

Two Ca Current Components of the Receptor Current in the Electroreceptors of the Marine Catfish *Plotosus*

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ABSTRACT In the isolated sensory epithelium of the *Plotosus* electroreceptor, the receptor current has been dissected into inward Ca current, I_{Ca} , and superimposed outward transient of Ca-gated K current, $I_{K(Ca)}$. In control saline (170 mM/liter Na), with $I_{K(Ca)}$ abolished by K blockers, I_{Ca} declined in two successive exponential phases with voltage-dependent time constants. Double-pulse experiments revealed that the test I_{Ca} was partially depressed by prepulses, maximally near voltage levels for the control I_{Ca} maximum, which suggests current-dependent inactivation. In low Na saline (80 mM/liter), I_{Ca} declined in a single phase with time constants similar to those of the slower phase in control saline. The test I_{Ca} was then unaffected by prepulses. The implied presence of two Ca current components, the fast and slow I_{Ca} 's, were further examined. In control saline, the PSP externally recorded from the afferent nerve showed a fast peak and a slow tonic phase. The double-pulse experiments revealed that $I_{K(Ca)}$ and the peak PSP were similarly depressed, i.e., secondarily to inactivation of the peak current. The steady inward current, however, was unaffected by prolonged prepulses that were stepped to 0 mV, the in situ DC level. Therefore, the fast I_{Ca} seems to initiate $I_{K(Ca)}$ and phasic release of transmitter, which serves for phasic receptor responses. The slow I_{Ca} may provide persistent active current, which has been shown to maintain tonic receptor operation.

INTRODUCTION

Ca ions play important roles in various cellular functions, not only in the membrane activity but also in the regulatory processes within the cell. Some of the Ca ions enter the cell through the Ca channels in the membrane (Hagiwara and Byerly, 1981; Reuter, 1983; Tsien, 1983). Recently, based on differences in the activation threshold, the inactivation time course, or the sensitivity to ions and blockers, several types of Ca channels have been identified in vertebrate sensory ganglia (Carbone and Lux, 1984a, b; Bossu et al., 1985; Fedulova et al., 1985; Nowycky et al., 1985), in GH₃ cells (Matteson and Armstrong, 1984, 1986), in hypolish ciliate

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(Deitmer, 1984), in cardiac atrial cells (Bean, 1985), and in ventricular cells (Nilius et al., 1985). Their functional roles, however, remain unclear.

In the electroreceptors of fish, the receptor potential is dependent on Ca ions, which serve for two processes, i.e., for sensory transduction and for synaptic transmission to the afferent nerve (Obara and Bennett, 1972; Zipser and Bennett, 1973; Clusin and Bennett, 1977, 1979*a, b*; Sugawara and Obara, 1984*a, b*). The preceding report has described presence of the Ca current and the Ca-gated transient K current in the *Plotosus* receptor, both in the basal membrane of the receptor cells (Sugawara and Obara, 1989). The present study further dissects the Ca current into two types, based on differences in sensitivity to Na concentration and also in current decay. In control saline, the Ca current clearly decays in two exponential phases with widely separated time constants. The component for the fast decay phase is abolished by low Na saline, and inactivated in a current-dependent fashion. The fast component seems to initiate the transient K current and the phasic release of transmitter. The component for the slow decay phase is less Na-sensitive, and little inactivated. The slow component seems to maintain the sensory epithelium depolarized close to zero potential for tonic receptor operation (Sugawara and Obara, 1984 *a, b*).

A part of this study has been reported elsewhere as a meeting abstract (Sugawara and Obara, 1985).

METHODS

The ampullary electroreceptor of the marine catfish *Plotosus anguillaris* and isolation of the receptor unit have been described in the preceding paper (Sugawara and Obara, 1989). The receptor currents were recorded from the sensory epithelium (ampulla) under voltage clamp, and were stored on a magnetic data recorder (R-80; TEAC, Tokyo, Japan). In some figures, the net inward currents were shown by subtracting the leakage currents from the receptor currents on successive command steps of reversed polarity. The holding potential was generally set at the resting DC level.

The decay of the Ca currents was examined in the presence of K blockers, completely abolishing the transient outward current as shown previously. Each current record was enlarged on a digitizer, whereby a hundred points were obtained for processing (M223; SORD, Tokyo, Japan). First, a slower component was fitted by an exponential with the least-squares method, and then extrapolated to the onset of the command step for subtraction. The remainder was again fitted by another exponential as a fast component.

The composition of control saline was (in millimoles/liter): 170 NaCl, 3 CaCl₂, 1.5 MgCl₂, 10 glucose, and 8.5 HEPES buffer adjusted to pH 7.2. The Na concentration was altered by isosmotic substitution with choline chloride. The K current was blocked by 4-aminopyridine (4-AP) of 5 mM/liter, and by both 4-AP and tetraethylammonium chloride (TEA) of 20 mM/liter in later experiments. 5×10^{-6} g/ml tetrodotoxin (TTX) was added to saline for recording the postsynaptic potentials (PSP) extracellularly from the afferent nerve. All experiments were carried out at a room temperature of 20–22°C.

