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Inward Rectification of Resting and Opiate-Activated Potassium Currents in Rat Locus Coeruleus Neurons

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Intracellular recordings were made from rat locus coeruleus neurons *in vitro*, and membrane currents were measured at potentials from −50 to −130 mV. In the absence of any applied agonists, the slope conductance of the cells increased 3-fold when the cell was hyperpolarized from −60 to −120 mV. This conductance increase was complete within 5 msec of the onset of a hyperpolarizing command and was subsequently independent of time for several seconds. The conductance increase was blocked by cesium chloride (1–2 mM), rubidium chloride (1–2 mM), or barium chloride (1–100 μM). The membrane potential range over which the conductance increased was centered at the potassium equilibrium potential (E_K; extracellular potassium concentration, 2.5–10.5 mM): the current/voltage (I/V) relation of the cell could be well described by supposing that there were 2 potassium conductances, one voltage independent (G_1) and the other (inward rectifier, G_{ir}) activated according to the expression $G_{ir} = G_{ir,max}/\{1 + \exp[(V - E_K)/k]\}$, where k ranged from 15 mV in 2.5 mM potassium to 6 mV in 10.5 mM potassium. The additional membrane potassium conductance that developed when agonists at μ-opioid and α_2-adrenoceptors were applied also became larger with membrane hyperpolarization, and this voltage dependence was also reduced or blocked by rubidium, cesium, and barium; in the presence of these agonists the current also reached its final value within 5 msec. However, the conductance increased by the agonists (G_{ag}) was not well expressed by simply increasing the values of G_1 and $G_{ir,max}$. It was best described by a potassium conductance that increased according to $G_{ag,max}/\{1 + \exp[(V - V_m)/k]\}$, where V_m (the potential at which the conductance was half-maximum) was close to the resting potential of the cell. It is concluded that the locus coeruleus neurons have a typical inward rectifier potassium conductance evident at membrane potentials negative to E_K. Agonists at μ-opioid and α_2-adrenoceptors increase a potassium conductance that becomes maximal at less negative potentials (about 10–20 mV hyperpolarized from the resting level); this serves to amplify the hyperpolarization caused by these ligands and provides for more effective inhibition of cell firing.

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The neurons of the rat locus coeruleus express a number of receptors that couple to a membrane potassium conductance. Membrane hyperpolarizations result from activation of μ-opioid, α_2-adrenaline, somatostatin, and $GABA_B$ receptors (Williams et al., 1982, 1985, 1987; Osmanovíc and Shefner, 1987); the α_2-adrenaline receptor can also be activated by transmitter released from other neurons, resulting in an inhibitory postsynaptic potential (i.p.s.p.) (Egan et al., 1983). The effectiveness of a potassium conductance increase to inhibit the firing of a neuron depends on the voltage dependence of the conductance itself and on the presence or absence of other membrane conductances which open or close as the membrane hyperpolarizes. The purpose of the experiments described in this paper was to investigate these 2 factors so that we could more reliably interpret inhibitory transmission operating by this mechanism. Preliminary reports of some aspects of this work have been published (North et al., 1987).

Materials and Methods

Intracellular recordings were made from locus coeruleus neurons in tissue slices from the pons of adult rats. A full description of the methods has been published previously (Williams et al., 1984). Briefly, slices (300 μm) were cut in a vibratome in cold (4°C) physiological saline and placed in a tissue bath through which flowed physiological saline (1.5 ml/min) at 37°C. The content of the physiological saline solution was (mM): NaCl, 126; KCl, 2.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $CaCl_2$, 2.4; glucose, 11; $NaHCO_3$, 25; gassed with 95% O_2, 5% CO_2 at 37°C. Electrodes were filled with potassium chloride (2 M). Membrane currents were recorded with a single-electrode voltage-clamp amplifier (Axoclamp II) using a switching frequency of 3–5 kHz. The potential at the headstage of the amplifier was monitored at all times with a separate oscilloscope. Experiments were carried out only when the potential at the headstage was flat for 20% of the cycle prior to sampling the voltage for the next cycle. Currents could be measured satisfactorily with 30–40 MΩ electrodes and by reducing the level of physiological saline above the slice to less than 300 μm. Current/voltage plots were constructed directly on an X/Y recorder using slow ramp potentials (−140 to −40 mV). The speed of the ramp potential (usually 1–2 mV/sec) was chosen so that the currents recorded were not different from those flowing at the end of 1 sec voltage step. The time course of the currents was studied by measuring the currents at the onset and offset of rectangular voltage command pulses.

Drugs and solutions of different ion content were applied by changing the superfusing solution by means of 3-way taps. Drugs and salts were barium chloride, cesium chloride, cocaine, [Met^5]enkephalin, kelatorphan (gift from B. Roques), noradrenaline bitartrate, rubidium chloride, tetraethylammonium chloride, tetrodotoxin (TTX), and Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO).

Results

Inward rectification of the cell membrane

Steady-state current/voltage plots had 2 linear portions, one between −60 and −80 mV and a second at potentials more

