Effects of Morphine on Internally Perfused Squid Giant Axons[1] (36160)

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(Introduced by L. L. Boyarsky)

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Experiments on nerves *in situ* and on isolated nerves have provided little evidence that morphine interferes with impulse transmission in myelinated or nonmyelinated nerve fibers. Kosterlitz and Wallis (1), utilizing several mammalian nerve preparations, reported that the resting potential, action potential, positive after-potential, and conduction velocity were relatively unaffected by concentrations of morphine known to depress transmission at autonomic nerve–effector cell junctions. Recently, however, Simon and Rosenberg (2) have reported a weak local anesthetic action of morphine when applied externally to the squid giant axon. Morphine, at 10^{-3} *M*, caused about a 40% decrease in spike height after about 30 min of exposure. The ability of tritiated levorphanol, a morphine congener, to penetrate into the axonal envelope and the axoplasm correlated well with the effects on electrical activity and was enhanced at the higher pH. These experiments suggest that the action of these agents is on the internal surface of the axonal membrane. The present paper describes the results of experiments in which morphine was applied directly to the internal surface of the squid axonal membrane. Voltage clamp techniques were employed to determine the effect of morphine on membrane ionic conductances.

Methods. Giant axons of the squid, *Loligo pealei*, available at Marine Biological Laboratory, Woods, MA, were used. The method of internal perfusion has been described in detail previously (3). In brief, a giant axon was isolated and partially cleaned, its axoplasm was squeezed out by means of a small roller, and the crushed axon was inflated with an internal perfusate. In all experiments the perfusion was continuous. The internal perfusate contained 400 mmole/liter of K^+, 370 mmole/liter of glutamate, 15 mmole/liter of $H_2PO_4^-$, and 333 mmole/liter of sucrose; the pH was adjusted to 7.3. Artificial sea water was used as the external bathing medium; it contained 449 mmole/liter of Na^+, 10 mmole/liter of K^+, 50 mmole/liter of Ca^{2+}, 30 mmole/liter of tris(hydroxymethyl)aminomethane, and 559 mmole/liter of Cl^-, and the pH was adjusted to 8.0. A capillary microelectrode was used to sense the resting and action potentials of the membrane. The maximum rate of rise of the action potential (dv/dt) was used as a measure of excitability because it is proportional to the inward current at that moment and is a more sensitive index than the height of the action potential. In those cases in which the resting potential slowly changed, the measurements of the action potential and its rate of rise were made after the potential was brought back to its original level by application of an appropriate current through a separate platinum wire electrode. At least four axons were tested with morphine at each of the following concentrations: 10^{-3}; $5 \times$

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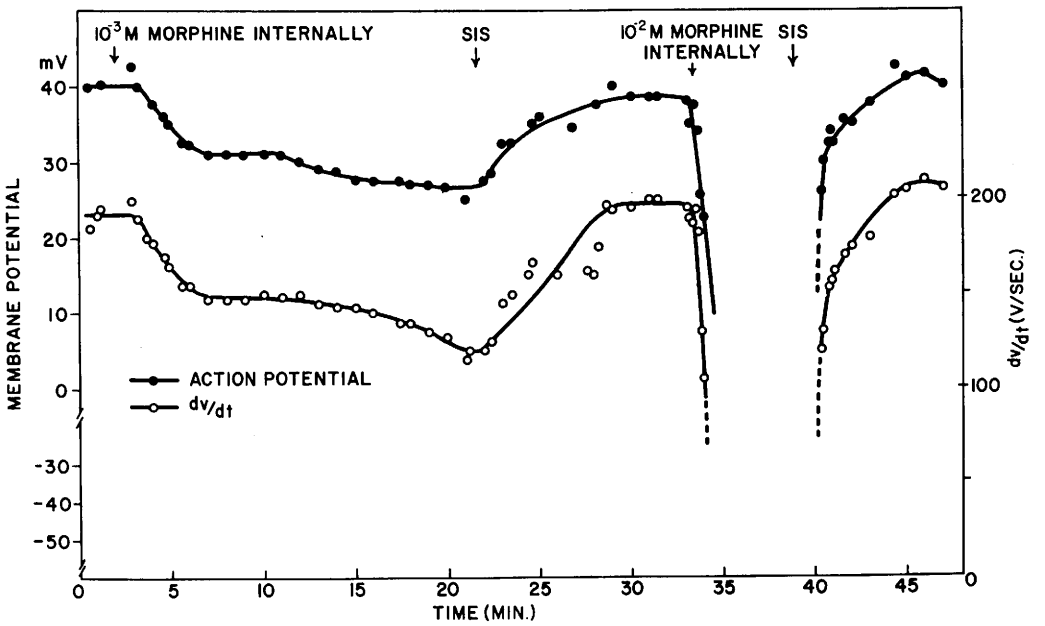