RESULTS

The receptor current consists of the Ca current, I_{Ca} , and the Ca-gated K current, $I_{K(Ca)}$, both in the basal membrane of receptor cells, as shown in the preceding paper (see also Figs. 6–8). During the preliminary survey, it was found that I_{Ca} was grad-

ually depressed in low Na saline, while the positive dip identified as $I_{K(Ca)}$ was depressed completely in time (Fig. 1). Since this occurs in the absence of K blocker, the Na-sensitive depression may involve either both I_{Ca} and $I_{K(Ca)}$, or I_{Ca} alone with a secondary depression of $I_{K(Ca)}$. In the present experiments, first with the K current fully blocked, the effects of low Na saline on I_{Ca} was studied, and I_{Ca} was dissected into two components. Secondly, their possible roles in receptor operation were examined in control Na saline.

Decrease of the Ca Current in Time in Low Na Saline

Fig. 2 A shows sample records of the receptor Ca current in the presence of K blockers. In each record, the inward current (larger trace) was superimposed over the leakage current (smaller trace). Holding potential was at the resting DC level. In control saline (170 Na), the inward current remained nearly constant after an initial decrease in 15 min, with no change in leakage. In low Na saline (40 Na), the inward

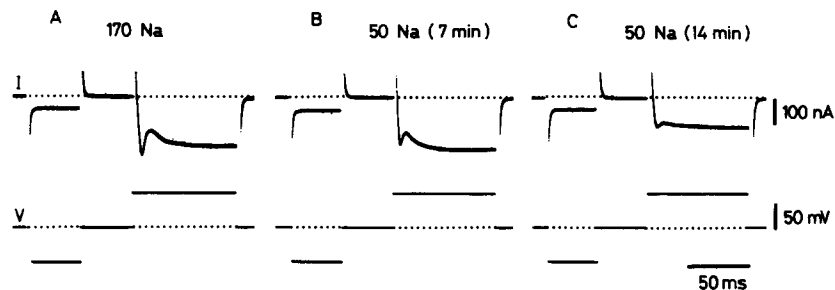


FIGURE 1. Depression of receptor current in low Na saline in the *Plotosus* electroreceptor under voltage clamp. Trace I is the receptor current, and trace V the potential in the ampulla. A negative command step (40 ms) induced the leakage current, and a positive step (80 ms) at an interval of 40 ms induced the receptor current. In A, the positive step to -3 mV induced a typical current pattern; the Ca current as an inward deflection superimposed by the K current as an outward transient. In B and C, the medium was changed to low Na saline (50 mM/liter). Note the gradual depression of both the Ca and the K currents. The leakage current remained unaffected. The holding potential was -52 mV.

current rapidly decreased by about one half within 15 min. The current during the command pulse decayed more slowly. In addition, the rising phase also seemed slower. The time to peak increased to 13.3 ms from the control of 10.2 ms, while those in control saline, though variable among receptors, only changed by ~ 1 ms after 60 min.

Fig. 2 B summarizes the effects of low Na saline on the inward current among receptors, with each peak current normalized to respective control. The peak current decreased in time, clearly dependent on Na concentration. Even in control saline (170 Na), it first slowly decreased to $\sim 80\%$ of the initial value within 10 min, but then became nearly constant to reach $\sim 75\%$ in 1 h. In Na-free saline, the inward current was rapidly abolished after a transient increase.

Such run-down, or wash-out, has been well documented on the Ca current in internally perfused cells, such as the dorsal root ganglion (Kostyuk et al., 1981), the snail neuron (Byerly and Hagiwara, 1982), the chromaffin cell (Fenwick et al.,

1982), and the chick hair cell (Ohmori, 1984). A line of evidence has suggested that certain intracellular molecules are required to maintain Ca channel activity. In the present case, however, the following observations seem to suggest that some Na-dependent metabolic processes are involved.

Similar Current-Voltage Relations for the Peak and Steady Currents

Various Ca currents have been discerned by the difference in activation voltage or in ionic selectivity. In the *Plotosus* receptors, the current-voltage relation in different Na concentrations had to be obtained in each different receptor, because of the Ca current run-down. The receptors isolated from one fish, however, proved to show fairly uniform current-voltage relations. Care was taken that measurements were

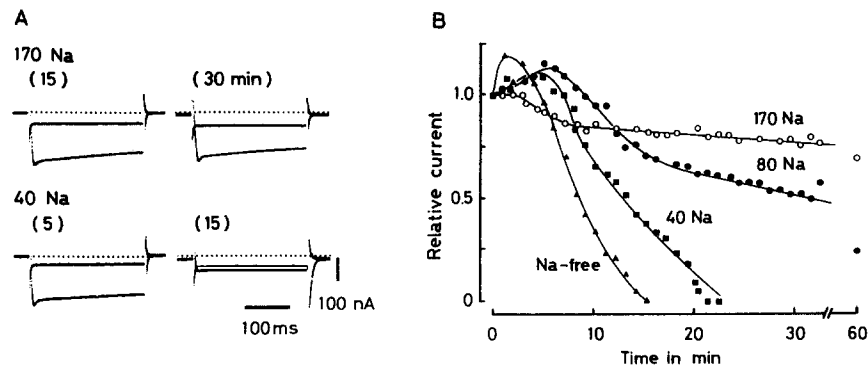


FIGURE 2. Decrease of the Ca current in time in low Na saline, in the presence of K blockers. In A, sample records show inward currents (larger trace) superimposed over leakage currents (smaller one). Upper records (170 Na) were obtained in control saline (170 mM/liter Na) by a voltage step to +2 mV from the holding potential of -33 mV. Lower records (40 Na) were from another ampulla in low Na saline (40 mM/liter) by a step to +1 mV from the holding potential of -23 mV. The inward current is nearly steady from 15 to 30 min in control saline, but it decreases to half the initial value within 15 min in low Na saline. The leakage current remains unchanged in both. The current decay during the command is slower in low Na saline than in control saline. In B, the inward current in different receptors was plotted against time. Each peak current was normalized to the initial value in control saline. Note that decrease of the current is dependent on Na concentrations.

made in similar periods of time after solution exchange. Fig. 3, A and B compare current patterns and the current-voltage relations in control and low Na saline. Sample records show that the Ca current decayed rapidly in control (170 Na), but more slowly in low Na saline (80 Na). The difference in decay will be further discussed in the next section.

