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# Axonal Microtubules Necessary for Generation of Sodium Current in Squid Giant Axons: II. Effect of Colchicine upon Asymmetrical Displacement Current

Gen Matsumoto, Michinori Ichikawa* and Akira Tasaki*
Electrotechnical Laboratory, Tsukuba Science City, Ibaraki 305, Japan

**Summary.** Effect of internal colchicine on asymmetrical displacement currents was studied by internally perfusing squid giant axons with a solution containing colchicine. It was found that (1) asymmetrical displacement currents were composed of two parts; colchicine-sensitive and colchicine-resistant; that (2) the colchicine-sensitive part had a definite rising phase while the colchicine-resistant one showed an instantaneous jump, followed by exponential decay; and that (3) the colchicine-sensitive part related to normal Na channels.

**Key Words** Na gating currents · Na currents · colchicine

## Introduction

Since the first discovery of the asymmetrical displacement current in squid giant axons, attempts to quantitatively correlate the voltage and time dependence of the asymmetrical displacement current with the predictions of the Hodgkin-Huxley activation parameter $m$ (Hodgkin & Huxley, 1952) have met with unsatisfactory results (Armstrong & Bezanilla, 1974, 1977; Bezanilla & Armstrong, 1974, 1975; Keynes & Rojas, 1974, 1976; Meves, 1974, 1976; Meves & Vogel, 1977*a*, *b*; Bezanilla & Taylor, 1978; Armstrong & Gilly, 1979; Kimura & Meves, 1979; Bezanilla, Taylor & Fernández, 1982; Gilly & Armstrong, 1982).

In the present paper, we will describe effects of internal colchicine upon asymmetrical displacement currents related to the voltage-dependent sodium channels in squid giant axons. It will be shown that asymmetrical displacement currents ('Na gating currents') consist of two parts; that is, colchicine-sensitive and -resistant parts. It will also be shown that the colchicine-sensitive part exactly correlates to the appearance of Na currents and that the colchicine-resistant part does not. Time course and voltage dependence of both parts of asymmetrical displacement currents will be studied in detail.[1]

## Materials and Methods

### Materials, Intracellular Perfusion and Voltage-Clamp

Materials and methods of intracellular perfusion and voltage-clamp are basically the same as those described in our preceding paper (Matsumoto et al., 1984). Giant axons of squid *(Doryteuthis bleekeri)* were used. The axon was intracellularly perfused under slow flow rate of perfusion (0.5 to 2 μl/min).

In order to reduce the error caused by the resistance in series with the membrane, we used compensated feedback and the 1/5 to 1/10 Na sea water (*see* Table 1), i.e., sea water with a fifth to a tenth of the normal Na. The amount of compensation for the series resistance was adjusted for critical damping (Katz & Schwartz, 1974). The linear capacitive transient left after the above adjustment was reduced in size as far as possible by means of a transient generator (*see* Fig. 1 of our preceding paper by Matsumoto et al., 1984). As a result, the linear capacitive transient returned to the baseline within 2 μsec without any overshoot or ringing when a test potential of 100 mV in amplitude was applied to the control or colchicine-treated axon. The pulse program was mainly the $P/4$ procedure: 16 positive pulses of amplitude $P$ and 64 *positive* pulses of amplitude $P/4$ were applied. Sometimes, the $P/-4$ procedure was adopted: 16 positive pulses of amplitude $P$ and 64 *negative* pulses of amplitude $P/4$ were applied. The holding potential was the resting potential of the axon. The positive pulses were preceded by a conditioning pulse with membrane potential of $-100$ or $-150$ mV (beginning 30 msec before the test pulse and outlasting it by 20 msec). The $P/4$ or $P/-4$ pulses were also preceded by a prepulse with membrane potential of $-150$ or $-170$ mV (starting 30 msec before the $P/4$ or $P/-4$ pulse and outlasting it by 20 msec), i.e. superimposed on $-150$ or $-170$ mV. One cycle time of the above pulse program was 540 msec. The pulse width was 0.6 msec when we measured asymmetrical displace-

* *Permanent address:* Institute of Applied Physics, The University of Tsukuba, Tsukuba Science City, Ibaraki 305, Japan.

