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CHANGES IN EXTRACELLULAR POTASSIUM ACCUMULATION PRODUCED BY OPIOIDS AND NALOXONE IN FROG SPINAL CORD: RELATION TO CHANGES OF NA–K PUMP ACTIVITY

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Superfusion of the isolated spinal cord of the frog with Ringer solution containing ouabain or naloxone elevated resting $[K]_e$ and depolarized the dorsal roots, while the application of enkephalins or morphine decreased $[K]_e$ and hyperpolarized the dorsal roots. During repetitive electrical stimulation (10–100 Hz) ouabain and naloxone increased the transient changes of $[K]_e$ and enhanced dorsal root potentials. When stimulation stopped, the clearance of K^+ was slowed, the poststimulation K^+ undershoot disappeared and poststimulation hyperpolarization of dorsal roots was diminished. The opposite effects were found during the application of enkephalins or morphine. Our results imply that the activity of the membrane Na–K pump is reduced after application of naloxone, while opioids enhance it.

Changes in membrane active transport, particularly of the Na–K pump, alter the concentration of ions in extracellular space and affect the nervous system function. Repetitive stimulation of peripheral nerves by adequate stimuli or by electrical pulses at 30–100 Hz leads to an increase of $[K]_e$ and to facilitation of Na–K pump activity in both mammals and amphibians [3, 5–7, 11]. The $[K]_e$ rises from the 'resting' level (about 3 mM) to the so-called 'ceiling' level (about 8–9 mM). With continued stimulation $[K]_e$ falls from the 'ceiling' level during stimulation. When stimulation stops $[K]_e$ decreases below 'resting' level, i.e. there is poststimulation K^+ 'undershoot'. Both the decrease of $[K]_e$ during the stimulation and the 'undershoot' reflect the participation of active processes in the clearance of accumulated K^+ (for review see ref. 10). Moreover, the depolarization of the dorsal root fibers, which accompanies the increase of $[K]_e$ during the stimulation, is followed by dorsal root hyperpolarization (DRH) when stimulation ceases. This hyperpolarization has also been considered to result from the activation of the Na–K pump [2, 3, 8].

Several studies suggest that neurotransmitters may alter neuronal activity and

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transmitter release by an action on electrogenic ion transport (for review see ref. 14). We recently found that enkephalins and morphine enhanced Na^+,K^+-adenosine triphosphatase activity in spinal cord membrane fractions from frogs, while naloxone blocked it [4]. The aim of this study was to determine if opioids and naloxone can affect membrane active transport in 'intact' spinal cords. We studied the effects of opiates and naloxone on the 'resting' level of $[K]_e$, stimulation-evoked K^+ transients and on polarization of primary afferent fibers in the isolated spinal cord of the frog. The effects were compared with those of ouabain, a known inhibitor of the Na–K pump [9].

Experiments were performed on isolated spinal cords of frogs (*Rana temporaria*). The cords (n = 35) were maintained in a chamber and superfused with oxygenated (95% O_2–5% CO_2) Ringer solution containing (in mM): NaCl 114.0, KCl 3.0, $CaCl_2$ 1.8, $NaHCO_3$ 10.0, glucose 1 g/l, at 17–19°C, pH 7.2 ± 0.1. DC recordings of the dorsal root potentials (DRPs) were made from either the 8th or 9th dorsal root with bipolar platinum electrodes. K^+ activity was measured by means of double-barrelled K^+-selective microelectrodes (Corning Code 477317) at a depth 400–500 μm from the dorsal spinal surface. The construction and calibration of the K^+-selective microelectrodes have been described elsewhere [3, 6].

Superfusion of isolated spinal cords with Ringer solution containing ouabain elevated the resting $[K]_e$ and depolarized the dorsal roots. At concentration of 10^{-7} or 10^{-6} M ouabain increased the resting $[K]_e$ by about 0.5 mM and at 10^{-5} M by about 2 mM. These effects were found even when synaptic transmission was blocked by the addition of 2 mM $MnCl_2$ or 20 mM $MgSO_4$. At low concentrations (from 10^{-8} to 10^{-6} M) (−)-naloxone (Endo) but not (+)-naloxone increased the resting $[K]_e$ by 0.1–0.6 mM and depolarized the dorsal roots. These effects were also found after adding Mn^{2+}, or Mg^{2+} to normal Ringer or to Ringer with low $[Ca^{2+}]$ (0.1 mM) (Fig. 1A). Application of Leu-enkephalin or Met-enkephalin (Sigma) as well as morphine from 10^{-6} to 10^{-4} M decreased the resting level of $[K]_e$ (by about 0.1–0.2 mM). These changes were accompanied by DRH. The hyperpolarization, but only small changes in resting $[K]_e$ (less than 0.05 mM) were found when enkephalins and morphine were applied with Mn^{2+} or Mg^{2+}.

