Nature of the Morphine Receptor Present in the Squid Axon[1] (37210)

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(Introduced by L. L. Boyarsky)

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Recently we reported that morphine applied inside the squid axon was effective in blocking the action potential (1). This block was reversible and not accompanied by depolarization of the nerve membrane. Voltage clamp experiments revealed that both components of ionic conductances were equally depressed by morphine. These results supported the notion of Simon and Rosenberg (2) that the receptor for morphine was located on the internal surface of the squid axon membrane. The question remained, however, whether the morphine-receptor in squid axon membrane possessed properties similar to receptors which produce analgesia in higher animals. The present paper describes results of experiments designed to test the effect of two known specific antagonists of morphine, naloxone hydrochloride and M5050 hydrochloride Reckitt, and one potent morphine-like agent, etorphine hydrochloride (M99 Reckitt) on ionic conductances in squid axon membrane. The two morphine antagonists were chosen because they have been shown to be competitive inhibitors of morphine with no analgesic properties of their own (3, 4). Etorphine is a morphine-like compound which was shown to be 2000 times more potent than morphine in producing analgesia in the rat (4, 5). It was hoped that the results of these pharmacological tests would provide a better understanding of the nature of the morphine receptor.

Methods. Giant axons of the squid, *Loligo pealei,* available at the Marine Biological Laboratory, Woods Hole, MA, were used in the study. The experimental drugs were added to the internal surface of the axonal membrane utilizing the method of internal perfusion described in detail previously (6). In all experiments the perfusion both internal and external was continuous. The standard internal solution contained 50 mmole/liter of Na^+, 350 mmole/liter of K^+, 320 mmole/liter of glutamate, 50 mmole/liter of F^-, 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^+, 100 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.

Membrane ionic currents were measured utilizing the axial wire-capillary electrode voltage clamp method (1). Current–voltage relationships were plotted for both peak transient sodium current and steady-state potassium current. The leakage component of conductance (0.5 mmho/cm^2) was extremely small and not affected by any of the drugs employed in the study (see Fig. 2). Therefore, the current–voltage curves presented are not corrected for leakage. Chord conductances were calculated for the peak transient and steady-state currents by the standard equations.

All experiments were performed at approximately 10°. The significance of the difference between experimental results was determined using the Student's *t* test.

The chemical structure of the compounds used in the study are shown in Fig. 1. All of

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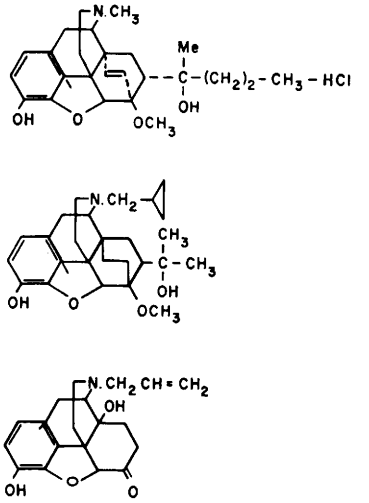

FIG. 1. The chemical structures of M99, M5050, and naloxone.

the drugs were obtained through the courtesy of Dr. Terry Christian, University of Alabama.

Results. Figures 2 and 3 are examples of what happens to membrane ionic currents when the two morphine antagonists, naloxone and M5050 (1×10^{-3} *M*) are added to the inside of the squid axon. The peak amplitude of the transient sodium current (I_p) and the steady-state amplitude of the late potassium current (I_{ss}) were plotted as a function of the membrane potential to draw the current–voltage curves. As is evident from these figures, both peak transient and late steady-state current are reduced almost equally in the presence of these two morphine antagonists (open triangles). Recovery, which is represented by the line connecting the open square symbols, was almost complete after washing.