[1] A short communication of these results was presented at the Annual Meeting of Biophysics in Japan, October, 1982.

**Table 1.** Components of the internal and external solutions

A) Internal solution (mM)

| Name | K | TEA | TMA | F | glutamate | Cl | HEPES[c] | Tris[c] |
|---|---|---|---|---|---|---|---|---|
| TEA-SIS (standard internal solution)[a] | 380 | 25 | – | 355 | – | 25 | 25 | – |
| 200 TMA[b] | – | – | 200 | 50 | 150 | – | – | 10 |

B) External solution (mM)

| Name | Na | Ca | Tris[c] | Cl | K |
|---|---|---|---|---|---|
| 1/5 NaSW | 112 | 50 | 378 | 212 | – |
| 1/7.5 NaSW | 75 | 50 | 415 | 175 | – |
| 1/10 NaSW | 56 | 50 | 434 | 156 | – |
| ASW | 400 | 44 | 10 | 498 | 10 |

[a] Glycerol was used to adjust osmolality of 980 mosmol/Kg
[b] Sucrose was used to adjust osmolality of 980 mosmol/Kg
[c] HEPES and Tris were used as buffers. pH was adjusted to 7.3 for HEPES and 7.35 for Tris, respectively

ment currents. Charges carried by the displacement currents were calculated with digital data of the currents; zero base-line was determined by averaging 10 successive data collected very close to but not beyond 600 μsec after the rise (as for calculation of charges $Q_{on}$ of turn-on asymmetrical displacement currents) or fall (as for that of the charges $Q_{off}$ of the turn-off displacement current) of test pulse. All data were sampled for 1.25 μsec unless otherwise specified. To improve signal-to-noise ratio in membrane currents, we used a two-pole Bessel filter (220 or 50 kHz at 3 dB). Experiments were performed at either 12 or 16 °C. Temperature of the external solution surrounding the axons was kept constant within ±0.05 °C.

### Solutions

The compositions of both internal and external solutions used in the present experiments are listed in the Table 1.

### Reagents

Colchicine, podophyllotoxin, vinblastine and aconitine were used. Podophyllotoxin was purchased from Aldrich Chemical Co. (Milwaukee, Wis.) and the other reagents from Sigma Chemical Co. (St. Louis, Mo.). Twenty-five millimolar aconitine was dissolved into ethyl alcohol and prepared as a stock solution. It was stocked at −20 °C until use.

## Results

### Asymmetrical Displacement Currents Consist of Colchicine-Sensitive and -Resistant Parts

Internal colchicine affected the Hodgkin-Huxley activation parameter $m$ as described in our preceding paper (Matsumoto et al., 1984), and as such should give some effect on an associated 'gating current.' We have studied the effect. A typical experiment is illustrated in Fig. 1*A*, where the axon immersed in 1/5 NaSW was internally perfused with the 200 TMA (control; record 1), the 200 TMA containing 2.5 mM colchicine (record 2), 7.5 mM colchicine (record 3) and 20 mM colchicine (record 4), in succession. The record # 1 for the control axon shows an instantaneous jump in asymmetrical displacement currents, followed by either plateau or a rise to a peak as had been studied by other groups (Armstrong & Gilly, 1979; Kimura & Meves, 1979). On the other hand, the axon after the 20 mM colchicine treatment (records # 4), where Na currents were almost suppressed, showed an instantaneous rise and exponential decay for asymmetrical displacement currents. Records # 2 and # 3 for the 2.5 mM and 7.5 mM colchicine-treated axon showed intermediate characteristics between those for the control and the 20 mM colchicine-treated axon. Time course, voltage dependence and size of asymmetrical displacement currents for the 20 mM colchicine-treated axon were almost unchanged even after the axon was internally perfused with 200 TMA containing 20 mM colchicine for 60 min, as compared with those for the 20 mM colchicine-treated axon for 10 min (record # 4); that is, one part of asymmetrical displacement currents (Colchicine-resistant part), as illustrated in record # 4 in Fig. 1*A* or record # 2 in Fig. 5, definitely remained when Na currents were blocked by internal application of colchicine. The other one, which disappeared by the colchicine treatment, is the colchicine-sensitive part of the currents.