Superfusion of isolated spinal cords with naloxone, enkephalins or morphine enhanced transient changes in $[K]_e$ and DRPs induced by single electrical stimuli (Figs. 1B and 2A). These effects on $[K]_e$ and DRPs were also found in the presence of Mn^{2+} or Mg^{2+} (Figs. 1A and 2B) when an increase in $[K]_e$ and DRPs were produced by K^+ released from primary afferents themselves [12, 15]. However, during repetitive electrical stimulation of dorsal roots, when the activity of membrane Na–K pump was stimulated, the naloxone and opioids acted differently. In the presence of enkephalins or morphine the 'ceiling' level of $[K]_e$ was always lower, the clearance of accumulated K^+ resulting from stimulation was faster, and poststimulation DRH was enhanced (Fig. 2C). These effects of opioids were also found in the presence of Mg^{2+} or Mn^{2+}. After application of naloxone the original 'ceiling' level of $[K]_e$ was exceeded (Fig. 1C). The clearance of accumulated K^+ after stimulation was dramatically slowed, the poststimulation 'undershoot' disappeared and DRH was dimi-

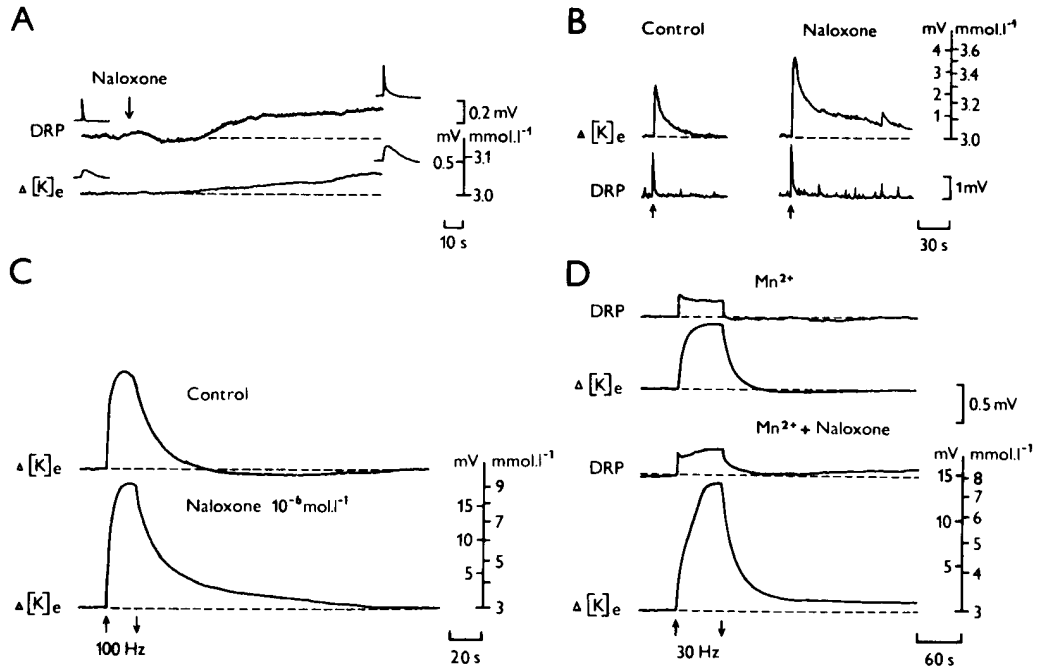

Fig. 1. Effects of 10^{-6} M naloxone upon resting $[K]_e$ in spinal dorsal horn and upon $[K]_e$ and DRPs evoked by electrical stimuli to dorsal root. A: depolarization of dorsal root and increase of resting $[K]_o$ after application of naloxone. Insets: responses to single electrical pulse (arrow). Time calibrations, 10 s for insets equals 120 s for continuous trace. B: $[K]_e$ and DRPs evoked by electrical pulse before (control) and after superfusion of spinal cord with naloxone in the presence of 20 mM Mg^{2+}. C: effects of naloxone on $[K]_e$ evoked by tetanic electrical stimulation at a frequency of 100 Hz. D: effects of naloxone on DRPs and $[K]_e$ evoked by tetanic stimulation in the presence of 2 mM Mn^{2+}.