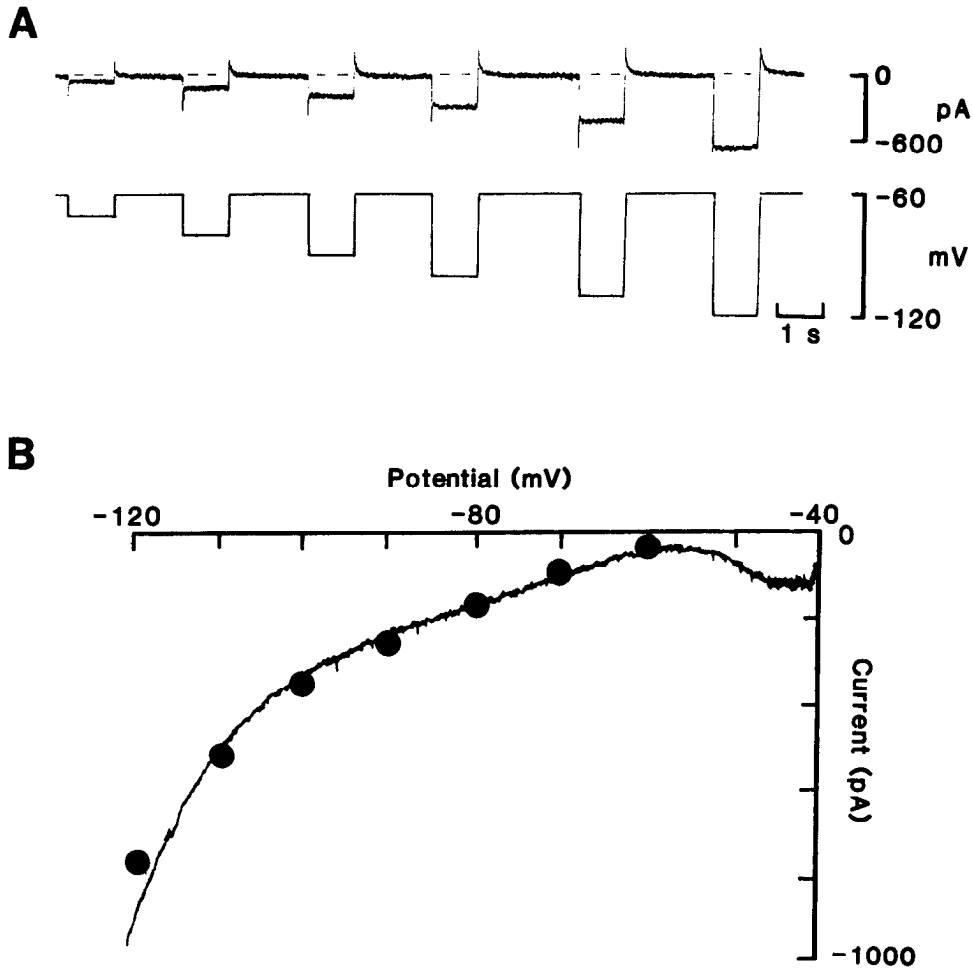

Figure 1. Inward rectification in a locus coeruleus neuron. *A*, Hyperpolarizing voltage commands were applied from a holding potential of −60 mV, and the resultant currents were measured. A transient outward current (A-current) flowed on return to the holding potential. *B*, Steady-state *I/V* plot from the same neuron as shown in *A*. The continuous line was plotted directly on an *x/y* plotter using a ramp depolarization from −120 mV to −40 mV. Points indicate the currents evoked by step hyperpolarizations from a holding potential of −60 mV.

negative than −110 mV (Fig. 1). (The same plots were obtained when the current was measured at the end of 1 sec hyperpolarizing steps from a holding potential of −60 mV, or by a slow ramp potential from −130 mV.) All locus coeruleus neurons displayed this rectification ($n > 200$). The conductance measured between −60 and −90 mV was 8.3 ± 0.5 nS ($n = 38$; range, 2.5–18.8 nS), and the slope conductance at potentials more negative than −110 mV was 24.5 ± 1.7 nS ($n = 27$; range, 10.2–41.6 nS). The additional current that developed with hyperpolarization was measured by extending a linear portion of the *I/V* plot between −60 and −80 mV and subtracting the current indicated by this extrapolation from the current actually observed at more negative potentials (see Fig. 2*A*).

The inward rectification reached a steady state within 5–7 msec of the onset of hyperpolarizing steps (settling time of the single electrode clamp), and the amplitude of the membrane current did not change during a 1–2 sec hyperpolarizing step command. With longer voltage steps from −60 to −120 mV the current declined by 10% or less in 5–15 sec. At the return of the membrane potential to the resting potential (−60 mV), a transient outward current (A-current) dominated other currents for a period of about 200 msec (see Williams et al., 1984). If the holding potential was maintained at −90 mV (where the A-current was not activated), step hyperpolarizations to more negative membrane potentials caused membrane currents that did not change with time.

Increasing the potassium concentration of the superfusing solution increased the conductance of the cell throughout the voltage range tested (−50 to −130 mV) and shifted to a less negative value the potential at which the inward rectification occurred (Fig. 2). In normal potassium solutions (2.5 mM), the *I/V* plot always had 2 distinct linear portions, one between −60 and −80 mV and the second at potentials more negative than −110 mV. In 4.5 or 6.5 mM potassium, this distinction between the linear portions of the *I/V* plots became less clear, and the conductance of the cell increased progressively between −60 mV and the potassium equilibrium potential; at more negative potentials, the conductance again became linear (Fig. 2*A*). At even higher potassium concentrations, the potential at which the inward rectification became prominent was less negative, within the range (about −60 mV) at which other inward calcium and sodium currents begin to activate (Fig. 2*A*; Williams et al., 1984); this made it difficult to estimate the linear portion accurately, and for this reason the inward rectifier current was not measured in potassium concentrations greater than 6.5 mM (Fig. 2*B*). In the limited range of potassium concentrations studied, it was clear that when the concentration was higher, the potential at which the cell conductance increased became less negative by an amount similar to the change in the potassium equilibrium potential (E_K; Fig. 2*B*).

Cesium chloride (1–2 mM), rubidium chloride (2 mM), and barium chloride (1–100 μM) each blocked the inward rectifica-

tion of the membrane with little effect on membrane current at −60 mV. Of the 3 blocking agents tested, barium provided the simplest picture. Barium appeared to remove the inward rectification with little change in the leak conductance (−60 to −80 mV) of the cell; thus, it made linear the *I/V* relation (Fig. 3*C*). Even at the low concentrations used (1 μM), barium also usually produced a small (5–40 pA) net inward current at −60 mV. The blockade of inward rectification by these low concentrations of barium was time dependent during the applied voltage command, complete blockade requiring about 300 msec from the onset of a step to −120 mV (Fig. 3*D*). At 100 μM, the blockade by barium occurred more quickly than could be resolved with the single-electrode voltage clamp (<5 msec).