### The Colchicine-Sensitive Part Directly Relates to Generation of Normal Na Currents

The colchicine-sensitive part was obtained by subtracting current records for the 20 mM colchicine-treated axon from those for the control axon since this part was lost by internal application of 20 mM

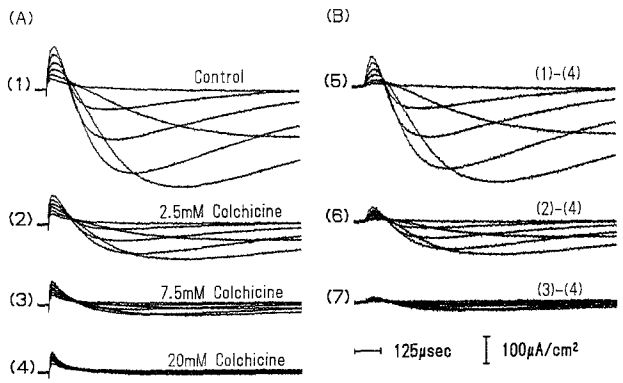


**Fig. 1.** Effect of internal colchicine upon Na currents and asymmetrical displacement currents at 16 °C. (*A*) The axon immersed in the 1/5 NaSW was internally perfused, firstly with the 200 TMA (record # 1), then with the 200 TMA containing 2.5 mM colchicine for 10 min (record # 2), with the 200 TMA containing 7.5 mM colchicine for 10 min (record # 3), and finally with the 200 TMA containing 20 mM colchicine for 10 min (record # 4), in succession. The pulse program *P*/4 was used. Sixteen positive pulses of the amplitude *P* were superimposed on −100 mV, and 64 positive pulses of the amplitude *P*/4 were superimposed on −170 mV. In each set of records, six currents are illustrated for the potentials of 60, 40, 20, 0, −20 and −60 mV, respectively. To improve signal-to-noise ratio in the currents, a two-pole Bessel filter (220 kHz at 3 dB) was used. (*B*) A part of asymmetrical displacement current ("the colchicine-sensitive part") and Na currents obtained by subtracting the currents in record # 4 from those in record # 1 (record # 5), from those in record # 2 (record # 6), and from those in record # 3 (record # 7), respectively. Each set of records stands for the currents corresponding to membrane potentials of 60, 40, 20, 0, −20 and −60 mV, respectively

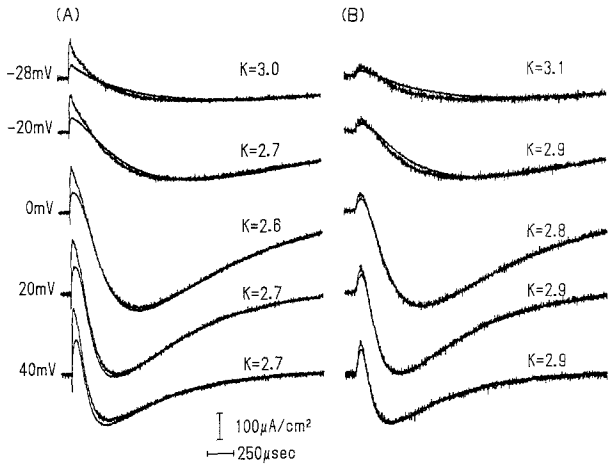


**Fig. 2.** (*A*) Comparison between the currents in records # 1 and 2 in Fig. 1*A* when the currents in record # 2 (for the 2.5 mM colchicine-treated axon) were expanded *K* times just to fit those in record # 1 (for the control axon). The expansion parameter *K* was voltage-dependent. (*B*) Comparison between the currents in records # 5 and 6, made by the same procedure as that in (*A*). Expansion coefficient *K* was also voltage-dependent

colchicine for over 10 min. Typical records of the colchicine-sensitive part thus obtained, followed by Na ionic currents, are illustrated in Fig. 1*B*, where records # 5 through 7 were obtained by subtracting record # 4 from records # 1 through 3, respectively.