nished. These effects of naloxone were even more marked when synaptic activity was blocked. While the $[K]_e$ under high Mn^{2+} or Mg^{2+} increased only to about 4 mM during the perfusion with naloxone, the 'ceiling' level of $[K]_e$ of about 8 mM was again attained. This large increase in $[K]_e$ during stimulation in the presence of naloxone also produced greater depolarization of primary afferents (Fig. 1D). A similar increase of 'ceiling' level of $[K]_e$, slowed K^+ clearance, enhancement of DRPs and decrease of DRH were found after spinal cord superfusion with ouabain at concentrations 10^{-6} or 10^{-5} M (Fig. 3). All the effects of enkephalins described above were fully reversible after superfusion of isolated cords with normal Ringer solution. On the other hand, the effects of morphine, naloxone and ouabain persisted even after 60 min.

The changes in the kinetics of $[K]_e$ as measured by K^+-selective microelectrodes agree with the effect of opioids and naloxone on Na^+,K^+-adenosine triphosphatase activity found on isolated membrane fragments [4]. The observed increase in resting $[K]_e$ after relatively low concentrations of naloxone and ouabain agrees qualitatively with changes in K^+ and Na^+ distribution, which can be expected during the inhibi-

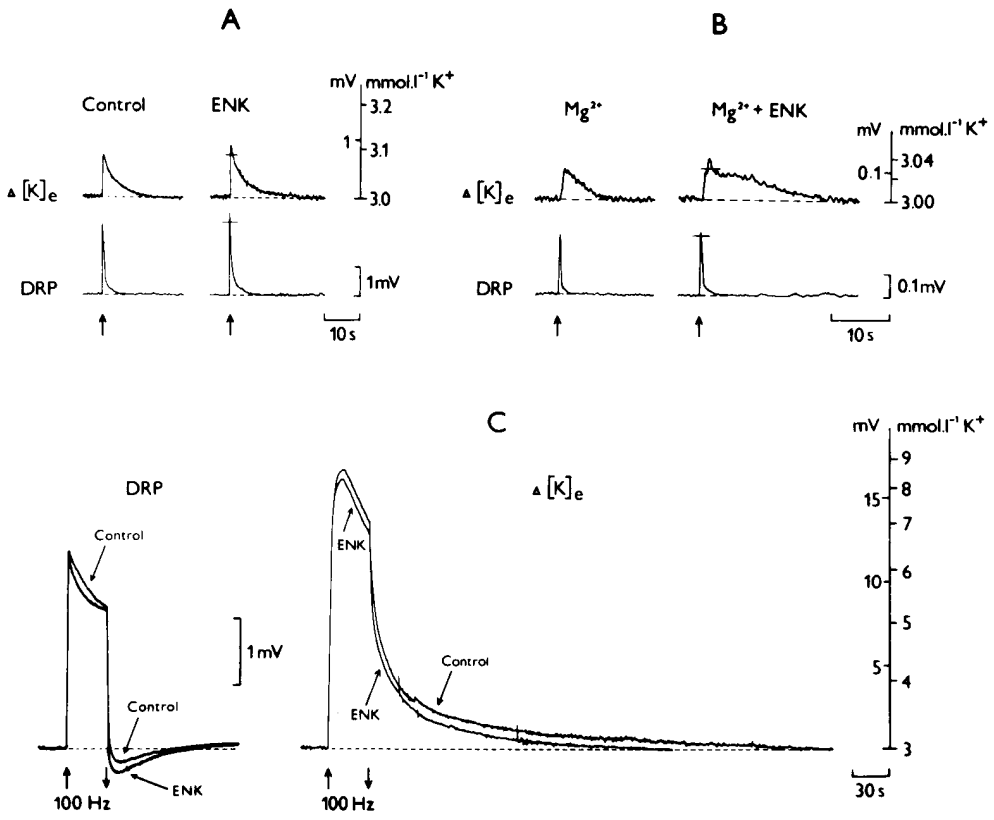

Fig. 2. Effects of Met-enkephalin (5×10^{-6}M) upon $[K]_e$ in dorsal horn and DRPs. A and B: responses to single electrical stimulus applied to adjacent dorsal root. Control, before; ENK, 15–30 min after superfusion of spinal cord by Ringer solution with enkephalin. Mg^{2+}, in the presence of 20 mM Mg^{2+}; Mg^{2+} + ENK, after addition of enkephalin. C: DRPs and $[K]_e$ during electrical stimulation of dorsal root at a frequency of 100 Hz. Duration of stimulation indicated by arrows.