Cesium prevented the increase in conductance that occurred at potentials negative to −105 mV (in 2.5 mM potassium) (Fig. 3*A*). With stronger hyperpolarization beyond E_K, cesium caused a progressively greater reduction in the membrane current, and also depressed the remaining leak conductance of the cell. A similar effect of cesium was also seen in 6.5 mM potassium. Rubidium also reduced or blocked the inward rectification, but this was generally associated with an increase in the membrane conductance between −60 and −80 mV; the effect of 2 mM rubidium on the leak conductance was similar to that of increasing the potassium concentration from 2.5 to 4.5 mM (compare Fig. 3*B* with Fig. 2*A*).

The cAMP analogs dibutyryl-cAMP (2–20 μM) and 8-bromo-cAMP (up to 1 mM), forskolin (1–10 μM), forskolin plus isobutylmethylxanthine (300 μM), and caffeine (10 mM) were applied in test of the hypothesis that the inwardly rectifying conductance was altered by intracellular cAMP levels (see Benson and Levitan, 1983). Of these, only caffeine reduced the inward rectification. We thus conclude that this inwardly rectifying conductance is unaffected by cAMP. The inward rectification was also apparently unaffected by intracellular injection of the nonhydrolyzable GTP analogs guanosine 5′-*O*-(3-thiotriphosphate; GTP-γ-S) and guanosine 5′-*O*-(2-thiodiphosphate; GDP-β-S) (Fig. 7*B*). Prolonged (up to 7 hr) soaking of the slice in a magnesium-free solution also did not change the inward rectification.

Potassium current evoked by agonists

μ-Opioid and α_2-adrenoceptor agonists increase potassium conductance in locus coeruleus neurons (Fig. 4*A*; see North and Williams, 1985). The voltage dependence of this conductance was studied by subtracting the current obtained in the presence of agonist from that obtained in the absence of agonist at a given voltage. The resultant current was plotted as a function of the membrane potential (Fig. 4*B*). The *I/V* plots for μ-opioid and α_2-adrenoceptor agonists were identical, as would be expected since occlusion experiments indicate that they operate the same conductance (North and Williams, 1985). The majority of experiments in the present study were carried out with [Met^5]enkephalin as the agonist, usually in the concomitant presence of kelatorphan to inhibit its degradation (Williams et al., 1987). The opioid conductance at −60 mV was about 6 nS and increased progressively with hyperpolarization to about 11 nS at −120 mV (Fig. 4*C*). The opioid conductance was dependent on the extracellular concentration of potassium, increasing at a given voltage as the potassium concentration increased (Fig. 4*C*). The steepness of the voltage dependence of the opioid conductance also increased as the extracellular potassium content was raised (Fig. 4*C*).

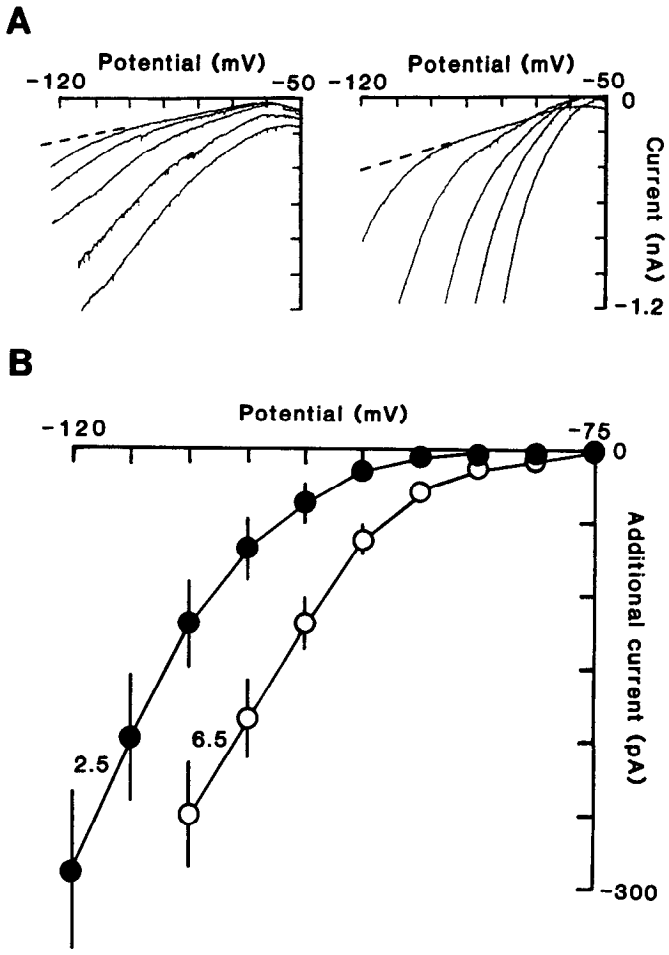

Figure 2. Dependence of membrane current on extracellular potassium concentration. *A*, Experiments are shown from 2 separate cells to illustrate the range observed. In each cell, *I/V* plots were constructed at 5 different potassium concentrations (2.5, 4.5, 6.5, 8.5, and 10.5 mM; *top left* to *bottom right traces*). The *broken line* is an extrapolation of the linear part of the *I/V* plot in 2.5 mM potassium. The difference between the actual current and the current indicated by this extrapolation is plotted in *B*. *B*, Additional (inward rectifier) current determined as described above. Observations are pooled for 10 cells in 2.5 mM potassium (*filled circles*) and for 8 cells in 6.5 mM potassium (*open circles*). *Vertical bars* are SEM.

The time course of development of the membrane current was not obviously affected by the presence of an opioid or adrenoceptor agonist; the current reached its steady-state value within 5–10 msec (see Fig. 5).