Current-voltage relations are plotted for the peak and the current at 450 ms. In control saline (Fig. 3 A), the activation voltage was nearly the same for both the peak and the steady current (within 5 mV or less), as were those for current maxima and for reversal levels. In low Na saline (Fig. 3 B), two current-voltage relations were identical. Furthermore, both the peak and the steady current occurred at similar voltage, between -20 and -10 mV in control and low Na saline. Changes in the

current decay might suggest that Na current contributed to the peak. Such differential contribution seems unlikely, however, since the reversal levels remain the same in control and in low Na saline.

Decay Time Constants in Control and Low Na Saline

The current decay, however, behaved quite differently. In control saline, the Ca current clearly decayed in two exponential phases, as shown in the semilogarithmic

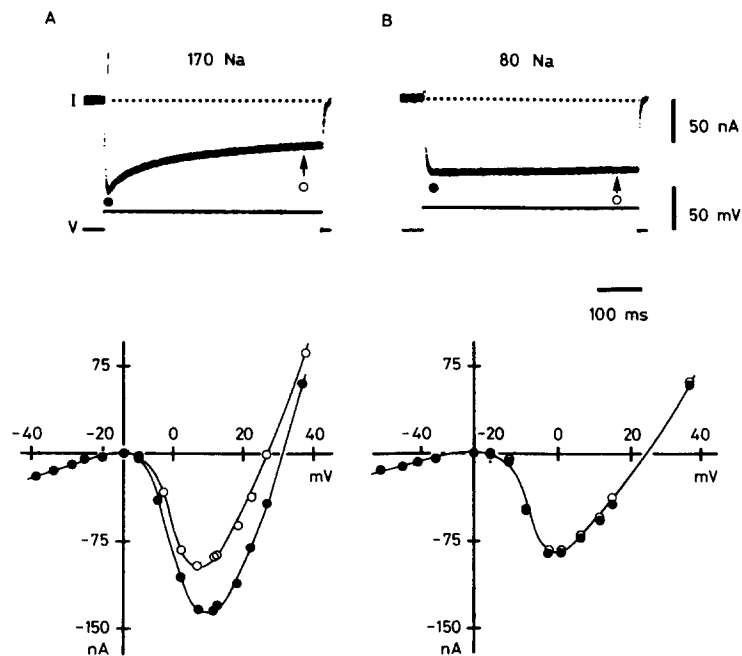


FIGURE 3. Similar current-voltage relations in control and low Na saline, recorded in the presence of K blockers, TEA (20 mM/liter) and 4-AP (5 mM/liter). Sample records were obtained from different ampullae, both at 15 min after solution exchange; (A) the inward current evoked by a step to +6 mV from the holding potential of -14 mV in control saline (170 Na), and (B) here it was evoked by a step to -1 mV from -24 mV in low Na saline (80 Na). The current-voltage relations are for the peak current (closed circles) and for the steady current at 450 ms (open circles). The ordinate axis is placed each at the holding potential. Note similar voltage levels for activation, for current maxima and for apparent reversal levels both for the peak and for the steady current, each in control saline (A) and in low Na saline (B). In low Na saline (B), the two current-voltage relations were identical. The inward current is activated at -20 and -10 mV among ampullae.

plot against time (Fig. 4 A). Upon a positive step to +2.5 mV, the time constant for the fast phase was 41.6 ms, and that for the slow phase was 1,300 ms (b_F and b_S). Upon a step to +7.5 mV, the two time constants were 40.6 and 1,160 ms (a_F and a_S). In low Na saline, the current decayed in a single exponential with slower time constants, 3,110 ms upon a step to 0 mV and 4,190 ms upon a step to +5.5 mV (Fig. 4 B, d and c).

The decay time constants in control saline are shown plotted against voltage steps with circles of different size for the two receptors (Fig 4 C). The time constants for the fast phase were <100 ms and changed in a voltage-dependent fashion (closed circles). Those for the slow phase were >1 s and seemed less dependent on voltage except at $>+20$ mV (open circles). The difference in the time constants was at least 30-fold in Fig. 4 C, with a mean value of 97.6-fold (12–560) in 12 cases. The difference in the voltage-dependency was confirmed in two receptors out of five exam-