FIG. 1. The effect of morphine (10^{-3} and 10^{-2} M) applied internally on the amplitude (●); and the maximum rate of rise [dv/dt (○)] of the action potential: resting potential, −60 mV; SIS, standard internal solution.

10^{-3} and 10^{-2} M. In an attempt to elucidate the mechanism of action of morphine the experiments were repeated under voltage clamp conditions. Membrane ionic currents were measured as follows: A 120 μ glass capillary (outer diameter) containing a 25 μ silver wire to decrease the impedance, and filled with 0.6 M KCl solution was used as the internal potential electrode. The internal current electrode was 75 μ platinum wire plated with platinum black. The wire was loosely twisted around the potential capillary electrode. The external reference electrode was a capillary (about 200 μ in diameter) filled with artificial sea water. The external current electrodes were composed of six platinum plates coated with platinum black. Each plate was 4 mm wide in the longitudinal direction, with three plates being arranged along each side of the axon. The central plates were used to measure the membrane current, while the others served as the guard electrodes.

Leakage currents were subtracted from the observed membrane currents to obtain net peak transient sodium current and steady-state potassium current in the manner described previously (4). Current–voltage relationships were then plotted for both components of membrane current. The chord conductance was calculated for the peak transient sodium current by the following equation: $G_p = I_p/E\text{-}E_p$, where G_p and I_p refer to the peak transient sodium conductance and current, respectively; E refers to the membrane potential; and E_p refers to the membrane potential where I_p reverses its polarity or the equilibrium potential for I_p. Because of the difficultly in measuring the equilibrium potential for the steady-state potassium current (I_{ss}), the slope conductance, g_{ss}, was calculated for I_{ss} by the equation, $g_{ss} = dI_{ss}/dE$. These conductances increase to a constant value with increasing magnitudes of the step-depolarizing potential. The maximum values were used as a measure to compare the effectiveness of morphine.

Experiments were performed at a temperature of about 7°. Three axons were tested at 10^{-3} and 10^{-2} M concentrations.

Results. Figure 1 shows an example of the effect of 10^{-3} M morphine when applied inside the squid axon. The solid circle represents the absolute magnitude of the overshoot of the action potential; the open circle