The inward rectification of the conductance increased by the agonists was blocked in solutions containing cesium chloride (2 mM), rubidium chloride (2 mM), or barium chloride (1–100 μM) (Figs. 5 and 6). None of these treatments changed the outward current caused by opioids at −60 mV (Fig. 6*C*). In normal potassium solution (2.5 mM, E_K = −110 mV), cesium blocked the inward current caused by the agonists at potentials more negative than E_K (Fig. 6). When the potassium concentration was raised to 6.5 mM (E_K = −84 mV), the opioid agonist induced conductance became voltage independent in the presence of cesium (Fig. 6*C*).

Rubidium (2 mM) also blocked the rectification in the agonist-induced current. The block of rectification was seen in all the cells examined, but there was also a reduction in the opioid

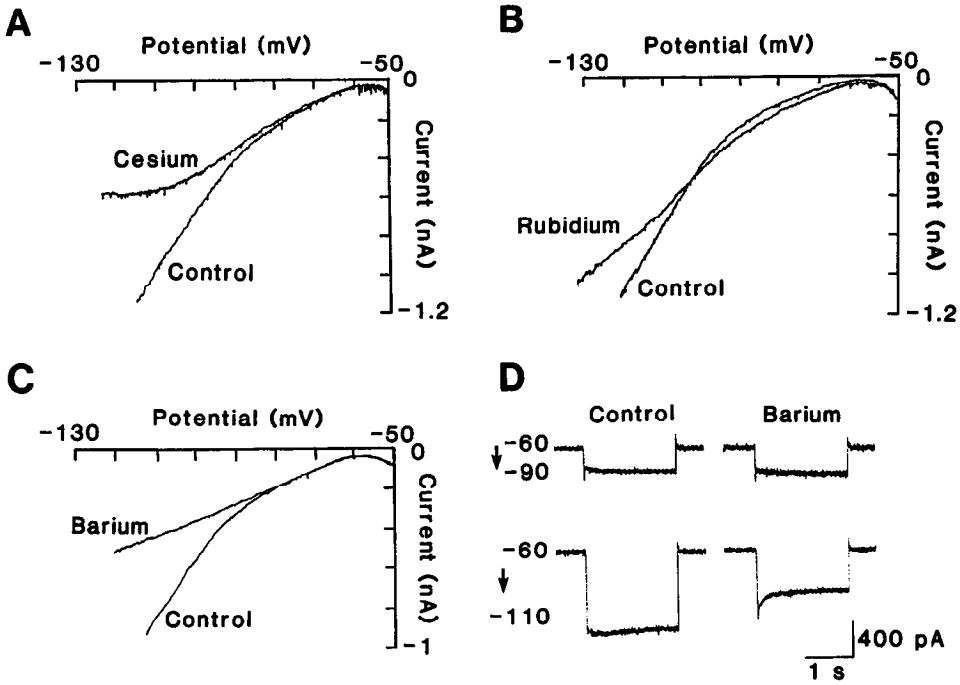

Figure 3. Block of inward rectification by barium, cesium, and rubidium. *A–C,* Steady-state *I/V* obtained from depolarizing ramp potentials before and after addition of cesium (2 mM), rubidium (2 mM), and barium (10 μM). All experiments were in 6.5 mM potassium. *D,* Time dependence of block of inward rectification by barium (10 μM). *Left side,* control; *right side,* in barium (10 μM). *Top,* Voltage step was from −60 to −90 mV. *Bottom,* Voltage step was from −60 to −110 mV. Barium did not affect the current evoked by stepping from −60 to −90; no inwardly rectifying current is evoked by the step to −90 mV. During the voltage step to −110 mV, barium caused at time-dependent block of the inwardly rectifying component.

induced *outward* current, which was variable from cell to cell (Fig. 6*C*). The reversal potential was shifted to a less negative value by up to 10 mV. Addition of rubidium might be expected to shift the reversal potential depending on the relative permeability of rubidium and potassium. Barium (1–10 μM) reduced but did not abolish the rectification of the agonist induced conductance, even in the same cells in which the inward rectification before the addition of agonist was completely blocked.

Cells impaled with electrodes containing GTP-γ-S became progressively hyperpolarized by 2–10 mV. Opioids caused an outward current at −60 mV, which was similar to that seen in control cells in all respects except for its smaller magnitude. The *I/V* relation before and after adding opioid agonist in such a cell is shown in Figure 7*B*. It can be seen that opioid current remains outward at all potentials examined. We interpret this to indicate that the opioids were acting predominately at elec-