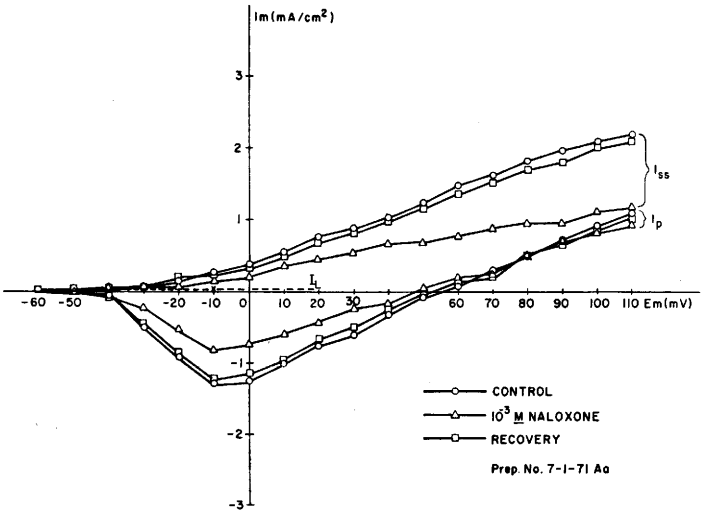

FIG. 2. Current–voltage relations for the peak amplitude of the transient (sodium) current (I_p) and the steady-state amplitude of the late (potassium) current (I_{ss}) before (○) and during application of 1×10^{-3} *M* naloxone (△), and after washing with a standard internal solution (□). I_L refers to the leakage current.

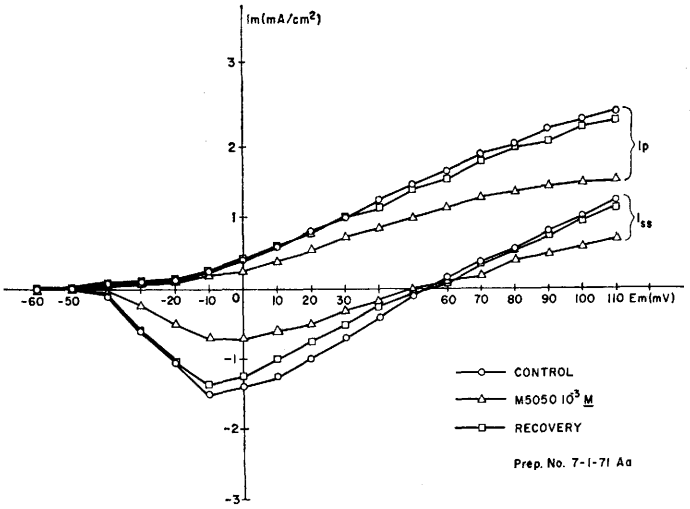

FIG. 3. Current–voltage relations for the peak amplitude of the transient (sodium) current (I_p) and the steady-state amplitude of the late (potassium) current (I_{ss}) before (○) and during application of 1×10^{-3} *M* M5050 (△), and after washing with a standard internal solution (□).

The effect of M99 is shown in Fig. 4. Again, both components of membrane ionic currents are reduced almost equally with good recovery following washing. The magnitude of the effect (30–40% reduction) is practically identical to what was obtained with the two antagonists and with morphine as reported in a previous publication (1).

Figure 5 demonstrates what occurs when M5050 and M99 are perfused simultaneously. In order to maintain a total concentration of 1×10^{-3} *M* the mixture contained M5050 and M99 both at a 5×10^{-4} *M* concentration. As is evident from this current–voltage curve the mixture produced the same relative block as either of the two compounds alone at

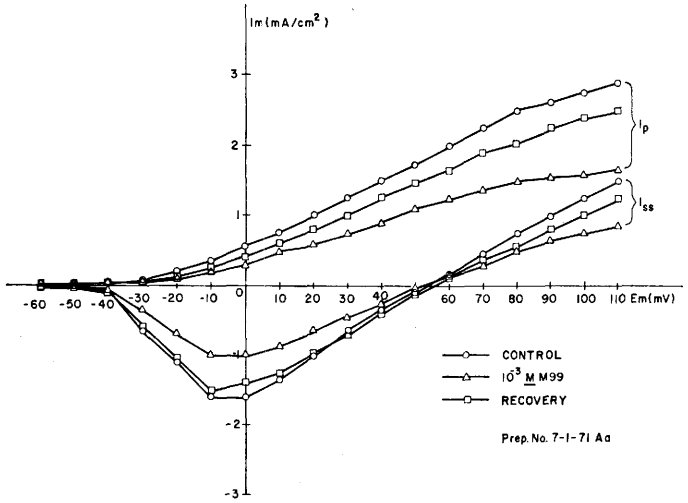

FIG. 4. Current–voltage relations for the peak amplitude of the transient (sodium) current (I_p) and the steady-state amplitude of the late (potassium) current (I_{ss}) before (○) and during application of 1×10^{-3} *M* etorphine (M99) (△), and after washing with a standard internal solution (□).

