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INHIBITION OF IONIC CURRENTS IN FROG NODE OF RANVIER TREATED WITH NALOXONE

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1 Myelinated nerve fibres of frog sciatic nerve were investigated under current and voltage clamp conditions. In the presence of 68 μM external naloxone, the action potential was completely, though progressively, blocked within 15 min of drug superfusion. The resting potential remained constant.

2 Under voltage clamp conditions both peak Na^+ and steady-state K^+ currents were decreased reversibly by external naloxone. Both currents were reduced in a dose-dependent manner but, whereas sodium current was affected by the smallest concentrations of naloxone (1.3 up to 12.5 μM), potassium current was decreased only by higher concentrations (25 up to 112 μM).

3 The time-course of development of the effect on both Na^+ and K^+ currents after exposure to 112 μM naloxone (a concentration giving more than 50% of decrease) showed that the effect develops quickly within the first 2 min of exposure to the drug, but afterwards both currents continue to fall more slowly, though progressively.

4 Experiments with constant test pulses to $E_m = -10$ mV and conditioning prepulses of various amplitudes, showed that the Na inactivation curve, $h_\infty (E_m)$, was shifted in a negative direction along the potential axis; the shape of the curve was also slightly changed in the presence of naloxone since the shift was larger near the top of the curve. All the observed effects were reversible after returning to the standard Ringer solution.

5 Internal naloxone (< 0.2 mM) reduced the amplitude of the action potential as well as peak Na^+ and steady-state K^+ currents; the sodium inactivation curve, $h_\infty (E_m)$, was shifted to more negative potentials.

6 A possible anaesthetic-like activity of naloxone on the nodal membrane is discussed.

Introduction

Frazier, Ohta & Narahashi (1973) described the effects of naloxone on the membrane ionic currents of the squid giant axon. The effects were examined on the internal surface of the axonal membrane. Both the peak amplitude of the transient sodium current and the steady-state amplitude of the late potassium current were reduced when naloxone was directly applied to the internal surface of the axonal membrane; all the observed effects were reversible after returning to the standard internal solution. These effects were apparently not due to an interaction of naloxone with a specific receptor, since additive, rather than antagonistic effects were observed with mixtures of morphine and naloxone.

The aim of the present research was to investigate the effects and possible mechanism of action of naloxone on the node of Ranvier in frog myelinated nerve fibres

Methods

The experiments were carried out on sensory and motor myelinated nerve fibres isolated from the sciatic nerve of the frog, *Rana esculenta*. The action potential and membrane currents were recorded under current clamp and voltage clamp conditions using the method of Nonner (Nonner, 1969). At the beginning of each experiment, the internodes bordering the test node were bathed in an isotonic (117 mM) KCl solution and were cut to allow displacement of the axoplasm by this solution before any measurements were made.

The resting potential was adjusted to give a steady-state inactivation of the sodium system of 30%; under these conditions the holding potential was assumed to be -70 mV. Votage clamp pulses were applied from this holding level. Symmetrical leakage and capacity currents were subtracted from the total current (Dubois & Bergman, 1977). The current amplitudes were calculated assuming an axoplasmic resistance of 10 MΩ. Under voltage clamp conditions, the resting sodium inactivation was removed by 45 ms hyperpolarizing prepulses to -110 mV.

The node under investigation was continuously superfused with normal Ringer solution. During the experiments the temperature was held constant at about 14°C. When studying the sodium current, the

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potassium current was blocked with caesium fluoride (90 mM) in the side pools in place of potassium chloride (Dubois & Bergman, 1975). Tetrodotoxin (100 nM) was used externally to block the sodium current, when studying the potassium current. The standard Ringer solution had the following composition (mM): NaCl 111.5, KCl 2.5, $CaCl_2$ 1.8 and $NaHCO_3$ 2.4. Naloxone hydrochloride (Endo) was added to the external Ringer solution; doses in the text refer to the concentration in the bathing medium.

To investigate the effects of internal naloxone, the drug was added to the isotonic KCl solution bathing the two cut ends of the fibre; in this case the drug reached the nodal membrane by diffusion into the axoplasm over a distance of 500–1000 μm.

Results

Current clamp measurements

Action potentials were elicited with 0.1 ms current pulses at a frequency < 0.2 Hz. After addition of 68 μM naloxone to the Ringer solution, the amplitude of the action potential decreased drastically and the threshold potential increased. Complete block of the action potential was reached within 15 min of drug superfusion (Figure 1). The resting membrane potential was not changed. Complete reversal was achieved after 10 min wash out of the drug.