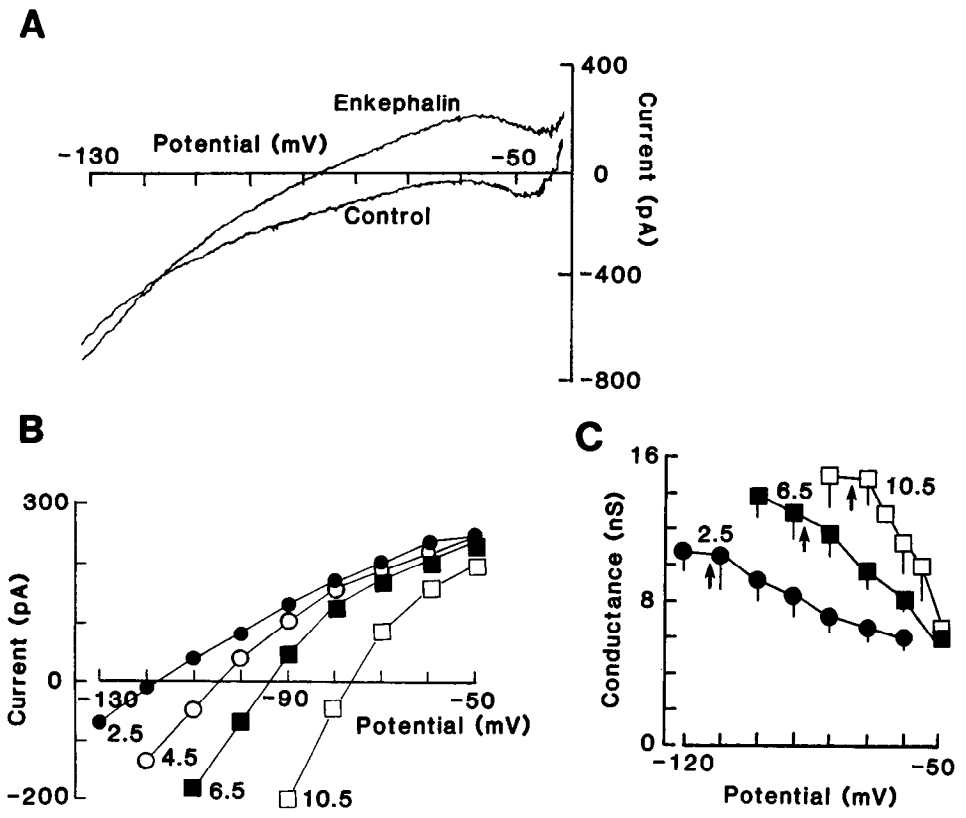

Figure 4. Enkephalin increases a potassium conductance that is voltage dependent. *A,* Steady-state *I/V* plots in control and after superfusion with [Met^5]enkephalin (10 μM). *B,* Enkephalin current was determined by subtraction and plotted as a function of membrane potential. This was repeated in 4 different potassium concentrations (indicated by trace, mM). In higher potassium concentrations, the slope of the *I/V* plot increased, the reversal potential shifted to less negative potentials, and the current became increasingly nonlinear. *C,* Enkephalin-induced conductance was determined as a function of membrane potential by dividing the current (from experiments such as shown in *B*) by the difference between the potential and the reversal potential. Reversal potentials (indicated by *arrows*) were −112.0 ± 1.3 mV (n = 21) in 2.5 mM potassium, −87.3 ± 0.8 mV (n = 15) in 6.5 mM potassium, and −74.0 ± 1.7 mV (n = 4) in 10.5 mM potassium. Values are means with SEM for 3–8 cells. Note that the enkephalin-induced conductance is already high near the resting potential (about −55 mV) (compare with Fig. 2*B*).

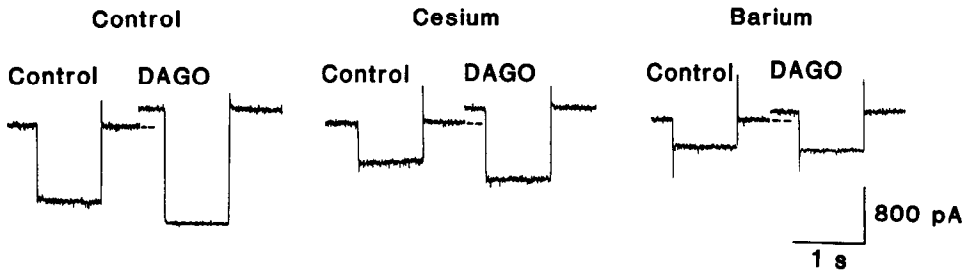

Figure 5. Conductance increased by opioids shows no significant time dependence. The experiment was carried out in 6.5 mM potassium. The holding potential was −60 mV, and the currents evoked by hyperpolarizing steps to −110 mV are illustrated. *Left side,* control. Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO) produced an outward current at −60 mV; at −110 mV (negative to E_K; see Fig. 4 legend), DAGO produced an inward current. *B,* Same experiment in the presence of CsCl (2 mM). *C,* In the presence of $BaCl_2$ (100 μM), the inward rectification of the resting membrane and the DAGO induced current was blocked within the settling time of the voltage clamp (compare to Fig. 3*D*).

trotonically distant parts of the cell unaffected by diffusion of GTP-γ-S and that such remote regions could not be sufficiently polarized to reverse the current flow. The electrotonically proximal regions are presumed to have the potassium channels already activated by the intracellular GTP-γ-S. Opioid actions were normal in cells impaled with electrodes containing GDP-β-S (Fig. 7*C*).

In 9 of 45 cells the opioid *I/V* plots showed little or no increase in current with membrane hyperpolarization; that is, no reversal of the opioid current was observed at potentials (Fig. 7*A*) less negative than −130 mV and the parallelism of the *I/V* plots before and after opioid implied an extremely negative reversal potential. All of these cells were typical locus coeruleus neurons in that they fired action potentials spontaneously, were hyperpolarized by opioids and α_2-adrenoceptor agonists, and showed inward rectification with hyperpolarization. The inward rectification of the membrane occurred at a membrane potential similar to that observed in the other cells centered around E_K, suggesting that the membrane potential was adequately (or equally adequately) clamped. These cells were exceptional with respect to other locus coeruleus neurons in 2 aspects; the slope conductance measured from −60 to −80 mV was larger (11.6 ± 1.1 nS, n = 9; range, 7.5–18.8 nS; control, 7.3 ± 0.4 nS, n = 36) and the opioid-induced outward current measured at −60 mV was also large (384 ± 34 pA, n = 9; range, 210–500 pA; control, 259 ± 17 pA, n = 36). It is considered that these recordings were from cells that had unusually large dendritic loads or from cells electrotonically coupled to other cells.