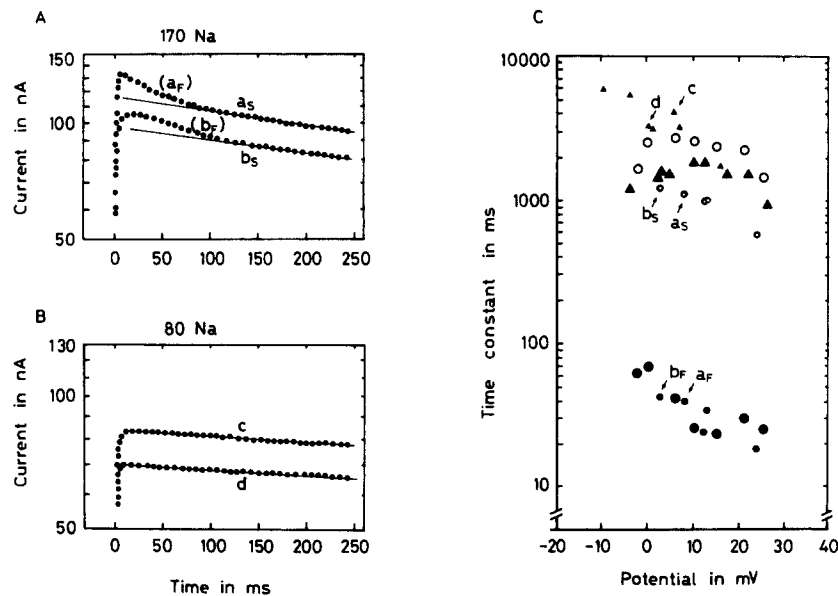


FIGURE 4. Different decay of the Ca current in control and low Na saline. In A, the Ca current in control saline (170 Na) decayed in two exponential phases, as shown in semilogarithmic plots against time. The two current traces, *a* and *b*, were obtained by command steps to +7.5 and +2.5 mV, respectively, from the holding potential of -24 mV. The time constants for the trace *a* were 40.6 (a_F) and 1,160 ms (a_S), and those for the trace *b* were 41.6 (b_F) and 1,300 ms (b_S). In B, the Ca current in low Na saline (80 Na) decayed in a single phase. The traces, *c* and *d*, were for steps to +5.5 and 0 mV from the holding potential of -24 mV. The time constants were 4,200 (*c*) and 3,100 ms (*d*). In C, the decay time constants are plotted against potential in the ampulla. The time constants in control saline (circles, in different sizes for each of the two cases) are in two groups, i.e., one <100 ms and the other $>1,000$ ms. The faster time constants are clearly more voltage-dependent than the slower ones. The decay time constants in low Na saline (triangles, in different sizes for each of the two cases) are similar to the slower ones in control saline.

ined in control saline. In the remaining three, the difference was less evident due to scatter, particularly for the slow phase.

The time constants in low Na saline (triangles) were long, similar to those for the slow phase in control saline, which would suggest abolition of the fast phase. Thus, the component for the fast phase appeared to be more sensitive to Na concentration external to the basal membrane, than to that for the slow phase. The two components will be tentatively referred to as the fast and slow I_{Ca} 's.

Difference in Inactivation Mode of the Two I_{Ca} 's

The inward current in control saline is partially inactivated in a current-dependent fashion, as shown in the preceding paper. In contrast, the slow current decay in low Na saline seems to suggest an absence of inactivation. Double-pulse experiments confirmed such difference of the two I_{Ca} 's.

The left-hand column in Fig. 5 shows current-dependent inactivation of the peak, or the fast I_{Ca} , in control saline. Conditioning pulses (P1) were 500 ms in duration.

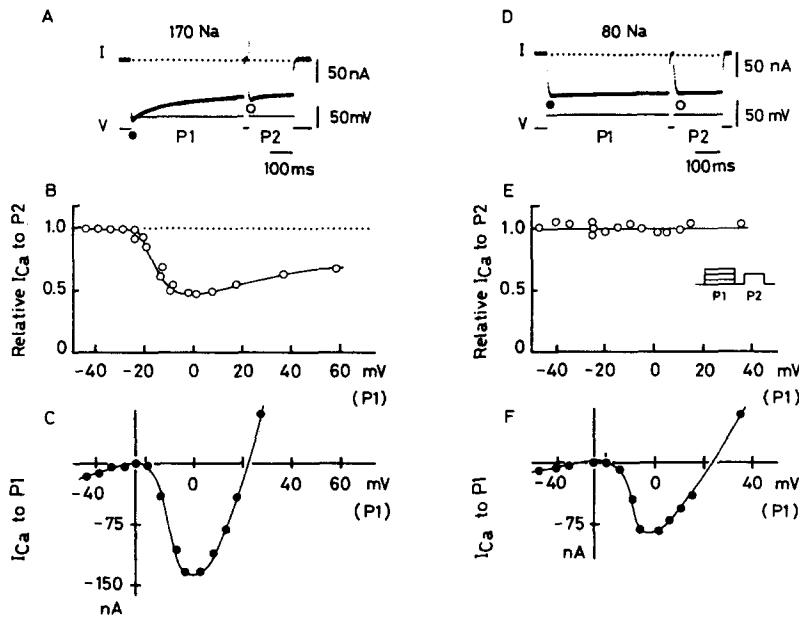


FIGURE 5. Differences in inactivation mode of the fast and slow I_{Ca} 's. The left- and right-hand columns were obtained from different receptors, in control saline (170 Na) and in low Na saline (80 Na). The uppermost row shows sample records (A and D). The conditioning pulses (P1) of 500 ms were applied before the test pulse (P2) of 100 ms at a 20-ms interval. Both P1 and P2 were steps to +1 mV from the holding potential of -24 mV (A), and to +1 mV from the holding -25 mV (D). The middle row shows the peak currents to test P2 (open circles) plotted against conditioning P1. In control saline (B) the test I_{Ca} is partially depressed by conditioning P1, whereas in low Na saline (E) it is hardly affected. The lowermost row (C and F) shows the current-voltage relations for the peak currents to conditioning P1 (closed circles). Note that in control saline (B) the I_{Ca} to test P2 is maximally depressed near the voltage levels for the inward current maximum to P1 (C). Thus, the fast I_{Ca} seems to inactivate in a current-dependent fashion, while the slow I_{Ca} is hardly inactivated.