Potential clamp measurements

Current-potential relation Positive potential steps of increasing amplitude were applied to the membrane. Each positive potential step was preceded by a 45 ms negative conditioning pulse to $E_m = -110$ mV. The peak Na^+ and steady-state K^+ currents were plotted in relation to membrane potential (Figure 2). After addition of 25 μM naloxone to the Ringer solution, both initial Na^+ (I_{Na}) and delayed K^+ (I_K) currents were decreased; there was no shift of the $I_{Na} - E_m$ and $I_K - E_m$ relations along the potential axis; the reversal potential for the sodium current was not affected by naloxone, implying that the relative permeabilities of the node to Na^+ and K^+ ions were not altered (Hille, 1971). After 15 min wash out of the drug, I_{Na} and I_K recovered to their control values.

In all the nodes investigated, the time for the transient current to reach its peak value was used as a measure of the kinetics of the mechanism which turns on the sodium current; in no case did naloxone affect the time required for the sodium current to reach its peak.

Steady-state inactivation of the sodium system The steady-state inactivation curve for I_{Na} was determined by a pulse programme in which a 7 ms constant test pulse to $E_m = -10$ mV was preceded by 45 ms conditioning pulses of various amplitudes. Potassium currents were blocked with 90 mM internal caesium. In the control Ringer solution, 30% of the Na permeability was inactivated, i.e. $I_{Na}/I_{Na\ max} = 0.7$. Naloxone (25 μM), applied outside the fibre produced a shift of the h_∞ curve to more negative potentials. The negative shift of the h_∞ curve, measured at the point $h_\infty = 0.7$, was 17 mV (Figure 3). The negative shift induced by naloxone could be partially removed by prolonging the duration of the hyperpolarizing prepulses. In addition to moving the entire h_∞ curve in the negative direction on the voltage axis, naloxone slightly changed the shape of the curve; the shift at large negative potentials (near $h_\infty = 1$) was

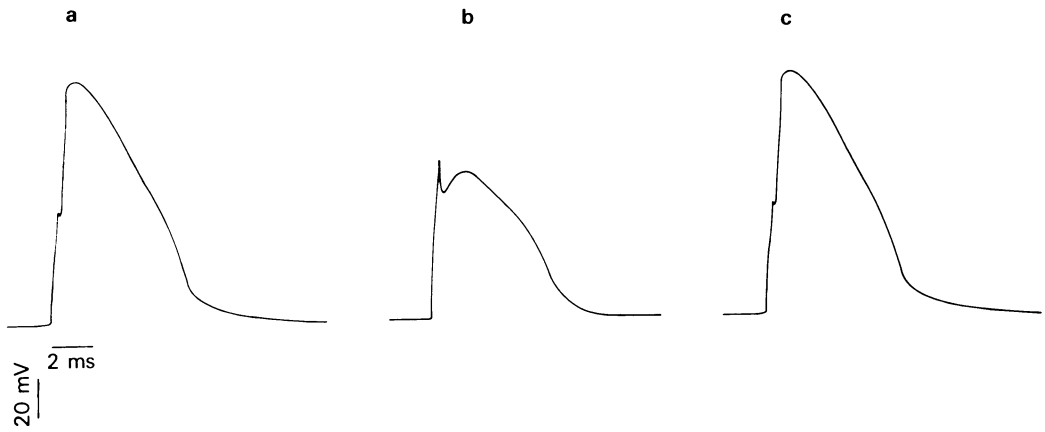

Figure 1 Effect of naloxone on current clamp measurements. The current clamped fibres were stimulated at < 0.2 Hz with 0.1 ms current pulses in control Ringer (a) and (c), Ringer + 68 μM naloxone (b). Motor fibre. Temperature: 14°C. Time between (a) and (b) = 3 min; between (b) and (c) = 10 min.