Discussion

Potassium conductances without agonists

In our first analysis of the *I/V* curves (Fig. 2) we chose to measure the currents with respect to a zero current potential of −60 mV. This method makes no assumptions regarding the equilibrium potentials for the ions carrying the current; the main finding was that the cell conductance negative to −60 mV could be well described by the sum of 2 currents, one with a linear dependence on voltage and the other increasing steeply around E_K. A dif-

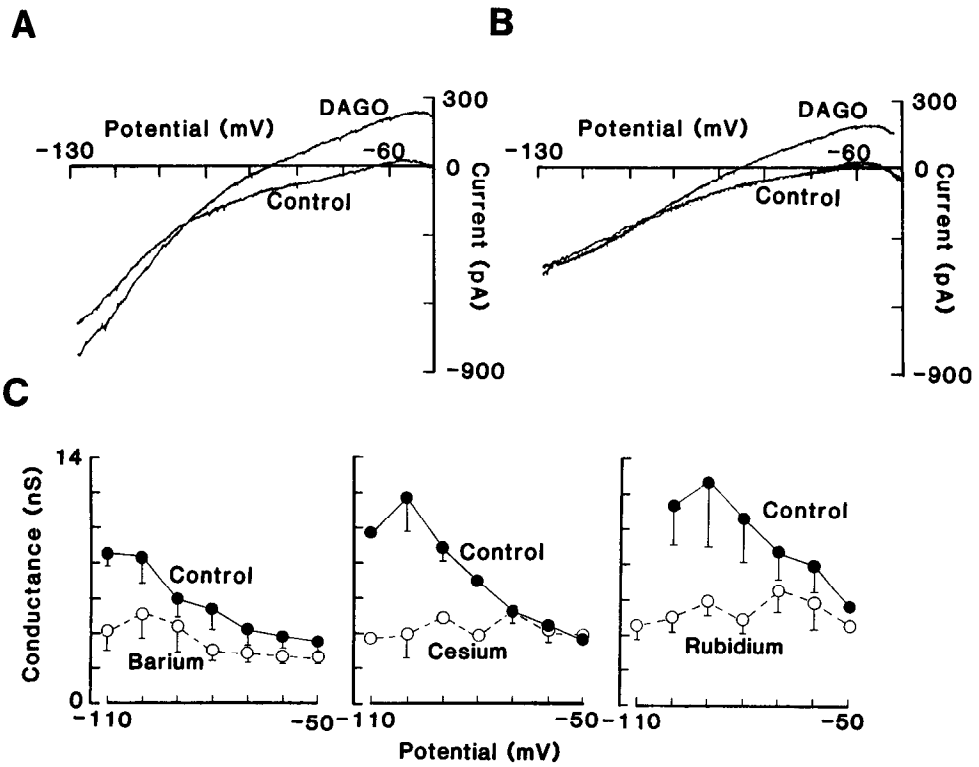

Figure 6. Rectification of the opioid conductance with membrane hyperpolarization is blocked by barium, cesium, and rubidium. *A, I/V* plots in the absence and presence of Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO, 3 μM; potassium concentration, 2.5 mM). *B,* Same experiment in a solution which contained CsCl (2 mM). The inward rectification of the resting membrane was reduced and the *inward* opioid current was abolished. *C,* rectification of opioid conductance is blocked by barium (1 or 10 μM), cesium (2 mM), and rubidium (2 mM). Opioid conductance plotted as a function of membrane potential in control solutions and in solutions containing potassium channel blockers. All of these experiments in *C* were carried out in 6.5 mM potassium. Points are mean ± SEM for x observations (x = 2-6, $\bar{x}$ = 3.8). Reversal potentials for current caused by opioid were as follows: control, −91.8 ± 2.4 mV; in barium, 89.0 ± 2.8 mV (n = 5); control, −86.0 ± 0.6 mV; in cesium, −83.7 ± 2.4 mV (n = 3); control, −87.5 ± 1.7 mV; in rubidium, −80.0 ± 4.3 mV (n = 4).

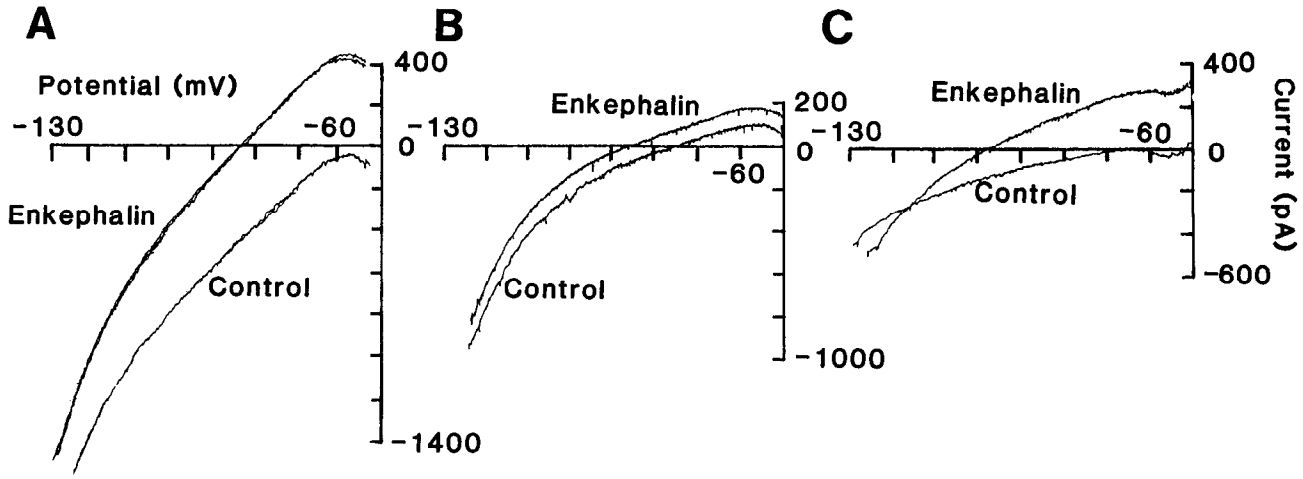

Figure 7. Electrotonically distant action of opioids. *A*, Steady-state *I/V* plot from an atypical cell in which the opioid current did not reverse. Two control traces (almost superimposed) are shown, and 2 traces are shown that were recorded in the presence of [Met^5]enkephalin (30 μM) and noradrenaline (30 μM) (labeled Enkephalin). Note the high conductance of the cell and the unusually large agonist-induced current at −60 mV. *B*, Recording from a cell impaled with an electrode that contained GTP-γ-S (10 mM). The inward rectification that occurred near E_K was similar to that found in control cells. Enkephalin caused an outward current that was almost independent of the membrane potential. *C*, Experiment in which the recording electrode was filled with GDP-β-S (10 mM). There is no difference between this experiment and one in which a KCl-filled electrode was used (see Figs. 4*A* and 6*A*).