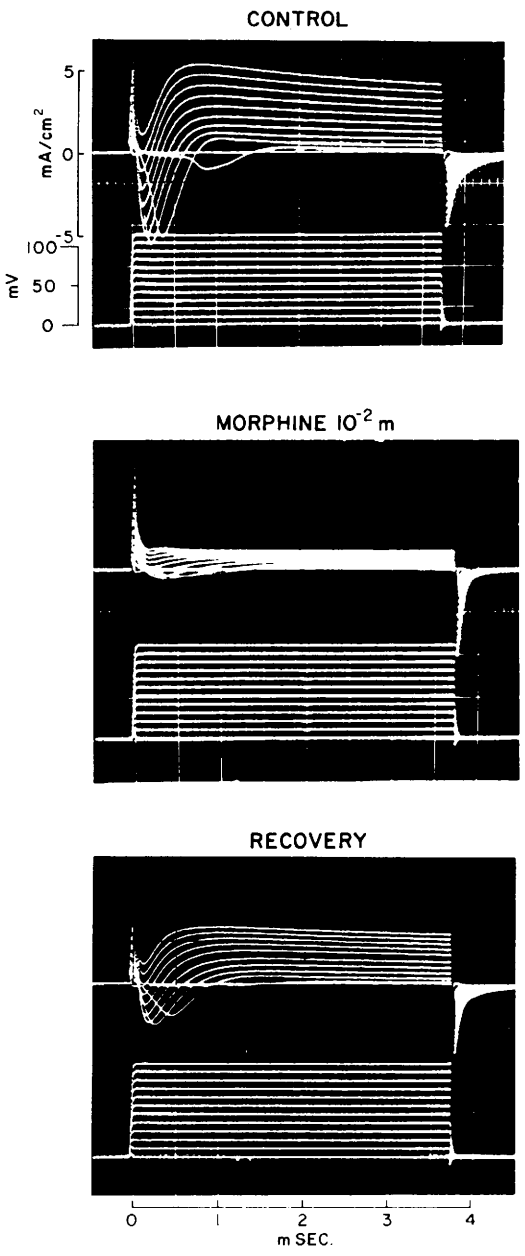

FIG. 2. Membrane currents associated with 10 mV step depolarizations from the holding membrane potential of —60 mV; (upper) a family of control membrane currents; (middle) a family of membrane currents obtained 5 min after treatment with 10^{-2} *M* morphine; (lower) a family of membrane currents obtained 5 min after returning to control solutions.

its maximum rate of rise (dv/dt). Morphine (10^{-3} *M*) partially depressed the magnitude, the rate of rise, and the rate of fall of the action potential ($\overline{X} = 30\%$) with the effect being readily reversible upon returning to the standard internal solution. The maximum effect was reached within the first 5 min after application. In all cases, 10^{-2} *M* morphine completely abolished the action potential with the effect again readily reversible. 5×10^{-3} *M* morphine had an intermediate effect causing about a 60% depression in the magnitude of the action potential. The resting potential was not appreciably affected by these concentrations of morphine.

Figure 2 represents three families of curves obtained from one of the voltage clamp experiments. In the upper panel are the ionic currents (upper tracings) associated with various step depolarizations (lower tracings). The membrane potential was held at —60 mV and subsequently depolarized in 10 mV steps. In the middle panel, a similar family was taken following application of 10^{-2} *M* morphine. It is evident that both components of ionic conductances [*i.e.*, peak transient (sodium) and late steady state (potassium)] are suppressed. The bottom panel is a third family of curves taken 5 min after returning to control solutions. From these families of membrane currents, one can plot, as in Fig. 3, ionic conductances g_p (peak transient or sodium conductance) and g_{ss} (steady-state or potassium conductance) against the membrane potential (E_m). Both g_p and g_{ss} are suppressed in magnitude by morphine but are not significantly shifted along the potential axis.

The kinetics of sodium conductance was apparently affected by morphine as shown in Fig. 4. In Fig. 4 control values are represented by open circles, morphine (10^{-2}) by open triangles, recovery by closed circles. The time for sodium current to reach its peak was slowed about 50% by application of morphine at all membrane potentials studied.

Voltage clamp experiments with 10^{-3} *M* morphine revealed similar results, with the magnitude of the affects being somewhat less than those demonstrated by 10^{-2} *M* morphine.

Discussion. Morphine applied inside the giant squid axon depressed the action poten-