Test pulses (P2), given at 20-ms intervals, were stepped to +1 mV from a holding potential of -24 mV, and induced near maximal inward currents. In Fig. 5 A, P1 and P2 were equal sized, and the current to test P2 was clearly depressed. The test current was normalized, and plotted against conditioning P1 (Fig. 5 B). The maximum depression to ~50% occurred to P1 near -3 mV, which was close to the voltage level for the current maximum to P1 (Fig. 5 C). The test current recovered upon larger P1 values (over +10 mV) with depression to ~30% at +60 mV.

The right-hand column in Fig. 5 shows similar experiments in low Na saline. The sample record (Fig. 5 *D*) was obtained when both P1 and P2 were stepped to +1 mV from the holding potential of -25 mV, and the current to test P2 was hardly changed. In Fig. 5 *E*, plots of data in low Na saline clearly indicate a virtual absence of inactivation for the slow I_{Ca} .

Fast I_{Ca} Initiates the Transient K Current

Low Na saline depresses the transient $I_{K(Ca)}$ without K blockers (Fig. 1). The $I_{K(Ca)}$ depression may have been caused secondarily by depression of the fast I_{Ca} in low Na saline. The correlation was further examined by double-pulse experiments in control saline.

The $I_{K(Ca)}$ appears as a positive dip after the peak current (Fig. 6 *A*). If the fast I_{Ca} induces the $I_{K(Ca)}$, the positive dip should be depressed simultaneously with inactivation of the fast I_{Ca} . Fig. 6 *A* shows sample responses to test P2, with and without conditioning P1 of various durations. The positive dip was clearly depressed by P1. Effects on the fast I_{Ca} were more difficult to assess, since in control saline the peak current would be contaminated by the onset of the slow I_{Ca} and the $I_{K(Ca)}$. Inactivation of the fast I_{Ca} was therefore tentatively assessed by the time to peak, which was expected to increase upon the abolition of the fast I_{Ca} (see Fig. 2).

The $I_{K(Ca)}$ was estimated as deviations of the positive dip from the steady inward current (See Fig. 6 *A, a*), which were normalized to control and plotted against P1 in Fig. 6 *B*. The $I_{K(Ca)}$ was maximally depressed by P1 near +20 mV, but recovered upon further depolarization. On increasing the P1 duration from 20 to 100 ms, depression of the $I_{K(Ca)}$ became more pronounced (*a-c*). The fast I_{Ca} seemed to be correspondingly depressed. The time to peak increased to 11.0 ms by 20 ms P1 from control 6.8 ms (Fig. 6 *C, a*), and further to 15.0 ms by 100 ms P1, though from a slightly prolonged control of 8.5 ms (Fig. 6 *C, c*). The time to peak thus can serve as a measure in assessing the I_{Ca} inactivation (see also Fig. 7 *E*).

Therefore, the $I_{K(Ca)}$ is depressed in a current-dependent fashion, and secondarily to inactivation of the fast I_{Ca} . Complete depression on longer P1 is partly due to the slower recovery of the $I_{K(Ca)}$, which may last longer than several seconds (unpublished observation). Depression, however, was observed even with a short P1 that barely covered the inward current peak. This may imply that the channels for the fast I_{Ca} and for the $I_{K(Ca)}$ are closely located for direct interaction.

Fast I_{Ca} Initiates the Phasic Release of Transmitter

Depression by conditioning pulses was also observed with the PSPs. Experimental protocol was the same as before, except that a small amount of 4-AP (2 mM) was added to reduce the K current, but to enhance the release of transmitter (Molgó et al., 1979). In Fig. 7, the conditioning P1s (40 ms) were applied 40 ms before the test P2 (80 ms) to +13 mV. The uppermost traces (*N*) show the PSPs to P2; those with negative P1 serving as control, and those with positive P1 as the test PSP (marked by arrows). The middle (*I*) and the lowermost traces (*V*) are similar to those in Fig. 6.

In Fig. 7 *A, b*, the conditioning P1 was stepped to +13 mV, which is the same as the test P2, and the PSPs to either P1 or P2 alone were identical and maximal. The PSP to test P2 was markedly depressed when preceded by positive P1. The test PSP,

however, gradually recovered upon more positive P1 (*c* and *d*). The smaller PSP may simply indicate a synaptic depression, such as caused by transmitter depletion. Evidence against such a possibility was obtained by observing OFF PSPs as marked by an asterisk after larger P1 (Fig. 7 *A, b-d*). Note that the test PSP immediately after a large OFF PSP showed a complete recovery (*d*), in contrast to the depression that followed a smaller P1 (*b*). Hence, the transmitter depletion could not account for the PSP depression.