tion of the Na–K pump. A rise in $[K]_e$ related to the inhibition of Na–K pump has previously been reported during hypoxia [6, 16], hypoglycemia [1] and after the application of cardioactive steroids [13]. Since naloxone also slowed K^+ dissipation and particularly because it inhibited poststimulation K^+ undershoot, which is the most specific indicator of Na–K pump activity [5–7], we conclude that naloxone, like ouabain, inhibits the Na–K pump. The opposite effects of opioids suggest that they may facilitate the activity of the Na–K pump. In this way opioids can help to restore the normal extracellular milieu more effectively, especially in situations when $[K]_e$ is substantially raised due to enhanced activity of spinal cord neurons and primary afferents.

It has been accepted that opioid peptides inhibit the discharge of nerve cells and that their action results from 'postsynaptic' inhibition of neuronal firing as well as from a 'presynaptic' reduction in the release of neurotransmitters. Both types of inhi-

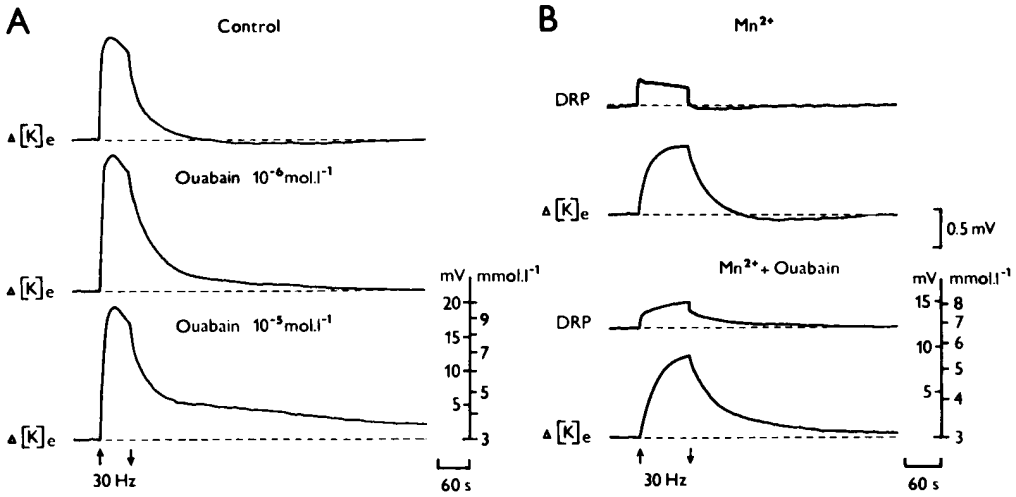

Fig. 3. Effects of ouabain upon $[K]_e$ and DRPs evoked in spinal dorsal horn by tetanic electrical stimulation. A: $[K]_e$ before (control) and 30 min after application of ouabain (10^{-6} and 10^{-5} M). B: effects of ouabain (5.10^{-5} M) upon $[K]_e$ and DRPs in the presence of Mn^{2+}.

bition may result from an increase in membrane K conductance and membrane hyperpolarization [17]. However, the increased rate of the Na–K pump may also account for membrane hyperpolarization [14]. It remains to be seen, therefore, to what extent the activation of the electrogenic Na–K pump also plays a role in neuronal effects of opioids.

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