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17. This was not surprising. Levorphanol is complexed reversibly with the receptors. In the refractionation, levorphanol dissociates from the drug-receptor complex as it passes through the column. This process is promoted by the considerable retentive capacity of the dextran gel for free levorphanol. The resulting free receptor molecules are retarded, and finally emerge during the C-M 1 : 1 elution. Typical data were 2000 pmole per gram of tissue for total levorphanol binding capacity in the C-M 1 : 1 eluate from the first column; 1100 pmole/g in the levorphanol-receptor complex refractionated on the second column; and residual binding capacity of 400 pmole/g in the C-M 1 : 1 eluate of the second column.
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23. The two phases were equilibrated by three treatments on a Vortex mixer for 10 seconds each. Free drug equilibrated completely between the phases in less than 5 seconds. The tubes were centrifuged for 1 minute at 1000*g* at room temperature to separate the phases before sampling for radioactivity counting. Prolonged or vigorous mixing caused a loss of binding capacity in the organic phase, accompanied by the appearance of a fluffy layer at the interface, which retained bound opiate. The amount of extract used in the phase distribution procedure has a pronounced effect upon the total binding; with extract from increasing quantities of tissue, the binding per gram of original tissue increases greatly. However, SSB per gram does not change significantly; thus, the amount of tissue used is not critical for the measurement of SSB. Opiates labeled with ^{14}C or ^{3}H were used interchangeably with identical results. Since the ^{14}C compounds were labeled in the *N*-methyl group whereas the ^{3}H compounds were labeled in positions 7 and 8 of the ring system, the results indicate that binding involves the intact opiate molecule.
24. Except as otherwise noted, all reagents were obtained from J. T. Baker Co. Sephadex LH-20 was obtained from Pharmacia Fine Chemicals, Inc.; *n*-heptane (spectroscopic AR grade) and [*allyl*-1,3-^{14}C]naloxone (19.2 μg/mg) from Mallinckrodt Chemical Works; and L-leucine (A grade) from Calbiochem, Inc. Naloxone was a gift from Endo Laboratories.
25. Cerebellar and cerebral hemispheres were first removed. Then the brainstem was cut transversely just caudal to the posterior colliculus, so that section 1 contained the pons and medulla with most of the fourth ventricle. A second transverse cut just rostral to the pituitary stalk placed the mesencephalon and part of the diencephalon with all of the third ventricle in section 2. Finally, the olfactory lobes were removed, which left most of the diencephalic structures and the telencephalon in section 3. Material from eight mice was pooled, and 5 to 16 determinations were made on each pool. Mean weights of wet tissue (in milligrams) per brain were as follows: whole brain, 485; cerebellum, 53; cerebrum, 233; brainstem, 199; section 1, 45; section 2, 102; and section 3, 61.
26. We are indebted to R. Schulz for permission to use information derived from his experiments on the isolation of opiate receptor from guinea pig myenteric plexus.
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Gating Currents of the Sodium Channels: Three Ways to Block Them

Abstract. *Preceding the opening of the sodium channels of axon membrane there is a small outward current, gating current, that is probably associated with the molecular rearrangements that open the channels. Gating current is reversibly blocked by three procedures that block the sodium current: (i) internal perfusion with zinc ions, (ii) inactivation of sodium conductance by brief depolarization, and (iii) prolonged depolarization.*

The sodium and potassium channels of nerve membrane open and close in response to electric field changes within the membrane. This almost certainly means that charged gating structures change conformation in response to the field changes, and in theory the charge movement or "gating current" associated with these changes should be measurable. Currents with many of the properties expected of Na channel gating current have been reported by Armstrong and Bezanilla (*1*), and their measurements have since been repeated by Keynes and Rojas (*2*). Though quite suggestive, none of the previously reported evidence conclusively links the observed currents with the gating structures. Perhaps the strongest evidence of such a linkage would be the discovery of a way to block both sodium current (I_{Na}) and the presumed gating current simultaneously. Rather disappointingly, tetrodotoxin (TTX), which selectively blocks I_{Na}, does not affect the presumed gating currents: apparently it blocks the channels without significantly altering the gating process. We report here three procedures that reversibly suppress both I_{Na} and gating current, greatly strengthening the evidence for association of the two.