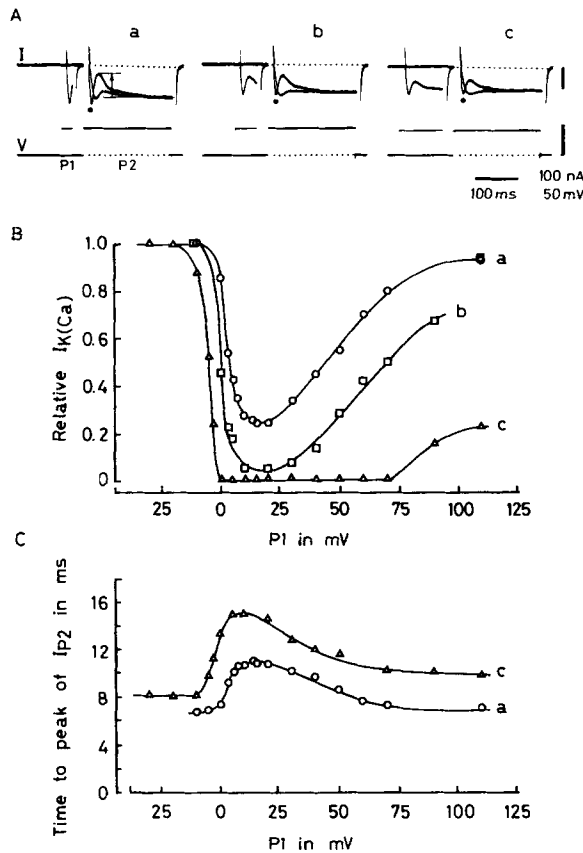


FIGURE 6. Depression of the $I_{K(Ca)}$ caused by inactivation of the fast I_{Ca} . The receptor current was recorded in control saline, without K blockers. In *A*, the control inward currents to test P2 (marked by dots) are shown superimposed over the test currents preceded by conditioning P1 of 20 (*a*), 50 (*b*), and 100 ms (*c*) in duration, at an interval of 20 ms. Both P1 and P2 were stepped to +10 mV from the holding potential of -30 mV. The $I_{K(Ca)}$ was estimated as a positive dip from the steady inward current, as shown on trace *I* in *a*. Note that conditioning P1 markedly depressed the $I_{K(Ca)}$, and also retarded the time to peak of the test currents (*a-c*). In *B*, the normalized $I_{K(Ca)}$ to test P2 was plotted against conditioning P1. Depression of the $I_{K(Ca)}$ was enhanced by increasing P1 duration, as shown by curves *a-c* which are similar in duration to those in the sample records. In *C*, the time to peak was plotted as a measure of

changes in the rate of rise of the test currents, or of inactivation of the fast I_{Ca} . The time to peak was retarded in a mirror image to the $I_{K(Ca)}$, and was dependent both on P1 level and on P1 duration (*a* and *c*). Thus, the $I_{K(Ca)}$ seems to be depressed by conditioning P1, secondarily to inactivation of the fast I_{Ca} .

The peak amplitude of the test PSP was normalized, and plotted against P1 (Fig. 7 *D*), with the peak current and the time to peak to test P2 (*E*). In *B* and *C*, the PSPs and the inward current to conditioning P1 were also shown as references. The test PSP was maximally depressed (*D*) near the voltage levels for the inward current maximum to P1 (*C*). The test PSP, however, completely recovered upon larger P1 (*D*) near the PSP suppression potential (*B*), at which OFF PSPs reached a maximum

that was the same as that of ON PSPs. The time to peak of the current to test P2 increased in a mirror image to PSP, again suggesting inactivation of the fast I_{Ca} even though the apparent decrease of the current peak was rather small in this preparation (E).