It was found that the colchicine-sensitive part had a definite rising phase but no instantaneous rise (Fig. 1*B*). This will also be illustrated later (Fig. 5). Further, the colchicine-sensitive part just correlated to the appearance of Na ionic currents (Fig. 1*B*); that is, Na currents were induced, following after the emergence of this part, and also for a fixed membrane potential the bigger the colchicine-sensitive part is the more amount of the Na current flows (compare record # 5 with records # 6 and 7). Further, we have examined effects of colchicine upon asymmetrical displacement currents by increasing the Na ionic currents in amplitude in the colchicine-treated axon to fit with those in the control axon. Typical examples are illustrated in Fig. 2, where Na currents in the 2.5 mM colchicine-treated axon (record # 2 or 6 in Fig. 1) were enlarged by *K* times in size as compared with those for the corresponding membrane potential in the control axon (record # 1 or 5 in Fig. 1), in order to fit the Na currents in the colchicine-treated axon with those in the control axon. Figure 2*A* illustrates original asymmetrical displacement currents and Na ionic currents in the control and the 2.5 mM colchicine-treated axon, while Fig. 2*B* shows the colchicine-sensitive parts and Na currents in the control and the 2.5 mM colchicine-treated axon. As seen in the Figure, it should be noted that both sizes and time courses of Na currents and asymmetrical displacement currents in the control and colchicine-treated axon quite well resemble those in Fig. 2*B* but not in Fig. 2*A*. However, values of the expansion coefficient *K* had to be large around −30 mV, which reflects the fact that voltage dependence of Na activation parameter shifted to the depolarization side along voltage axis after the colchicine treatment, as described in our preceding paper (Matsumoto et al., 1984).

Temporal relationship between the asymmetrical displacement currents and Na ionic currents was studied in more detail by measuring the currents when the axon internally perfused with the 200 TMA was firstly immersed in the 1/7.5 to 1/10 NaSW and subsequently in the 1/5 NaSW. Typical current records for the axon immersed in the 1/5 and 1/7.5 NaSW are illustrated in Fig. 3*A*. The currents are composed of asymmetrical displacement currents and Na ionic currents. It should be noted that the asymmetrical displacement currents were exactly the same for the axon immersed in the 1/5 and 1/7.5 NaSW. Therefore, Na currents were obtained simply by subtracting the currents