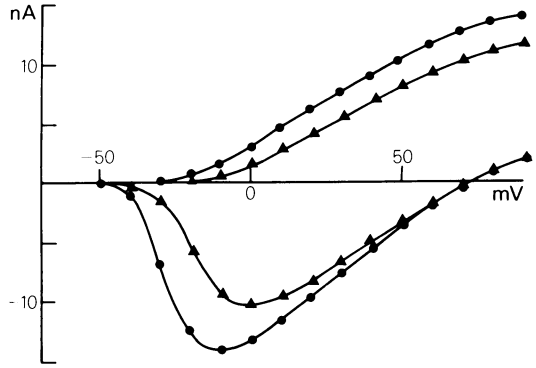

Figure 2 Reversible effects of naloxone on current-voltage curves. The peak Na^+ and steady-state K^+ currents were recorded during depolarizations of various amplitudes in normal Ringer solution (●) and in Ringer plus 25 μM naloxone (▲). Ordinate scale: current density in nA/cm^2. Abscissa scale: membrane potential (E_m) in mV. Temperature: 15°C.

greater than that at small negative potentials (near $h_\infty = 0$). The negative shift and the change of shape of the h_∞ curve were removed after a 15 min wash out of the drug.

Dose-response relation The effects of increasing concentrations of naloxone, ranging from 1.3 up to 112 μM, on the membrane currents were studied. The peak sodium and steady-state potassium currents were recorded with a double pulse programme; after removing sodium inactivation by negative prepulse (see Methods), a 7 ms depolarizing pulse was applied to $E_m = -10$ mV followed by a 68 ms depolarizing pulse to $E_m = +65$ mV. The decrease of the peak sodium and steady-state potassium currents was calculated as a percentage of the value in Ringer solution before drug application (Figure 4). The smallest concentrations of naloxone (1.3 up to 12.5 μM) reducing peak sodium current, did not affect steady-state potassium current.

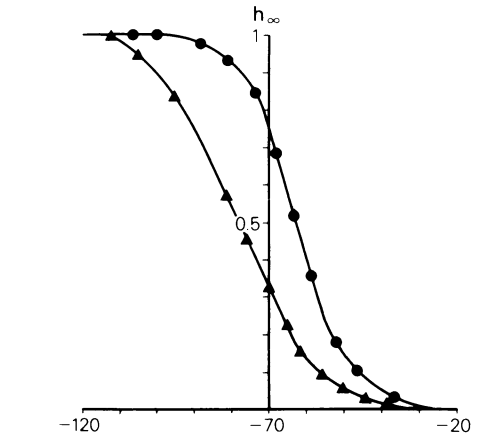

Figure 3 Effects of naloxone on the sodium inactivation system. Availability of the Na system in Ringer solution (●) and in Ringer plus 25 μM naloxone (▲). Sodium currents were recorded after 15 min internal perfusion with 90 mM caesium fluoride. The h_∞ curve is shifted in a negative direction along the potential axis. Temperature: 14°C.

Time-dependent block of Na^+ and K^+ currents

The time-course of development of the effect of naloxone on the peak sodium and steady-state potassium currents was examined (Figure 5). Both Na^+ and K^+ currents were recorded with the double pulse programme described above. A concentration of naloxone (112 μM) decreasing both I_{Na} and I_K more than 50% was used. Figure 5 shows that a large decrease of both Na^+ and K^+ currents was reached within the first 2 min of drug superfusion, but afterwards, during the remaining exposure to the drug, both Na^+ and K^+ currents continued to fall more slowly, though progressively.

Effects of internal naloxone

Naloxone was added in the side pools (see Methods), at a concentration of 0.2 mM, to allow diffusion into the axoplasm; with this procedure it was not possible to evaluate exactly its concentration at the nodal membrane, but it was certainly much smaller than

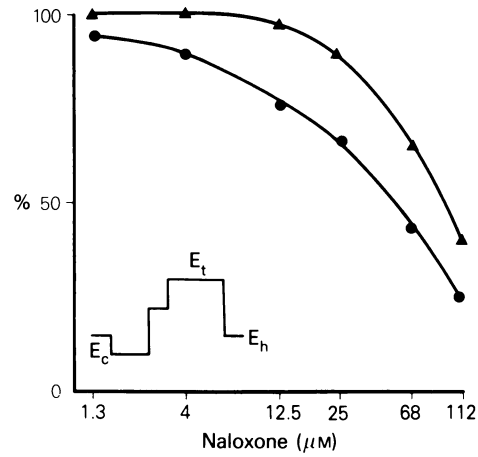

Figure 4 Dose-response relation: steady-state effects of increasing naloxone concentrations on peak sodium (●) and steady-state potassium (▲) currents; I_{Na} and I_K are given in % of the corresponding control value in Ringer solution. Abscissa scale: naloxone concentration plotted on a logarithmic scale. Inset, double pulse programme with E_c = conditioning prepulse, E_t = test pulse and E_h = holding potential. Temperature: 15°C.