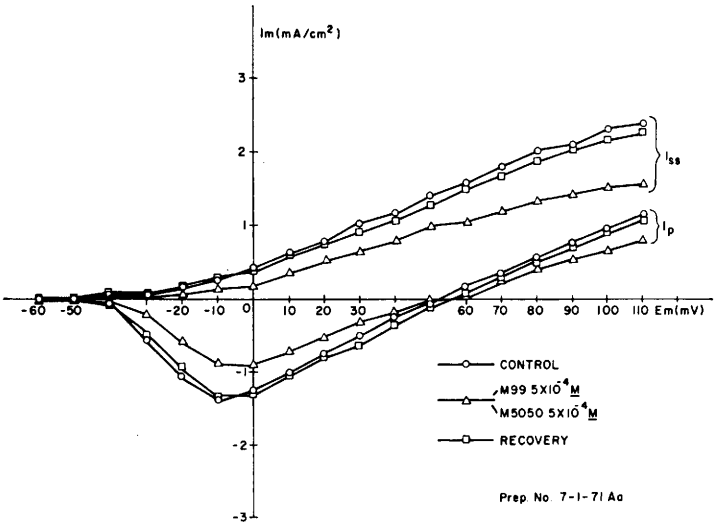

FIG. 5. Current–voltage relations for the peak amplitude of the transient (sodium) current (I_p) and the steady-state amplitude of the late (potassium) current (I_{ss}) before (○) and during application of 5×10^{-4} *M* etorphine (M99) + 5×10^{-4} *M* M5050 (△) and after washing with a standard internal solution (□).

the equivalent concentration. There was, therefore, no antagonistic interaction noted between the two drugs. The absence of antagonistic interaction was observed with a mixture of morphine and naloxone.

The effects of all the compounds on the maximum values of the peak (g_p) and steady-state (g_{ss}) conductances are tabulated in Table I. To normalize the results the values are expressed as a percentage of the control value. Table I lists the means with the standard errors of all experiments. Statistical tests revealed no significant difference ($p > 0.1$) if the results of either g_p or g_{ss} obtained with M99, M5050, the mixture M99 and M5050, or naloxone were compared.

The time for the transient current to reach its peak value (T_p) was used as a measure of the kinetics of the mechanisms which turns on the sodium current. The values of such measurements are given in the last column of Table I. The time (msec) to peak current for experiments with M99, M5050, or the mixture is almost the same as that found in the control experiments. There is no significant difference between any of these values. Naloxone, on the other hand, significantly shortens the time required for sodium cur-

TABLE I. The Effects of M99, M5050, and Naloxone on the Peak Transient Conductance (g_p) the Steady-State Conductance (g_{ss}), and the Time for the Transient Current to Reach its Peak (T_p).[a]

Drug	Concn (*M*)	No. of Expts	Mean % of control (mmho/cm²) g_p	Mean % of control (mmho/cm²) g_{ss}	T_p (msec)
Control	—	15	100	100	0.50 ± 0.04
M99	1×10^{-3}	4	63 ± 3.2	67 ± 3.3	0.45 ± 0.08
M5050	1×10^{-3}	4	57 ± 5.7	74 ± 4.3	0.46 ± 0.10
M99 and M5050	5×10^{-4} and 5×10^{-4}	4	71 ± 9.2	73 ± 2.8	0.44 ± 0.07
Naloxone	1×10^{-3}	3	74 ± 4.0	62 ± 4.1	0.32 ± 0.04

[a] Data are expressed as the mean percentage of control (±) the standard error.

rent to reach its peak. When compared to control the results with naloxone were highly significant ($p < 0.01$). This result is markedly different from that obtained with morphine which tends to prolong the time required for sodium current to reach its peak (1).

An interesting finding with respect to M99 and M5050 is their effect on potassium inactivation. As is shown in Fig. 6, M99 (Panel A) and M5050 (Panel B) produce marked potassium inactivation, whereas, naloxone (Panel C) and morphine (Panel D) apparently have no such action when compared to control.

