Facilitation of Ca^{2+} -channel currents in bovine adrenal chromaffin cells

(patch clamp/ Ca^{2+} current/ Ba^{2+} current)

T. HOSHI*, J. ROTHLEIN†, AND S. J. SMITH*

Departments of *Physiology and †Pharmacology, Yale University School of Medicine, New Haven, CT 06510

Communicated by Charles F. Stevens, April 12, 1984

ABSTRACT Ca^{2+} -channel currents in primary cultures of bovine adrenal chromaffin cells were studied using the whole-cell patch-clamp method. Parameters of a double-pulse protocol were systematically varied to characterize facilitation by a prepulse (P_1) of Ca^{2+} -channel current during a test pulse (P_2). The pulses were usually separated by 30 msec, an interval sufficient for decay of any measurable P_1 tail currents. The Ca^{2+} -channel current amplitude during P_2 increased when P_1 voltage was more positive than 0 mV. The effect became progressively greater with more positive P_1 voltage. With a 60-msec P_1 to +80 mV, the current amplitude typically increased by 25%–35% during a 60-msec P_2 . Comparison of facilitated and control inward Ca^{2+} -channel current $I(V)$ curves showed that facilitation was also strongly dependent on P_2 test voltage. Facilitation of Ca^{2+} -channel currents is a voltage-dependent phenomenon and is not dependent on Ca^{2+} entry. When short repetitive voltage-clamp pulses were applied, the Ca^{2+} -channel current amplitude increased with each pulse. This suggests that Ca^{2+} -channel facilitation could enhance release of catecholamines from chromaffin cells during a train of action potentials.

At many synapses, when two action potentials separated by a short interval arrive at the presynaptic terminal, the second action potential releases more transmitter than the first. This phenomenon is referred to as monosynaptic facilitation of transmitter release, and it represents one of the most common types of synaptic plasticity (1). Two major hypotheses for the mechanism of synaptic facilitation have been proposed. One states that Ca^{2+} channels facilitate in response to a prior depolarization. According to this hypothesis, the first action potential changes properties of the Ca^{2+} channels such that more Ca^{2+} ions flow through the channels during the second action potential, resulting in enhanced release. The other hypothesis states that Ca^{2+} channels do not facilitate and that residual Ca^{2+} ions remaining in the terminal after the first action potential are responsible for facilitation (2, 3). Studies to test these hypotheses have been hindered by the inaccessibility of most presynaptic terminals, because such studies require measurements of Ca^{2+} ion flux through presynaptic Ca^{2+} channels. A voltage-clamp study of the squid giant synapse, one of the few synapses in which direct measurements of Ca^{2+} currents are possible, supports the residual Ca^{2+} hypothesis because Ca^{2+} current amplitude was constant when two identical pulses were given (4). However, generality of this finding remains to be established.

Many of the properties of the presynaptic terminals, such as Ca^{2+} -dependent release, are shared by endocrine cells. Unlike the presynaptic Ca^{2+} channels, however, Ca^{2+} channels in endocrine cells can be studied using the patch pipette voltage-clamp technique (5). Fenwick *et al.* (6) found that Ca^{2+} current in bovine adrenal chromaffin cells was in-

creased by a prior depolarization pulse. This phenomenon is of special interest because this is one of the mechanisms hypothesized for synaptic facilitation. Thus, we have defined this effect of a prior depolarization pulse as facilitation of Ca^{2+} channels and systematically investigated the phenomenon using essentially the same chromaffin cell preparation used by Fenwick *et al.* We report conditions that enhance Ca^{2+} -channel facilitation and describe its dependence on time and voltage dimensions of paired-pulse commands. The results suggest that Ca^{2+} -channel facilitation is caused by intrinsic reactions of Ca^{2+} -channel molecules to changes in the membrane potential and that it could be an underlying mechanism of facilitation of transmitter release in some synapses during a train of action potentials.

MATERIALS AND METHODS

Cell Culture. Chromaffin cells were prepared from adult and young bovine adrenal medulla by the method of Kilpatrick *et al.* (7) as modified by Wilson and Viveros (8). The cells were plated on coverslips coated with collagen at a density of ≈ 1200 cells per cm^2 . The cells were kept up to 20 days in culture. Experiments were carried out only on undifferentiated round cells with diameters of 10–18 μm .