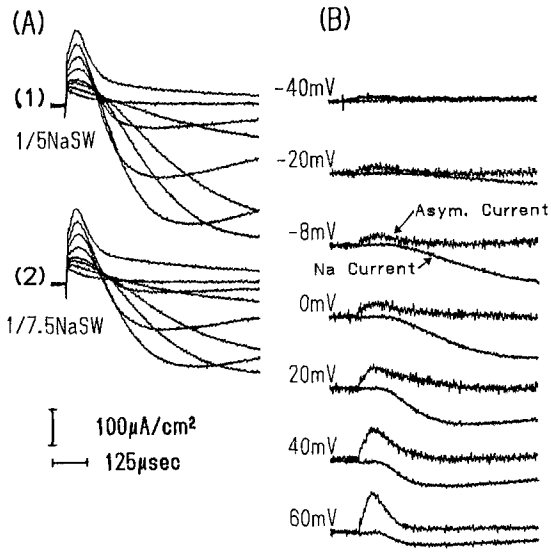


**Fig. 3.** Temporal relation between the colchicine-sensitive part of asymmetrical displacement currents and Na currents. (*A*) Asymmetrical displacement currents and Na currents at 16 °C when the axon immersed in the 1/5 NaSW (record # 1) or in the 1/7.5 NaSW (record # 2) was internally perfused with the 200 TMA. The pulse program *P*/4 was used. Sixteen positive pulses of the amplitude *P* were superimposed on −100 mV, and 64 positive pulses of the amplitude *P*/4 were superimposed on −170 mV. In each set of records, eight currents are illustrated for the potentials of 80, 60, 40, 20, 0, −8, −20 and −40 mV, respectively. A two-pole Bessel filter (220 kHz at 3 dB) was used to improve signal-to-noise ratio in the currents. (*B*) The colchicine-sensitive part (*upper* traces for each set of the current record) and Na currents (*lower* traces). Time course of Na currents was obtained simply by subtracting the currents in record # 2 of (*A*) from those in record # 1 The colchicine-sensitive part was obtained without using tetrodotoxin (TTX) in the external medium, as follows; the whole part of asymmetrical displacement current was obtained by subtracting the Na currents after they were expanded to fit those in record # 1 (*A*) in amplitude from the currents in record # 1 (*A*). Then, the colchicine-sensitive part was obtained by subtracting the currents after the axon immersed in the 1/5 NaSW was internally perfused with the 200 TMA containing 20 mM colchicine for 60 min from the whole part of asymmetrical displacement currents obtained just described above. The same axon was used throughout the experiments in this Figure

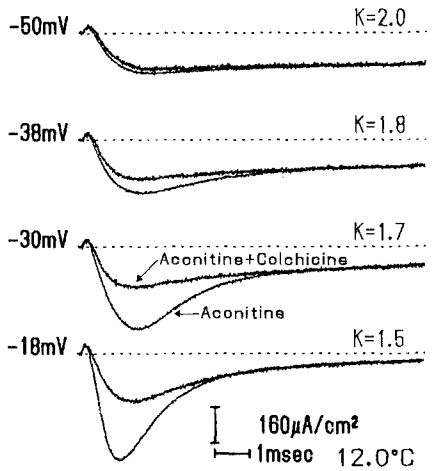


**Fig. 4.** Effect of colchicine upon peak transient (normal) Na currents and slow Na currents induced by internal application of aconitine at 12 °C. Na currents, obtained when the axon immersed in ASW was internally perfused with the TEA-SIS containing 250 μM aconitine for 40 min, were the control (*lower* traces for each set of the current record). Effect of colchicine upon the aconitine-induced Na currents was examined by internally perfusing the axon with the TEA-SIS containing both 250 μM aconitine and 3.5 mM colchicine for 20 min (*upper* traces for each set of the current record). The pulse program *P*/-4 was used. Sixteen positive pulses of the amplitude *P* were superimposed on −150 mV, and 64 negative pulses of the amplitude *P*/4 were also superimposed on −150 mV. A two-pole Bessel filter (50 kHz at 3 dB) was used so that the time course of asymmetrical displacement currents in these records was not accurate. Na currents after the colchicine treatment were expanded by *K* times to fit their slow currents to those for the control axon

for the axon immersed in the 1/7.5 NaSW (record # 2) from those of the one in the 1/5 NaSW (record # 1) for their corresponding membrane potentials (*lower* current traces in Fig. 3*B*). Asymmetrical displacement currents were also obtained by subtracting the Na currents thus determined from either of the current records # 1 or 2 after the Na currents were expanded in size just to fit those in Fig. 3*A*. The asymmetrical displacement currents thus obtained were very similar in their time course and voltage-dependence (*not shown*) to those obtained with using tetrodotoxin (TTX) as will be shown in record # 1 of Fig. 5*A*. The colchicine-sensitive part was obtained without using TTX by subtracting the currents for the axon internally perfused with the 200 TMA containing 20 mM colchicine for 60 min from the asymmetrical displacement currents just obtained above. The colchicine-sensitive part thus obtained without using TTX (*upper* current traces in Fig. 3*B*) was basically the same as the one obtained with using TTX in record # 3 of Fig. 5*A*. In Fig. 3*B*, the temporal relationship between the colchicine-sensitive part and Na currents is compared, where the time when the Na current starts to flow becomes shorter with bigger dipolarization steps and, at the same time, the time when the colchicine-sensitive part of the asymmetrical displacement current attains its peak also becomes shorter with bigger depolarization steps.