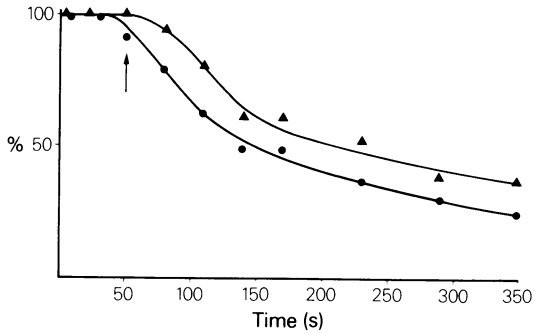

Figure 5 Time-dependent effect of naloxone on I_{Na} and I_K: the time-course of development of the effect of naloxone on peak sodium (●) and steady-state potassium (▲) currents after exposure to 112 μM naloxone. The node was continuously superfused with control Ringer solution before naloxone was added to the superfusate, which began at the arrow; I_{Na} and I_K are given as a % of the corresponding control value in Ringer solution. A double pulse programme was used to measure peak I_{Na} and steady-state I_K (see Figure 4). Abscissa scale: time (s) of exposure to naloxone. Temperature: 14°C.

0.2 mM. The effects of internal naloxone were consistent with those obtained with external superfusion. In fact, under current clamp conditions, the amplitude of the action potential was reduced progressively with ultimate complete block after 30 min of drug application; under voltage clamp conditions, both peak Na^+ and steady-state K^+ currents were reduced and the h_∞ curve was shifted to more negative potentials.

Discussion

External naloxone was found to reduce, with ultimate complete block, the amplitude of the action potential and to raise the threshold potential; the resting potential remained unchanged. Analysis of the ionic currents revealed that in the presence of naloxone, both peak Na^+ and steady-state K^+ currents were reduced, according to the observed effects on the action potential. The peak Na^+ current was reduced to a larger extent than the steady-state K^+ current; unlike peak Na^+ current, the smallest concentrations of naloxone tested (1.3 up to 12.5 μM) did not affect the steady-state potassium current. The time-course of development of the effect on both I_{Na} and I_K after exposure to 112 μM naloxone (concentration giving more than 50% of decrease) showed that the effect developed quickly within the first 2 min of exposure to the drug, but afterwards both currents continued to fall although more slowly. Naloxone also affected the steady-state inactivation of the sodium system; in the presence of the drug, the availability of the sodium system was reduced; the h_∞ curve was shifted to more negative potentials; the shape of the curve was also slightly changed since the shift at large negative potentials was larger near the top of the curve. Naloxone thus accentuates the asymmetry of the h_∞ curve about the point $h_\infty = 0.5$ (Chiu, 1977).

Internal naloxone (< 0.2 mM) produced effects consistent with those obtained with external superfusion. The effects of externally and internally applied naloxone on the ionic currents at the nodal membrane, in many respects resemble those of internal naloxone on the squid giant axon described by Frazier *et al.* (1973); however, whereas in the squid giant axon naloxone significantly shortens the time required for sodium current to reach its peak, in the node membrane this value is not affected. It is noteworthy that such unselective block of nodal ionic currents is also observed with external enkephalins (Carratù, Dubois & Mitolo-Chieppa, 1982); nevertheless, we must point out that unlike naloxone, enkephalins, either internally or externally applied, produce neither shift nor change of shape of the h_∞ curve of the node membrane.

It must be emphasized that a shift of the Na inactivation curve to negative potentials was first described for myelinated nerve fibres treated with local anaesthetics; in addition, it is well known that the inactivation induced by local anaesthetics can be relieved by a long hyperpolarization of the membrane (Khodorov, Shishkova, Peganov & Revenko, 1976; Hille, 1977); both effects are very similar to those produced by naloxone in our experiments

Therefore, it appears that naloxone shares with the local anaesthetics the following properties: unselective block of ionic currents, negative shift of h_∞ curve and removal of inactivation with prolonged hyperpolarizing prepulses. A degree of lipid solubility of the naloxone molecule could explain its anaesthetic-like activity. In conclusion, we suggest that naloxone, like local anaesthetics, may penetrate the nodal membrane to reach its site of action near the Na inactivation gate.

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