In order to see gating current, it is necessary to suppress ionic current and subtract from the records the linear component of capacitative current, both of which are large compared to gating current. Our procedure for doing this has been described (*1*), and we repeat here only its essential features. Most of our experiments were performed on axons perfused internally with CsF, which is essentially impermeant, and bathed in a medium in which the impermeant tris(hydroxymethyl)methyl ammonium ion (tris+) was substituted for all or most of the Na^+. The ionic current with Cs^+ inside and no external Na^+ is a time-invariant "leakage" current, which can easily be subtracted from the transient gating current. Ionic current through the sodium channels was further suppressed in some experiments by addition of $3 \times 10^{-7} M$ TTX to the external medium. To eliminate the linear portion of membrane capacitative current we algebraically summed the current produced by successive positive and negative pulses of exactly equal magnitude. Summation and averaging were performed by a digital averager of new design, which will be described elsewhere (*3*). The new averager made it possible to measure gating current with a small number of pulses.

Using these procedures, we recorded a transient current from axon membrane which from the electrical point of view is a slow and nonlinear component of capacitative current, and from the physiological point of view is, we think, gating current. The first way of blocking gating current is by internal perfusion with Zn^{2+}. Begenisich and Lynch (*4*) have reported that 10 m*M* $ZnCl_2$ in the internal medium suppresses both I_{Na} and I_K (potassium current) and, surprisingly, the effect is reversible. Figure 1 shows that $ZnCl_2$ also suppresses gating current. In this experiment, I_{Na} was completely eliminated by adding TTX to the external medium, and there was no Na^+ inside or out. The control record shows an outward current that continues until the termination of the pulses. This is what we have interpreted as the charge movement associated with the opening of the Na channel activation gates. When the pulses end, there is a tail of inward current which we associate with the return of the gating charge from "open" to "closed" position (*1*). Both inward and outward gating current were suppressed almost completely when 10 m*M* $ZnCl_2$ was added to the medium (Fig. 1b) and both recovered

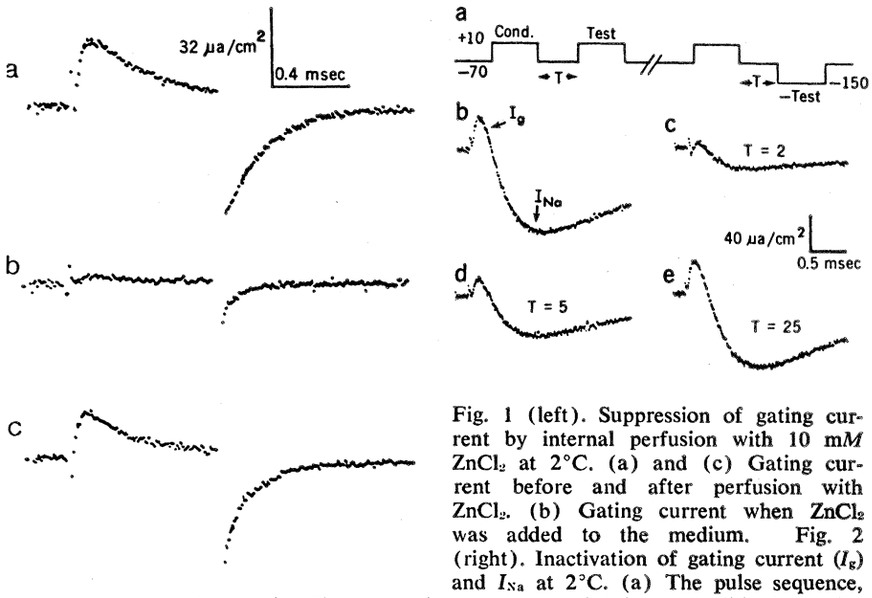

Fig. 1 (left). Suppression of gating current by internal perfusion with 10 m*M* $ZnCl_2$ at 2°C. (a) and (c) Gating current before and after perfusion with $ZnCl_2$. (b) Gating current when $ZnCl_2$ was added to the medium. Fig. 2 (right). Inactivation of gating current (I_g) and I_{Na} at 2°C. (a) The pulse sequence, on a compressed time scale. The test pulses were preceded by a conditioning pulse (*Cond.*) to +10 mv. Current during the positive and the negative test pulses were summed to eliminate the linear portion of capacitative current. (b) No conditioning pulse. (c) to (e) Conditioning pulse to +10 mv precedes each test pulse by *T* msec.

almost completely after it was washed away (Fig. 1c).