Action of colchicine on Na currents was found to be rather specific to normal Na channels but not to slow Na channels, as shown in Fig. 4. It was found by Seyama and Narahashi (1981) that, in the axon internally perfused with a solution containing grayanotoxin (GTX) I, peak transient Na current was followed by a secondary, slow Na current during a prolonged step depolarization lasting 100 to 200 msec. As the GTX I concentration in-

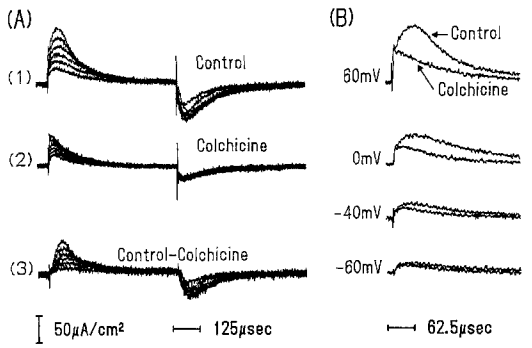


**Fig. 5.** Time course of asymmetrical displacement currents at 16 °C. (*A*) Asymmetrical displacement currents for the axon internally perfused with the 200 TMA (record # 1) and with the 200 TMA containing 50 mM colchicine for 120 min at the flow rate of perfusion of 1 μl/min (record # 2), in succession. External medium was 1/10 NaSW-TTX (300 nM). The pulse program *P*/4 was used. Sixteen positive pulses of the amplitude *P* were superimposed on −100 mV, and 64 positive pulses of the amplitude *P*/4 were superimposed on −170 mV. In each set of records, six currents are illustrated for the potentials of 60, 40, 20, 0, −20 and −40 mV, respectively. A two-pole Bessel filter (220 kHz at 3 dB) was used. Test pulse width was 600 μsec. The currents in record # 3 (the colchicine-sensitive part) were obtained by subtracting those in record # 2 from those in record # 1 for their respective membrane potentials. (*B*) The whole part of asymmetrical displacement currents (*upper* traces in each set of record) and the colchicine-resistant part (*lower* traces) on an expanded time scale. The whole part and the colchicine-resistant part are the same as those in records # 1 and 2, respectively, except the time scale

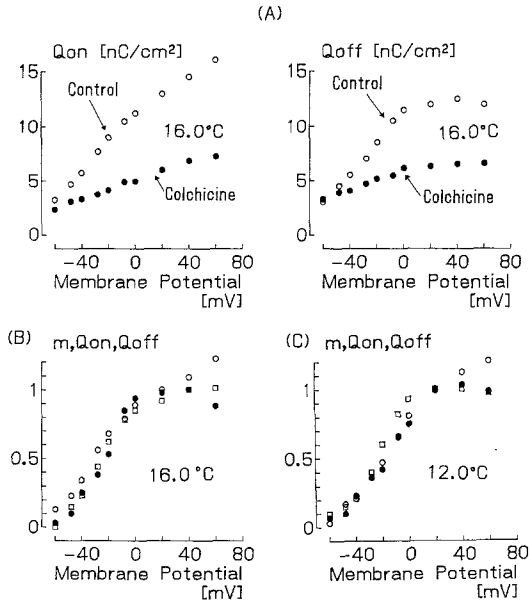


**Fig. 6.** Voltage dependence of charges, $Q_{on}$ and $Q_{off}$, accompanied by asymmetrical displacement currents and of Na activation parameter *m*. (*A*) *Left;* Voltage dependence of charges $Q_{on}$ forthe control axon (o) and for the colchicine-treated axon (•), at 16 °C. *Right;* Voltage dependence of charges $Q_{off}$ for the control axon (o) and for the colchicine-treated axon (•), at 16 °C. The same axon as in Fig. 5. (*B*) Voltage dependence of charges, $Q_{on}$ (o) and $Q_{off}$ (•), accompanied by the colchicine-sensitive part of asymmetrical displacement currents and Na activation parameter *m* (□). The charges, $Q_{on}$ and $Q_{off}$, were obtained by subtracting those carried by the colchicine-resistant part (• in (*A*)) from those by the whole part of asymmetrical displacement currents (o in (*A*)), respectively. Voltage dependence of *m* was determined, prior to the measurements of asymmetrical displacement current, when the axon immersed in the 1/10 NaSW was internally perfused with the 200 TMA. The charge $Q_{on}$ was normalized by the value at 20 mV, and $Q_{off}$ and *m* were normalized by their respective values at 40 mV. (*C*) Voltage dependence of charges, $Q_{on}$ (o) and $Q_{off}$ (•), accompanied by the colchicine-sensitive part of asymmetrical displacement currents and Na activation parameter *m* (□), at 12 °C. The axon was different from the one used in (*A*) and (*B*). Experimental procedures were the same as those in (*A*) and (*B*)