Voltage Clamp. The cells were voltage-clamped using the tight-seal whole-cell patch-clamp technique of Hamill *et al.* (5), which allowed dialysis of the cell interior. The patch pipettes made of borosilicate glass (Boralex, Rochester Scientific, Rochester, NY) had initial input resistances of 1–2 M Ω when filled with the internal solution described later. The seal resistance was usually 15–40 G Ω . The input resistance in the whole-cell configuration was >2 G Ω . The holding potential was usually -70 mV. Approximately 50% of series resistance was usually compensated. After the series resistance compensation, the voltage-error, because of the uncompensated series resistance of 5–10 M Ω for a 200 pA current was 1–2 mV and the capacitive current in response to a 15-mV hyperpolarizing step pulse settled within 100 to 150 μsec . Current signals were low-pass filtered through an 8-pole Bessel filter, digitized, and analyzed using a minicomputer. Ohmic leakage and capacitive currents were subtracted by scaling the average of 10 or more current responses to 15-mV hyperpolarizing voltage steps.

Ca^{2+} -Channel Current Separation. Ca^{2+} -channel currents were measured after pharmacological suppression of other ionic currents in the following way. Na^+ currents were eliminated by using no Na^+ in the solutions. Cl^- currents were minimized by replacing most of the Cl^- with aspartate ions. K^+ currents were blocked by an absence of K^+ in both internal and external solutions and by the presence of Cs^+ and tetraethylammonium (Et_4N^+) (internal), and Et_4N^+ , 3,4-diaminopyridine (DAP), and quinidine (external). The remaining voltage-dependent currents were inward with moderate

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Et_4N^+ , tetraethylammonium; $I(V)$, current-voltage; DAP, diaminopyridine.

depolarizations and were insensitive to external applications of tetrodotoxin (17 $\mu\text{g}/\text{ml}$), but they were blocked by 5 mM external Mn^{2+} . Thus, the voltage-dependent inward currents under these conditions were assumed to be Ca^{2+} -channel currents. These inward currents disappeared within 10 min after achieving the whole-cell configuration. A labile property similar to this has been found with Ca^{2+} -channel currents in many other internally dialyzed preparations and termed rundown or wash-out (6, 9). Most data were taken at least 1 min after achieving the whole-cell configuration to allow exchange of ions between the cell and the pipette interior.

Solutions. The external solution in most experiments contained 135 mM Et_4N^+ aspartate/10 mM CaCl_2 (or BaCl_2)/5 mM DAP/2 mM MgCl_2 /5 mM Hepes/40 μM quinidine sulfate, pH 7.2. The internal solution in most experiments contained 120 mM Cs aspartate/20 mM Et_4N^+ aspartate/11 mM EGTA/2 mM MgCl_2 /1 mM CaCl_2 (10 nM free Ca^{2+})/5 mM Hepes, pH 7.2. The solutions had milliosmolarities of 300–315. In most of the experiments, Ba^{2+} was used as the charge carrier because the Ba^{2+} current was larger than Ca^{2+} current.

Experiments were carried out at room temperature (22°C–26°C).

RESULTS

Ca^{2+} -channel currents were studied using a double-pulse protocol. A prepulse (P_1) was followed by a test pulse (P_2) after an interval of 30 msec in most experiments. Fig. 1A shows the effects of P_1 to +80 mV on Ca^{2+} -channel currents measured at P_2 voltage of +5 mV in Ca^{2+} or Ba^{2+} containing saline. P_1 to +80 mV increased the P_2 current amplitude when compared with the control in which P_1 was omitted. We refer to this increase in Ca^{2+} -channel current by P_1 as facilitation of Ca^{2+} -channel current. Facilitation of Ca^{2+} -

channel currents by P_1 was observed even though measurable tail currents after a P_1 prepulse were over well before the application of P_2 . Facilitation also affected the time course of Ca^{2+} -channel current activation. Normally, the currents continued to increase during a 60-msec pulse and they were still increasing at the end of the pulse (see control currents in Fig. 1A). Facilitated currents, however, reached the peak value more quickly (see facilitated currents in Fig. 1A). As shown in Fig. 1A, charge carrier species did not influence facilitation. Ca^{2+} , Ba^{2+} , and Sr^{2+} currents facilitated in a very similar fashion. Since Ba^{2+} currents were often larger than Ca^{2+} or Sr^{2+} currents and easier to analyze, Ba^{2+} was used as the charge carrier in most experiments.