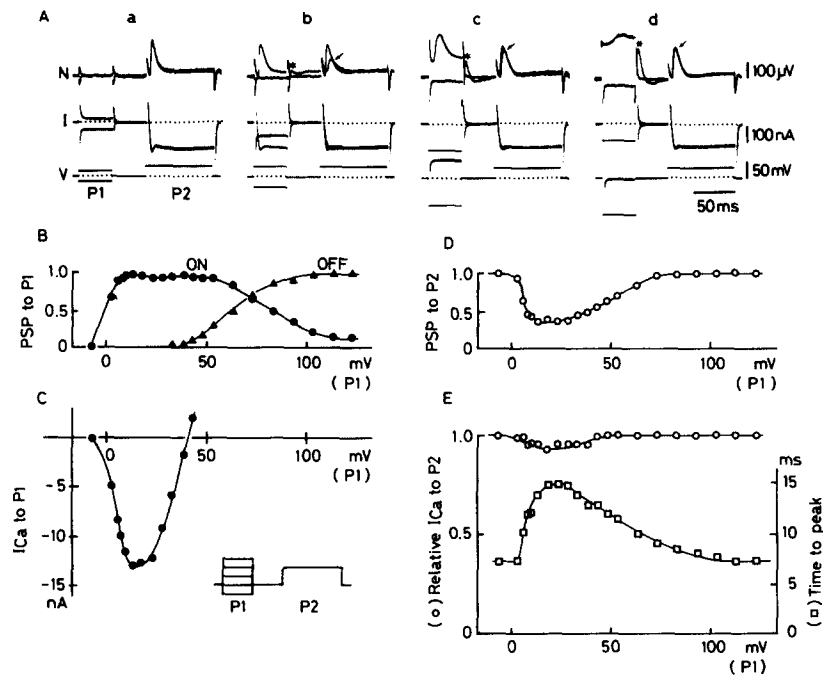


FIGURE 7. Depression of PSP in nerve due to inactivation of the fast I_{Ca} . Responses were recorded in control saline with the addition of 4-AP (2 mM), which reduced the $I_{K(Ca)}$ but enhanced transmitter release. In A, sample records to test P2 (80 ms) are preceded by conditioning P1 (40 ms) at a 40-ms interval. Test P2 was stepped to +13 mV from the holding potential of -17 mV. The trace N shows PSPs; those preceded by negative P1 served as control, and those preceded by positive P1 served as the test PSP (marked by arrows). The traces I and V are for current and potential. In A, b, both P1 and P2 were stepped to +13 mV, and PSPs to P1 or P2 alone were identical and maximal. Note that the test PSP is depressed when preceded by positive P1 (A, b), but recovers upon larger P1 (A, c and d). Note also that the test PSP after a full-sized OFF PSP (marked by the asterisk), which followed larger P1, shows a full recovery (A, d) in contrast to the PSP depression after small P1 (A, b). In B and C, responses to P1 are plotted as references, for ON and OFF PSPs and for the peak current. In D, PSPs to test P2 plotted against P1 show a maximum depression near P1 levels for the inward current maximum (C), and a recovery upon P1 near the PSP suppression potential (B). In E, the peak inward current to test P2 and the time to peak were plotted against P1, which imply a current-dependent inactivation of the fast I_{Ca} .

Therefore, the test PSP is depressed in a current-dependent fashion, again secondarily to inactivation of the fast I_{Ca} . To summarize, the fast I_{Ca} seems to initiate the phasic transmitter release, in addition to the $I_{K(Ca)}$, both of which would serve for phasic receptor responses.

The OFF PSP results from a brief tail current on termination of P1. As transmitter store is evidently available for phasic release at an interval as short as that shown in Fig. 7 A, *d*, the OFF PSP size (*b-d*) may reflect the fast I_{Ca} channels that remain not inactivated. The full-sized OFF PSP upon the disappearance of the ON PSP (*d*) would again suggest the current-dependent inactivation of the fast I_{Ca} . The tonic PSP phase, however, was little affected, as shown by later PSP traces (*b-d*). Whether the tonic release of transmitter is related to the slow I_{Ca} remains undetermined.

Possible Role of the Slow I_{Ca} in the Tonic Receptor Operations

The *Plotosus* receptor in situ shows resting afferent discharges that result from the tonic receptor activity (Obara, 1976). A possible role of the slow I_{Ca} in tonic receptor operation was examined.

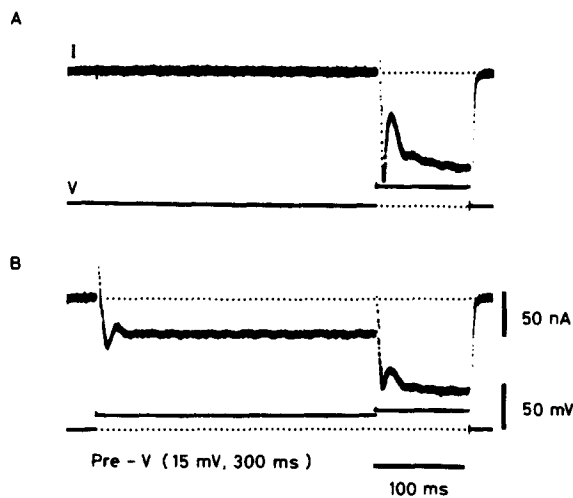


FIGURE 8. A possible role of the slow I_{Ca} in the tonic receptor operation. In *A*, the typical current pattern, the inward current peak followed by the transient $I_{K(Ca)}$, was evoked by a command step to +5 mV from the holding potential of -15 mV, in the absence of K blocker in control saline. In *B*, a prepulse of 300 ms was given to step the potential in the ampulla to 0 mV, which was close to the DC level in situ. The inward peak and the outward transient to test pulse were reduced, due to the inactivation of the fast I_{Ca} . The

steady current at the end of test pulse, however, was unaffected, presumably supplied by the slow I_{Ca} . Thus, the slow, noninactivating I_{Ca} probably serves to maintain the tonic receptor activity.

In control saline without K blockers, the sensory epithelium was held at a resting DC level of -15 mV. In Fig. 8 *A*, a test step to +5 mV evoked a typical current pattern. In *B*, the in situ conditions were simulated by adding a conditioning pulse to 0 mV, even though the pulse duration would be disproportionately short. The test response then showed both the inward peak and the outward transient clearly depressed, while the steady inward current was hardly affected. The data would suggest that the fast I_{Ca} , though partially inactivated, can nevertheless serve to modulate the phasic release of transmitter, while the slow I_{Ca} may provide the steady active current to maintain the tonic receptor operation in situ.

DISCUSSION

The Ca current in the *Plotosus* receptor has been subdivided into the fast and slow I_{Ca} 's on the basis of difference in (*a*) the sensitivity to Na concentration outside, (*b*)

the inactivation time constants, and (c) the modes of inactivation, even though their activation voltages are similar. Also functionally, (d) the fast I_{Ca} seems to be closely correlated to the transient $I_{K(Ca)}$ and the phasic release of transmitter, while (e) the slow I_{Ca} seems to provide the tonic active current in situ.