creased, the peak current decreased in amplitude while the slow current increased. Slow currents were observed in GTX I-treated axon even at −70 mV. These characteristics for the GTX I-treated axon have been confirmed in the present experiment and also observed for the axon treated with other lipid-soluble toxins such as batrachotoxin and aconitine (Matsumoto, Takemura, Ichikawa, Daly & Iwasa, *in preparation*). One of the typical examples for the aconitine-treated axons is illustrated in Fig. 4, where the axon immersed in artificial sea water (ASW) was internally perfused with the TEA-SIS (*see* Table 1) containing 250 μM aconitine for 40 min. Effect of colchicine upon the peak transient (normal) Na current was found to be more noticeable than that on the slow Na current (Fig. 4). In Fig. 4, Na currents in an axon internally perfused with TEA-SIS containing both aconitine and colchicine are shown, together with those in the aconitine-treated axon, where the Na currents in the aconitine- and colchicine-treated axon were *K* times in amplitude as compared to the original Na currents in order to fit with the slow currents in the aconitine-treated one. These results show that normal Na channels are colchicine-sensitive but that slow Na channels induced by lipid-soluble toxins are colchicine-resistant.

As a result, the colchicine-sensitive part in asymmetrical displacement currents directly correlates to normal Na channel.

## The Colchicine-Sensitive Part Has a Rising Phase but No Instantaneous Jump in the Current

The colchicine-sensitive part in asymmetrical displacement currents was obtained by subtracting the currents in the colchicine-treated axon after the Na currents were completely suppressed from those in the control axon, as described above. One

of the typical results is illustrated in Fig. 5*A*, where records # 1 and 2 stand for asymmetrical displacement currents for the control axon and for the axon internally perfused with 200 TMA containing 50 mM colchicine for 120 min, respectively, during step depolarization to membrane potentials of −40, −20, 0, 20, 40 and 60 mV with 0.6 msec in pulse width. In Fig. 5B, asymmetrical displacement currents in the control axon were compared with those of the axon treated with colchicine for 120 min, depolarized to 60, 0, −40 and −60 mV on an expanded time scale. The colchicine-sensitive part of asymmetrical displacement currents (record # 3 in Fig. 5*A*) was obtained by subtracting the currents in record # 2 from those in record # 1 in Fig. 5*A* for the corresponding membrane potentials.

The colchicine-sensitive part thus obtained (record # 3 in Fig. 5*A*) has a distinct rising phase but no instantaneous rise. The rising phase lasted a shorter time as membrane potentials were more depolarized; it lasted about 65 and 70 μsec for the depolarization of 60 and 20 mV, respectively.

### Voltage Dependences of Charges Carried by Asymmetrical Displacement Currents and Na Activation Parameter m

Charges accompanied by both turn-on and turn-off asymmetrical displacement currents were obtained as a function of depolarization potentials by integrating the respective currents. A typical example of the turn-on and turn-off charges, $Q_{on}$ and $Q_{off}$, for the control axon and the 50 mM colchicine-treated axon at 16 °C is illustrated as a function of membrane potentials in Fig. 6*A*; (1) both charges $Q_{on}$ and $Q_{off}$ had definite values even at −60 mV, the membrane potential which was low enough not to induce Na currents. This had been already pointed out (Armstrong & Gilly, 1979; Bezanilla et al., 1982). Further, $Q_{on}$ (or $Q_{off}$) values for the control axon and the colchicine-treated axon were almost the same at −60 mV. As a result, $Q_{on}$ (or $Q_{off}$) values of the colchicine-sensitive part were close to zero at −60 mV, as seen in Fig. 6*B* (at 16 °C) and in Fig. 6*C* (at 12 °C). (2) The values of $Q_{on}$ increased as a function of membrane potentials, both for the whole currents (Colchicine-resistant *plus* colchicine-sensitive parts) and for the colchicine-resistant part, while those of $Q_{off}$ increased with membrane potentials but attained the saturated levels after 0 mV, for both the whole and colchicine-resistant currents; the saturated level of the $Q_{off}$ value for the colchicine-resistant part is about half of that for the whole currents.