The tail current of the facilitated Ba^{2+} or Ca^{2+} current was greater in amplitude than the respective control tail current. Ba^{2+} tail currents on returning to –55 mV from P_2 of +5 mV are shown in Fig. 1B. A sum of at least two exponentials was required to fit the tail currents (see ref. 6). Any components of the tail currents faster than 100 μsec were not resolved because of the voltage-clamp settling time. P_1 to +80 mV increased the amplitude of the faster component and its time constant. The slower component was not usually affected by the P_1 in any consistent way. Ca^{2+} tail currents also showed similar facilitation.

The effect of a facilitating prepulse can persist for several hundred milliseconds during P_2 of long duration, but facilitated and control Ca^{2+} -channel currents finally reach equal levels. In Fig. 1C, P_2 duration was increased to 660 msec. After reaching a higher peak value, the facilitated current declined in amplitude to the same level as the control current at the end of the test pulse.

Variables That Affect Facilitation. With a pulse protocol as used in Fig. 1A, facilitated P_2 current was greater in amplitude than the control P_2 current, typically by 25% to 35%.

FIG. 1. Facilitation of Ca^{2+} -channel currents by a depolarizing prepulse. In each set, upper traces are voltage pulses and lower traces are current responses. Trace 1 is control without a prepulse and trace 2 is with a prepulse to +80 mV. (A) P_1 to +80 mV facilitates Ca^{2+} and Ba^{2+} currents measured at +5 mV. The scale bar represents 160 pA for Ca^{2+} current and 400 pA for Ba^{2+} current. In these traces, tail currents following P_2 are not resolved because of the low sampling rate used. (B) P_1 to +80 mV also facilitates Ba^{2+} tail current following a 60 msec P_2 to +5 mV. The tail currents were measured at –55 mV. (C) Facilitation of Ba^{2+} current with a long P_2 (660 msec) at +5 mV by a 60 msec P_1 to +80 mV. All traces are from different cells. P_1 duration was 60 msec. P_2 duration was 60 msec in A, 5 msec in B, and 660 msec in C. P_1 and P_2 were separated by 30 msec. Data were low-pass filtered at 4 kHz in A and C, and at 8 kHz in B. Sampling rate was 1 msec per point in A, 50 μsec per point in B, and 10 msec per point in C. Internal solution for A and C did not contain Et_4N^+ (substituted with Cs^+).

The degree of facilitation was influenced by the following variables. (i) Passage of time after achieving the whole-cell configuration. (ii) Prepulse and test pulse voltages. (iii) Prepulse duration. (iv) Separation of the prepulse and test pulse (interpulse interval). Each variable is described below in detail.

Washout of Facilitation. The degree of facilitation changed with time after establishing the whole-cell configuration. Strong facilitation was observed only during the first 2–3 min of the recordings and then gradually disappeared. The decline in facilitation usually occurred before the peak current amplitudes had declined appreciably because of washout. This property of facilitation severely limited the amount of data that could be collected from each cell. Disappearance of facilitation with time was accompanied by changes in the kinetics of control Ca^{2+} -channel currents. Control Ca^{2+} -channel currents that subsequently showed facilitation had a non-inactivating time course in that the currents continued to increase during a 60-msec depolarization pulse (see control currents in Fig. 1A). Ca^{2+} -channel currents that did not facilitate had an inactivating time course during a 60-msec pulse. During the course of an experiment, Ca^{2+} -channel kinetics changed from the noninactivating type to the inactivating type.

Dependence on Prepulse Voltage. Facilitation of Ca^{2+} -channel current is strongly dependent on P_1 voltage. When P_1 was more positive than 0 mV, peak P_2 Ca^{2+} -channel currents increased (Fig. 2). P_1 to +80 or +100 mV, where there was often a net outward current during P_1 , produced the greatest increase in P_2 current. P_1 that elicited large inward Ca^{2+} -channel currents were less effective in increasing the P_2 current amplitudes (see Fig. 3 for the voltage dependence of Ca^{2+} -channel activation). Hyperpolarizing prepulses to -100 mV for 60 msec did not change the control-current amplitude significantly. In some cells, a small inactivation of P_2 currents was observed after a P_1 prepulse to -10 or -5 mV even though P_2 currents facilitated when P_1 was more positive than 0 mV.

Dependence on Test Pulse Voltage. Peak current-voltage [I(V)] curves of facilitated and control Ca^{2+} -channel currents are compared in Fig. 3. Facilitation is strongly dependent on P_2 voltage, being largest at voltages where the Ca^{2+} -

FIG. 2. Facilitation is dependent on prepulse voltage. Increase in the peak amplitude of test Ba^{2+} current measured during a 60-msec P_2 to +5 mV compared with that of the control current after a 60-msec P_1 to different voltages, indicated on abscissa, is plotted. (Inset) Voltage commands used. The control current was obtained by omitting P_1 (i.e., P_1 to the holding potential, -70 mV). P_1 and P_2 were separated by 30 msec.

