Ren*, reuniens nucleus; *VL*, ventrolateral nucleus; *VM*, ventromedial nucleus; *VPL*, ventroposterior nucleus, lateral; *VPM*, ventroposterior nucleus, medial. Drawings adapted from Paxinos and Watson (1986).

of μ-opioids (Hescheler et al., 1987; Schroeder et al., 1991; Seward et al., 1991), and inhibition of calcium entry in thalamic ventrobasal neurons has already been reported (Formenti et al., 1995).

Characterization of I_{DAMGO}

Inwardly rectifying potassium channels are a common target of G-protein-coupled receptors and have been localized in the soma and dendrites of thalamic cells of the rat (Williams et al., 1988; North, 1989; Ponce et al., 1996). Furthermore, colocalization of these channels with μ-opioid receptors in several thalamic nuclei has been observed (Bausch et al., 1995). GK_{IR} is characterized by a voltage dependence that increases steeply at hyperpolarized potentials and is dependent on $[K^+]_{EXT}$, fast activation, and sensitivity to barium and cesium (Hagiwara et al., 1976, 1978; Hille, 1992).

The block of I_{DAMGO} by cesium was consistent with the blockage of GK_{IR} reported elsewhere, depending on both membrane voltage and extracellular cesium concentration (Hagiwara et al., 1976; Argibay et al., 1983; Sodickson and Bean, 1996). Nickel blocked the low-threshold calcium spike but also reversibly blocked GK_{IR} and its activation by DAMGO. Nickel blockade of GK_{IR} activation by adenosine has been reported previously, although an effect on unstimulated GK_{IR} was not demonstrated (see Alzheimer and ten Bruggencate, 1991).

μ-Opioid modulation of I_h

The hyperpolarization-activated cation current, I_h, is another target of G-protein-coupled receptors (Pape and McCormick, 1989). In thalamic cells, I_h is coupled both positively and negatively to G-proteins activated by the transmitters noradrenaline, serotonin, and histamine (positively coupled), and adenosine (negatively coupled), which shift the voltage range of its activation

in the depolarizing or hyperpolarizing direction through a cAMP-dependent mechanism (McCormick, 1992; Pape, 1992). This modulation of I_h has been found to control oscillatory activity, particularly sleep spindles (Bal and McCormick, 1996; Lee and McCormick, 1996). Opioid inhibition of forskolin-activated I_h has been demonstrated in guinea pig nodose ganglion neurons (Ingram and Williams, 1994), but in agreement with their results, μ-opioids had no effect on unstimulated I_h in our preparation. Therefore, it appears that opioid modulation of I_h in the thalamus may differ from that of other neurotransmitters. It is also possible that cellular components necessary for I_h regulation were washed out by dialysis of the intracellular solution.

Distribution of μ-opioid inhibition in the thalamus

Autoradiographic and mRNA studies have shown that opioid ligand binding sites and receptor mRNA transcripts are differentially distributed in the thalamus, with a dense and widespread concentration of μ receptors, a moderate and discrete localization of κ receptors (mainly in midline nuclei), and almost no δ receptors (Mansour et al., 1987, 1994).

The consistent response of centrolateral neurons to μ-opioids begged the question of the specificity of the response and the extent of opioid sensitivity in other nuclei of the thalamus. Was sensitivity confined to pathways regulating pain, or did it extend to areas processing other sensory modalities and motor information? We found that μ-opioids inhibited cells in relay, intralaminar, and midline nuclei throughout the thalamus, indicating that their action is widespread. However, because receptor densities in several nuclei such as the ventrobasal nuclei have been reported to be quite low, it is possible that the proportion of opioid nonresponsive cells is higher. Equally, the κ agonist should be more extensively tested in the midline nuclei, where receptor densities are greater (Mansour et al., 1987, 1994).

To date, *in vivo* studies have demonstrated opioid inhibition of cell firing in response to a variety of painful stimuli in the medial, ventrolateral, lateral, and ventrobasal nuclei and to both painful and rewarding stimuli in the CLN (Nakahama et al., 1981; Benoist et al., 1986; Carr and Bak, 1988). Furthermore, opioids appear to discriminate between nociceptive and non-nociceptive stimuli, blocking one but not the other. Our results in both young and adult rats (data not shown) suggest that if this discrimination occurs in the thalamus, it must occur at a presynaptic location.

At the postsynaptic level, morphine injected intravenously is likely to hyperpolarize not only thalamic relay cells but also thalamic reticular and cortical neurons, which influence thalamocortical activity. Thus, in the latter case, for instance, the tendency of thalamic cells to fire in bursts would be counteracted by a disinhibition of cortical excitatory inputs. As a result, it is difficult to reconcile the analgesic effect of morphine with *in vitro* thalamic burst activity, particularly because burst activity is associated with pain caused by deafferentation in spinal patients (Lenz et al., 1994).

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