Charges accompanied by the colchicine-sensitive part at both turn-on and turn-off were obtained as a function of membrane potentials by subtracting those of the colchicine-resistant part from those of the whole current. These were obtained and compared with voltage dependence of the Na activation parameter *m* for the same axon, at 16 °C (Fig. 6*B*) and at 12 °C (Fig. 6*C*), where the values of $Q_{off}$ and *m* were normalized by those at 40 mV while the values of $Q_{on}$ were normalized by those at 20 mV, respectively. Voltage dependence of charges $Q_{on}$, $Q_{off}$ of the colchicine-sensitive thus obtained was in fairly good agreement with that of *m* below 20 to 40 mV, but it deviated slightly from that of *m* above 20 to 40 mV. However, voltage dependence of charges $Q_{on}$, $Q_{off}$ of the colchicine-resistant part clearly disagreed with that of *m*, since the charges $Q_{on}$, $Q_{off}$ were far from zero at and below −60 mV where the steady-state activation parameter *m* was very close to zero.

### Effect of Podophyllotoxin and Vinblastine upon Asymmetrical Displacement Current

It has been well known that podophyllotoxin and vinblastine are more effective at low temperatures to specifically depolymerize microtubules than colchicine does (Wilson, 1970; Wilson et al., 1974). Effect of internal podophyllotoxin and vinblastine upon asymmetrical displacement currents was examined by making the experiments similar to those done with colchicine. We found that 1 mM podophyllotoxin or 5 mM vinblastine gave similar results at 16 °C as 20 to 50 mM colchicine did (Figs. 1, 2, 5 and 6).

## Discussion

Our experiments show the following facts: (1) Asymmetrical displacement currents are composed of two parts; colchicine-sensitive and colchicine-resistant. (2) The colchicine-sensitive part has a rising phase, while the colchicine-resistant part shows an instantaneous jump in the currents, followed by exponential decay. (3) The colchicine-sensitive part relates to normal Na channels, since colchicine suppresses both the colchicine-sensitive part of asymmetrical displacement current and Na ionic currents in the same fashion and, at the same time, since voltage dependence of charges accompanied by the colchicine-sensitive part is in fairly good agreement with that of the Na activation pa-

rameter $m$. On the other hand, the voltage dependence of the colchicine-resistant part is not in agreement with that of $m$, as seen in Fig. 6.

Turn-off charges $Q_{off}$ for the colchicine-sensitive part decreased with depolarizing potentials above 20 to 40 mV. This may relate to the charge immobilization (Armstrong & Bezanilla, 1977; Meves & Vogel, 1977*a*; Kimura & Meves, 1979) since colchicine did not give any effect on Na inactivation (Matsumoto et al., 1984; *also see* Fig. 2). Turn-on charges $Q_{on}$ for the colchicine-sensitive part still increased with depolarizing potentials above 20 to 40 mV, although the slope of the increase of $Q_{on}$ as a function of voltage became moderate as compared with that below 0 mV. It is left for future experiments to determine why the $Q_{on}$ values of the colchicine-sensitive part did not attain the saturated level above 20 to 40 mV. It may be possible to explain the unsaturation of the $Q_{on}$ above 20 to 40 mV by assuming that all the charges carried by the colchicine-sensitive part at the turn-on are not utilized to open normal Na channels since the Na ionic currents started to flow from the time when an appreciable amount of the colchicine-sensitive part of antisymmetrical displacement currents was still observed above 20 to 40 mV; that is, the Na currents started to flow when the colchicine-sensitive part arrived to the peak in the currents at 40 or 60 mV (Fig. 3*B*).

Our findings of effect of colchicine upon asymmetrical displacement currents could be explained in terms of physiological function of axonal microtubules and undercoat beneath the axolemma inside squid giant axons. This will be discussed elsewhere.[2]

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