ficulty with this method was that it could not be used when the potassium concentration was >6.5 mM because the straight portion of the *I/V* plot used to estimate the linear component disappeared. In some cells, a more complete method of analysis was used, taking advantage of the estimate of the potassium equilibrium potential (E_K) from the intersection of the *I/V* curves before and after adding enkephalin. It was assumed that the entire cell could be replaced by 3 conductances in parallel (see Fig. 8*C*). Two were potassium conductances, each in series with a battery having a value of E_K; one of these conductances was constant (G_l) and the other (G_{ir}) changed with voltage according to $G_{ir} = G_{ir,max}/\{1 + \exp[(V - V_m/k]\}$. The third was a conductance for inward current in series with a battery of +40 mV (G_{in}); the value of G_{in} was calculated for the cell so as to give zero net membrane current at −60 mV. An iterative least-squares curve-fitting program was then used to obtain the best estimates of G_l, $G_{ir,max}$, V_m, and k: in all cases an extremely good fit was obtained to the experimental data ($r^2 > 0.9998$; $n = 8$–15). This finding strongly supports the interpretation that the entire (steady-state) behavior of the neuron at potentials negative to rest can be accounted for by the sum of these 2 potassium conductances.

In the cell illustrated in Figure 8, the values were $G_l = 4.2 \pm 0.2$ nS, $G_{ir,max} = 13.3 \pm 1.3$ nS, $V_m = -116 \pm 3$ mV, and $k = 15 \pm 2$ mV. These values were obtained in 2.5 mM potassium; when the potassium concentration was raised to 4.5, 6.5, and 10.5 mM, the values for G_l increased progressively to 13.8 ± 0.2 nS, the value of $G_{ir,max}$ rose slightly to 17 nS, V_m declined progressively to −77 mV, and k declined to approximately 5 mV. In other words, increasing the potassium concentration shifted the voltage at which the inward rectifier was activated to a less negative potential and steepened its dependence on membrane potential. The actual value of V_m was dependent on the logarithm of the external potassium concentration. Such behavior is typical of inward rectifier potassium conductances in a variety of tissues (see Noble, 1985, for review).

A number of other observations support the interpretation that this potassium conductance (G_{ir}) additional to the leak potassium conductance is a "classical" inward rectifier (Katz, 1949; Hagiwara and Takahashi, 1974; Leech and Stanfield, 1981; see Hille, 1984). These are the very rapid kinetics of activation, the time-dependent block by low concentrations of barium, the block by rubidium, and voltage-dependent block by cesium. Although most extensively studied in skeletal muscle, cardiac muscle and tunicate egg cells, some of these features have also been reported previously for a neuronal potassium current in guinea pig olfactory cortex (Constanti and Galvan, 1983) and submucous plexus (Mihara et al., 1987). The findings also strongly support the inferences made by Osmanovíc and Shefner (1987) from voltage recordings that rat locus coeruleus neurons possess such a "classical" inward rectifier conductance.

Agonist-induced potassium current

The conductance increased by opioids and α_2-adrenoceptor agonists has previously been shown to be a potassium conductance that shows inward rectification (North and Williams, 1985; North et al., 1987). In the present study we sought to determine 2 things: first, whether the conductance increased by these agonists could be accounted for by simply increasing the values of the existing conductances without changing their properties and, second, the consequences for neuronal inhibition resulting from the particular properties of the conductance increased by the agonists.

The agonist-induced conductance was measured by subtraction of the *I/V* curves before and after adding the agonist (similar results were obtained with opioids or noradrenaline). This conductance had some similarities to the G_{ir} component present in the absence of agonist; it activated within 5 msec of a potential change, it showed inward rectification (Fig. 4), and the *I/V* relation was straightened by rubidium, cesium, or barium (see also North et al., 1987). However, there were fundamental differences between the properties of the potassium conductance increased by the agonists and those present at rest. The main one is obvious in many of the illustrations (e.g., Figs. 2*B* and 4*B*); the region of most curvature of the *I/V* relation is close to