Decay of the Ca Current in Two Phases

Decay of the Ca current in more than one exponential phases has been shown first in the *Helix* neuron (Brown et al., 1981), later in other molluscan neurons (Plant et al., 1983), and also in insect muscle (Ashcroft and Stanfield, 1981, 1982a, b). Two explanations have been so far proposed.

First, Brown et al. (1981) suggested a coexistence of populations of the Ca channels with a difference in inactivation processes, which was either current-dependent (Magura, 1977; Brehm and Eckert, 1978; Eckert and Tillotson, 1981) or voltage-dependent (Akaïke et al., 1978). This possibility will be considered in a later section.

Secondly, the current-dependent inactivation per se may also develop in two phases (Standen and Stanfield, 1982; Plant et al., 1983; Chad et al., 1984). In the binding-site model, the Ca current is described by a product of the second-order activation kinetics and P_n (the probability of the channel being not inactivated) that decreases in a rectangular hyperbola against the intracellular free Ca ions. Calculated current decay is well fitted by two exponential phases. In addition, it is shown that an increase in the intracellular free Ca ions would induce a preferential loss of the fast decay phase.

Such a mechanism, however, would hardly explain the present findings. In the *Plotosus* receptor, (a) the two decay time constants differ by 97.6-fold, which is much larger than the fivefold that has been calculated from the binding-site model (Ashcroft and Stanfield, 1982; Chad et al., 1984), (b) the time constants in low Na saline are similar to those of the slow phase in control, and (c) the slow I_{Ca} is unaffected by prepulses, in contrast to the fast I_{Ca} . These data simply suggest the abolition of the fast I_{Ca} .

Some Ca current, which normally shows a current-dependent inactivation in two phases, may change to those with slower decay phase and with less effects of prepulses, as shown in *Paramecium* (Brehm et al., 1980), *Helix* (Brown et al., 1981), and *Aplysia* (Chad et al., 1984). The change, however, was induced by an injection of EGTA, which increased the intracellular Ca buffering capacity, and hence the steady Ca current. In the *Plotosus* receptor, the Ca current was depressed by low Na saline, which would hardly be expected to increase the Ca buffering capacity.

Coexistence of Two Populations of the Ca Channels

Coexistence of two types of the Ca channel have been first shown in the starfish eggs (Hagiwara et al., 1975). In the mammalian inferior olivary neurons, Llinás and Yarom (1981) have shown two types of Ca spikes, the "low- and high-threshold," each in soma and in dendrites, respectively. Recently, three types, T, N, and L, have been classified in the dorsal root ganglia (Nowycky et al., 1985) and in other cells (cf. Tsien et al., 1987), in terms of (a) activation range, (b) inactivation processes, (c) relaxation rates, (d) single channel conductance, (e) channel kinetics, (f) tendency to wash-out, (g) sensitivity to agents, and (h) deactivation kinetics.

On the basis of these criteria, the receptor Ca current may be termed as either the N or the L types. The fast I_{Ca} resembles the N current in the activation range, relaxation rate, and suppression by Cd^{2+} , while the slow I_{Ca} is similar to the long-lasting L current. Both I_{Ca} 's are like the L current, however, in the irreversible run-down. As for inactivation, the N current inactivates completely in the range of -100 and -40 mV, while the L current shows little inactivation for about 100 ms, but an essentially complete inactivation for longer pulses at -60 to -10 mV. The L current in the vertebrate cardiac cell inactivates either in voltage- or current-dependent fashion (Mentrard et al., 1984; Lee et al., 1985). In the *Plotosus* receptor, however, so far only current-dependent inactivation has been observed.

The Ca channels sensitive to low Na saline have been rarely described. In the aquarín-injected squid axon, Na-free saline induces a rise in the resting glow, which suggests an increase in the free Ca ions (Baker et al., 1973), presumably due to the depression of the Na-dependent Ca pump (cf. Baker, 1984). In the *Plotosus* receptor, the receptor activity is depressed by ouabain or KCN (Sugawara, 1986). The Na sensitivity of the fast I_{Ca} may indicate that these Ca channels are associated with similar ionic pumps. In low Na saline, the slow I_{Ca} is also gradually depressed and eventually abolished. The tonic receptor operation with a continual Ca influx may require such association with metabolic processes.

Physiological Roles of the Two Ca Channels

The fast I_{Ca} , initiating the transient $I_{K(Ca)}$ and the phasic PSP, would contribute to phasic receptor responses. In the squid giant synapse, the Ca ions are assumed to enter the presynaptic fiber near the release sites (Llinás et al., 1976). The channels for the fast I_{Ca} in the *Plotosus* receptor may be located close to the active site.

The slow I_{Ca} is well suited to maintain the tonic receptor operation. The *Plotosus* receptor, when isolated or current-clamped, shows negative DC potential, which is presumably maintained by ionic pumps (Sugawara, 1986). The ampulla in situ, however, is electrically shunted by the duct, and the bias current flows outward in the basal membrane. The slow I_{Ca} , partially activated, further depolarizes the ampulla to 0 mV (Sugawara and Obara, 1984b). Even with maintained stimuli, the tonic afferent activity recovers in tens of seconds, resulting in sensory adaptation, which may represent an interplay of the slow I_{Ca} and the ionic pumps.

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