Discussion. It had been hoped that the squid axon membrane might serve as a useful model for studying the analgesic properties of morphine. However, two specific morphine antagonists, naloxone and M5050, had essentially the same effect on ionic conductances as did morphine and etorphine (M99). Apparently, the squid axon membrane could not distinguish between the morphine antagonists and morphine. The results reported here are in complete agreement with preliminary observations by Simon and Rosenberg (2) for external application for some morphine antagonists. These combined findings would tend to suggest that the morphine receptor described earlier for squid axon membranes (1, 2) is different from the morphine receptor which produces analgesia in other preparations.

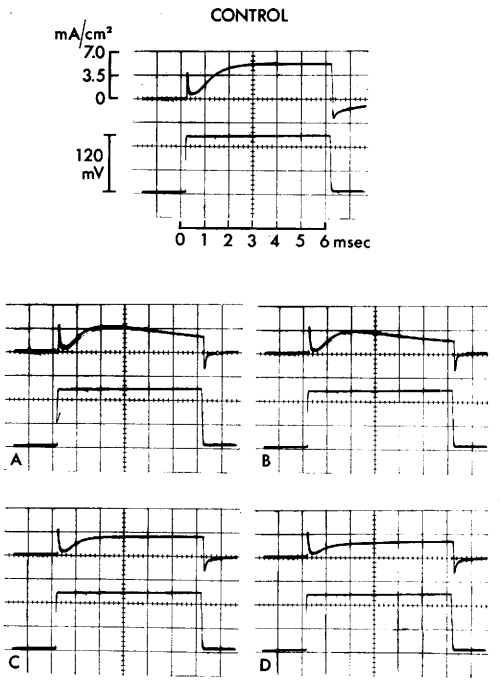

FIG. 6. Ionic currents associated with a 120 mV step depolarization. Control record is shown with calibrations; effect of etorphine (M99) (A); M5050 (B); naloxone (C); morphine (D).

As reported by Blane and Boura (3) M5050 and naloxone have a competitive action with respect to morphine but do not by themselves induce a state of analgesia. In the present study, both compounds effectively reduced both peak transient and steady-state ionic conductances. Their potency was almost identical to that previously reported for morphine (1). Thus, with regard to the parameters monitored, the antagonists had the same effect on the squid axonal preparation as did morphine. Similar results were also obtained with M99. This compound has been shown to possess narcotic properties 1000 to 80,000 times more potent than morphine (5). In the squid axonal preparation, however, the ability to block ionic conductances were essentially equivalent. Experiments designed to reveal interactions of the antagonist M5050 and the agonist M99 produced negative results. A mixture containing 5×10^{-4} M of each substance resulted in the same block as 1×10^{-3} M concentration of either one of the compounds alone. Additive, rather than antagonistic effects, were also observed with mixtures of morphine and naloxone. It appears obvious that the effects of morphine on the squid axonal membrane cannot be extrapolated to the production of analgesia as studied in most other preparations. It should be noted that similar agonist activity has been reported for naloxone in studies on the pigeon (7).

With respect to the kinetics involved with turning on the sodium current, only naloxone significantly shifted this measurement (see Table I). At a concentration of 1×10^{-3} M, naloxone has a tendency to shorten the amount of time required for sodium to reach its peak value following a step depolarization. However, it should be pointed out that in our previous publication (1), mor-

phine itself, at 1×10^{-2} and 1×10^{-3} *M*, increased the time to peak at all membrane voltages studied. Apparently, these two drugs affect the membrane kinetics in an entirely opposite manner.

Potassium inactivation was markedly affected by both M99 and M5050. This was in sharp contrast to the action of both morphine and naloxone. Potassium inactivation has been produced in squid axons when treated with other drugs such as dibucaine, tropine-*p*-tolylacetate, pentyltriethylammonium (8). This pharmacological property of M99 and M5050 may prove very useful in future experiments designed to characterize the mechanism underlying the process of potassium inactivation.

Summary. Two morphine antagonists, M5050 and naloxone, are equally potent as morphine in blocking peak transient and late steady-state currents in squid axons. Etorphine (M99), a very potent morphine-like analgesic, was no more potent in this preparation than was morphine. The reduction of ionic currents was completely reversible and not accompanied by depolarization of the nerve membrane. The results of these experiments tend to exclude the squid axonal preparation as a good model for studying the analgesic properties of morphine-like compounds. Two interesting findings were (a) naloxone, unlike all the other compounds, significantly shortens the amount of time required for sodium to reach its peak value following a step-depolarization and (b) M99 and M5050 produce a significant potassium inactivation.

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