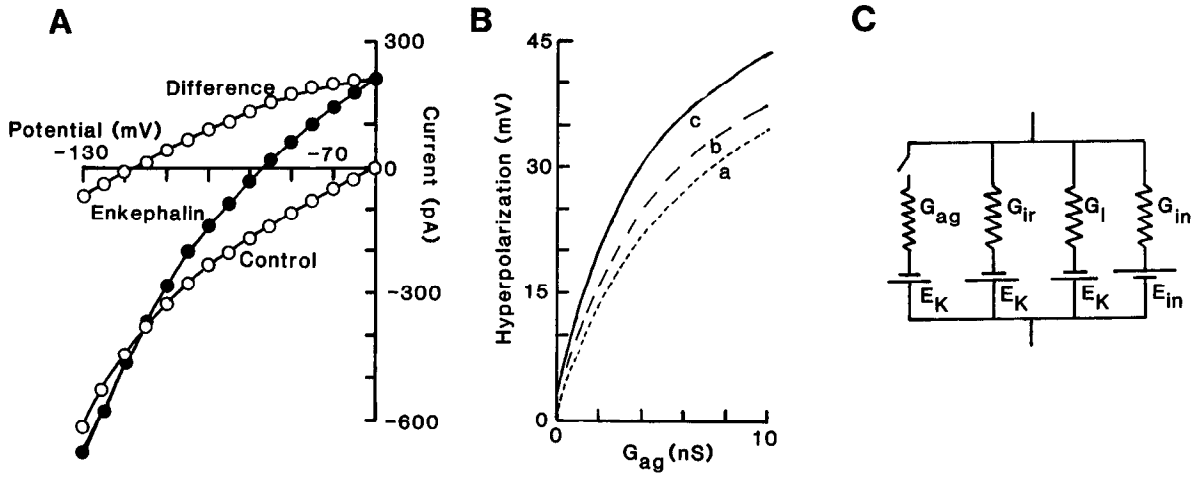

Figure 8. Inward rectification of the resting membrane potassium conductance and of the conductance increased by agonists serve to amplify the hyperpolarization caused by the agonist effect. *A*, *I/V* relation for a typical neuron and the effect of enkephalin. *Open circles* are membrane currents recorded at 5 mV increments of membrane potential; *filled circles* are the currents in the presence of Met^5-enkephalin (30 μM). The line passing through these points was fitted by a least-squares method to obtain the coefficients 13.3 for $G_{ir,max}$, 4.2 for G_l, −116 mV for V_m, and 15 mV for k (see text and *C*). E_K was taken from the intersection of the *I/V* relations before and after enkephalin (−116 mV). Thus, the line is $I = (13.3(V + 116)/\{1 + \exp[(V + 116)/15]\}) + [4.2(V + 116)] + [2.56(V - 40)]$. *Open circles* (difference) indicate the current induced by enkephalin, the difference between *open* and *filled circles*. The line passing through these points is given by $I = G_{ag,max}(V + 116)/\{1 + \exp[(V + 50)/15]\}$, where $G_{ag,max}$ = 5.5 nS. *B*, Hyperpolarization (mV, ordinate) resulting from a given increment in potassium conductance caused by agonist [G_{ag} (nS), abscissa]. Resting conductance at −60 mV was 4.2 nS (see *A*). See *C* for details of equivalent circuit. *a*, Linear membrane: potassium conductance is voltage independent (G_l) and its value is simply increased by G_{ag}. *b*, Inward rectifier membrane: potassium conductance is given by $G_{ir,max}/\{1 + \exp[(V + 116)/15]\} + \{G_l\}$ and enkephalin increases this by G_{ag}. *c*, Inward rectifier membrane and rectifying agonist conductance. Membrane conductance is as in *b*; enkephalin conductance is given by $G_{ag} = G_{ag,max}/\{1 + \exp[(V + 50)/15]\}$. The value of $G_{ag,max}$ was taken to be 1.51 nS because this scales to 1 nS at −60 mV for comparison with *a* and *b*. *C*, Equivalent circuit referred to in text and used for calculation of hyperpolarization in *B*. G_{ag} is conductance increased by agonist. G_{ir} is inward rectifier conductance of the cell. G_l is leak (voltage-independent) potassium conductance of the cell. G_{in} is a nonspecific conductance for all other ions, in series with a battery of E_{in}. For these calculations, G_l was taken to be the cell conductance between −60 and −80 mV, and E_{in} (= +40 mV) and G_{in} were computed to give zero membrane current at −60 mV; these values were used throughout subsequent computations.

the resting potential of the cell rather than nearer to the potassium equilibrium potential.

The effect of this was most clearly seen when the *I/V* relation for the cell in the presence of agonist was fitted by the least-squares method described above. The action of the opioid could not be accounted for by a simple increase in G_l or $G_{ir,max}$ when observations in all potassium concentrations were taken into account. The single model that provided the best fit to the experimental observations was obtained by adding to the resting conductances of the cell (G_l and G_{ir}) an additional potassium conductance (G_{ag}). G_{ag} was described by $G_{ag,max}/\{1 + \exp[(V - V_m)/k]\}$, where V_m, the midpoint for activation of the conductance, was approximately −50 mV and k was 15 (Fig. 8*A*): V_m did not change significantly when the potassium concentration was changed but k became smaller, as was seen for the resting "inward rectifier" (see above). One consequence of this behavior is that the opioid induced current does not decline greatly at −60 mV when the potassium concentration is increased (Fig. 4*B*); such an increase in extracellular potassium concentration could occur during the simultaneous hyperpolarization of locus coeruleus neurons by an agonist. A further prediction of this model is that in 2.5 mM potassium, the opioid conductance should be very low at −30 to −40 mV, as was previously observed (North and Williams, 1985). Rather similar conclusions have been made for the potassium conductance increased by ACh (Hartzell et al., 1977), adenosine (Trussell and Jackson, 1985), and baclofen (Gahwiler and Brown, 1985) in other neurons. This agonist-induced conductance thus shows inward rectification, around the resting potential, but should not be confused with the "inward rectifier" that is activated at potentials centered around E_K. The similarities between the conductances in terms of kinetics and sensitivities to blocking agents could be interpreted as suggesting that the same channels are involved, but that when they bind activated G-protein (see North et al., 1987), the midpoint voltage for their activation (V_m) is shifted from close to E_K to a value close to the resting potential of the cell. Satisfactory though this explanation is from the physiological point of view, it should be stressed that no evidence is available to substantiate the point.

It may be significant that the conductance increased by the agonists is already high at the resting potential of the cell and continues to increase with further hyperpolarization. Such a voltage dependence not only ensures that the transmitters will be effective at the resting potential (see Egan et al., 1983), but also leads to an amplification of the effects of transmitter on membrane potential. This is because any hyperpolarization will result in a further increment in conductance. Figure 8*B* illustrates the degree of amplification that would result from this effect. A conductance change of 2 nS caused by an agonist would hyperpolarize this neuron by about 13 mV if both the potassium conductances (i.e., those present in the cell and those opened by the agonist) were voltage independent. The *actual* hyperpolarization with conductances showing the voltage dependence observed is about 20 mV.

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