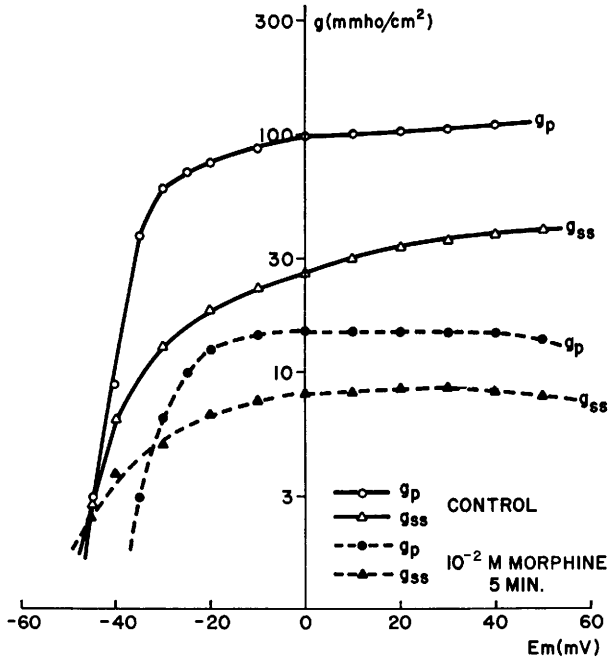

FIG. 3. The effect of 10^{-2} M morphine applied internally on peak transient sodium conductance g_p and steady-state potassium conductance g_{ss}: (○△) control; (●▲) following morphine.

tial very rapidly with the maximum effect occurring within 3–5 min after administration. It should be noted that a small part of this delay is related to the dead space in the internal perfusion system. The effect was dose dependent with 10^{-3} M resulting in approximately 30% reduction; 5×10^{-3} M, 60%, and 10^{-2} M, complete blockage of the action potential. This block was reversible at all concentrations and was not accompanied by depolarization of the resting membrane potential.

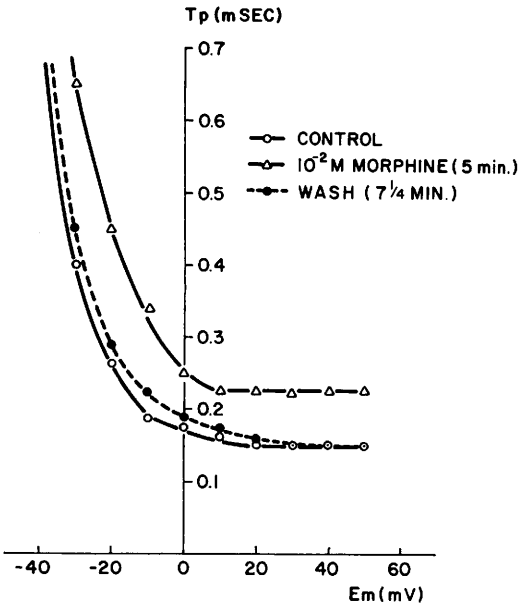

FIG. 4. The effect of 10^{-2} M morphine on the time required to reach peak current (T_p) for sodium: (○) control; (△) after morphine; (●) recovery.

Voltage clamp data revealed that both components of ionic conductances (g_p and g_{ss}) were equally depressed by morphine. One of the more interesting findings was that morphine apparently slows the kinetics required for the sodium conductance changes seen with depolarization (Fig. 4). The actual mechanism by which morphine depresses the sodium and potassium conductance still remains rather obscure.

Simon and Rosenberg (2) have reported that morphine has a weak local anesthetic action when applied to the outside of a squid axon. Morphine (10^{-3} M) caused approximately a 40% decrease in amplitude of the action potential after about a 30 min exposure. Additional pH and radioactive labe-

ling experiments with congeners of morphine (levorphanol, levallorphan, *etc.*) indicated that the ability to block the action potential correlated well with the ability of the compound to penetrate the membrane. These observations of Simon and Rosenberg (2) coupled to the present results, in which morphine was seen to have a very rapid onset of action when applied inside (3 min inside as compared to 30 outside), give strong support to the notion that the receptor for morphine is on the internal surface of the membrane. The action of morphine thus resembles that reported for local anesthetics in that: (i) it works better from inside the membrane than from the outside (5); and (ii) it depresses both sodium and potassium conductances (6).

Summary. Morphine, when applied inside the squid axon depressed the action potential within a few minutes, with 10^{-3} *M* resulting in a partial block (30%) and 10^{-2} *M*, a complete block. This block was reversible and not accompanied by depolarization of the resting membrane potential. Voltage clamp experiments revealed that both components of ionic conductances (g_p, g_{ss}) were equally depressed by morphine, and the curves relating g_p and g_{ss} to the membrane potential were not significantly shifted along the potential axis. These actions of internally applied morphine resemble those reported for local anesthetics. The results of these experiments support the concept that the receptor for morphine is located on the internal surface of the membrane.

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