The second procedure for blocking I_{Na} and gating current is to inactivate them by depolarizing the fiber for a short period. In the description by Hodgkin and Huxley (*5*), each Na channel is governed by two factors or gates: an activation gate that opens on depolarization, and an inactivation gate that closes on depolarization. After a few milliseconds of depolarization most of the channels are inactivated, and the membrane potential V_m must be returned to a negative value for some milliseconds to restore their ability to conduct. Figure 2 shows that depolarization inactivates gating current as well as I_{Na}. The fiber was perfused with CsF, and 95 percent of the external Na^+ was replaced by $tris^+$ (no TTX). In this medium, I_{Na} is greatly reduced but not absent, and it is possible to record both gating current and I_{Na} at the same time. The control record (Fig. 2b) shows the sum of current for five positive and five negative steps with an amplitude of 90 mv. There is a distinct initial peak of outward current, the gating current, which is followed and partially obscured by inward sodium current. In Fig. 2, c to e, the test pulses were preceded by a 5-msec depolarization to + 10 mv, followed by a recovery interval of 2 to 25 msec at $V_m = -70$ mv (see Fig. 2a). After a 2-msec recovery interval (Fig. 2c) both gating current and I_{Na} are almost completely absent. The next two records show partial and complete recovery after longer intervals at −70 mv, and it is clear that gating current and I_{Na} recover together. After removal of inactivation by Pronase (*6*) neither I_{Na} nor gating currents are depressed by a conditioning depolarization.

The third method involves prolonged depolarization of an axon, a procedure that reversibly decreases I_{Na}. In order to follow I_{Na} and gating current simultaneously, we left a small concentration of Na^+ in the external medium, as in the experiment just described. We found that I_{Na} and gating current were almost completely eliminated by holding V_m at + 56 mv for 2 minutes (2°C), and they recovered together, with a half-time of roughly 50 seconds.

The experimental findings just described show that I_{Na} and what we call gating current are closely related. A fourth series of findings makes the case for calling them gating currents still stronger. There is no simple kinetic relation between outward gating current and the turn-on of I_{Na}, but there is a simple relation at turn-off. We find that the inward gating currents at the end of a pulse decay with the same or almost the same time course as I_{Na}. Further, the time course of both varies in the same way with membrane potential, becoming faster at more negative voltages.

It is remarkable that Hodgkin and Huxley (*5*) predicted gating currents 20 years before they were discovered; and their equations give good qualitative predictions of some properties of gating currents. There are, however, two significant points of difference between their predictions and our results. In the particle interpretation of the Hodgkin and Huxley equations, each Na activation gate is governed by three charged mutually independent activation particles. The fraction of these particles that are in the open position is given by the variable *m*. Gating current is produced by the movement of the activation particles, and is proportional to dm/dt. All three of the activation particles must be in the open position in order for the channel to conduct, and return of any of the three particles to the closed position closes the channel. The result is that I_{Na} should decay three times faster than gating current, but we find instead that they decay with almost the same time course. We conclude that, when closing, a channel does not behave as though it were governed by several independent particles.

The second difference from the Hodgkin and Huxley predictions is that activation and inactivation seem to be coupled rather than independent (*7*). In the Hodgkin and Huxley equations the activation process is completely independent of inactivation; or in paraphrase, the activation gate opens and closes with complete disregard for the state of the inactivation gate. Our results show that inactivation reduces gating current, which must mean that the gating charge of an inactivated channel is immobilized, either in "gate open" or "gate closed" position.

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