FIG. 3. Facilitation is dependent on test pulse voltage. Peak I(V) curves for the facilitated and control Ba^{2+} currents are compared. Δ and $\diamond$ represent control and facilitated I(V) curves, respectively. (Inset) Voltage commands used. P_1 to +80 mV was used to facilitate Ba^{2+} currents. Both P_1 and P_2 were 60 msec long and were separated by 30 msec.

channel current was largest. P_1 to +80 mV appears to increase the P_2 current amplitudes without any major shifts along the voltage axis, although small shifts were present in some experiments. Outward currents observed when P_2 was more positive than +70 or +80 mV were not affected by P_1 to +80 mV.

Effects of Prepulse Duration. P_1 duration affected the degree of facilitation of the Ca^{2+} -channel currents. This was examined by increasing P_1 duration from 10 msec to 80 msec while keeping the interval between P_1 and P_2 constant (30 msec). In the experiment shown in Fig. 4, the degree of facilitation increased with P_1 duration up to 50 msec, but it did not increase further with longer pulses.

Effects of Pulse Separation. The time interval between P_1 and P_2 also affected the degree of facilitation of the Ca^{2+} -channel currents (Fig. 5). Separation of the two pulses was increased from 30 to 210 msec in 30-msec steps. Increasing the interval between pulses decreased the degree of facilitation. The time course of decay of facilitation varied greatly among the cells tested.

Variables That Do Not Affect Facilitation. The following variables did not significantly affect facilitation. (i) Charge carrier species (Ca^{2+} , Ba^{2+} , or Sr^{2+}). (ii) Internal free Ca^{2+} concentration. Facilitation of the peak Ca^{2+} current was ob-

FIG. 4. P_1 duration affects Ba^{2+} current facilitation. Duration of P_1 to +80 mV was increased from 10 msec to 80 msec in 10-msec steps. The peak current amplitude is plotted against the prepulse (P_1) duration. (Inset) Voltage commands used. The interval between P_1 and P_2 was constant (30 msec).

FIG. 5. Interval between P_1 and P_2 affects Ba^{2+} current facilitation. Separation of P_1 and P_2 was increased from 30 msec to 210 msec in 30-msec steps. Change in the peak P_2 Ba^{2+} current amplitude is plotted against the interval between P_1 and P_2 . Dotted line indicates control current amplitude during a 90-msec pulse. (Inset) Voltage commands used. P_1 was a 60-msec pulse to +80 mV. P_2 was a 90-msec or longer pulse to +5 mV.

served with the internal solution buffered to contain 10 or 100 nM free Ca^{2+} and with the internal solution containing neither added Ca^{2+} nor EGTA. (iii) Amplitude of the control Ca^{2+} -channel current immediately after achieving the whole-cell configuration. Ca^{2+} currents as small as 40 pA in the peak amplitude and Ba^{2+} currents as large as 200 to 300 pA showed very similar facilitation. (iv) Holding potential. Although more negative holding potentials appeared to increase the control current amplitude slightly, the holding potential in the range tested (-50 to -120 mV) did not affect facilitation. (v) Voltage where the cells were clamped between prepulse and test pulse (interpulse voltage). The interpulse voltage was changed to either -180 , -70 , or -50 mV, and the holding potential was also changed to -120 mV to remove any steady-state inactivation of Ca^{2+} channels. When the interpulse voltage was -180 mV, a slow persistent inward tail current was observed after P_1 . However, the facilitated currents were not affected by the changes in the interpulse voltage. (vi) Presence or absence of internal Cs^+ or Et_4N^+ . Replacing the internal Cs^+ with Et_4N^+ or Et_4N^+ with Cs^+ did not affect facilitation. (vii) Presence or absence of DAP or quinidine. Facilitation was observed without DAP or quinidine or both. (viii) Number of days in culture. Ca^{2+} -channel currents from 1-day-old cells and 20-day-old cells showed similar facilitation.

FIG. 6. Facilitation of Ba^{2+} current by short repetitive pulses. Seven 9-msec pulses to +10 mV separated by 9 msec were applied to the cell. Dotted line represents peak amplitude of the first Ba^{2+} current. No ohmic leak or capacitive subtraction. Data were low-pass filtered at 6 kHz and were sampled at 0.4 msec per point.

Facilitation by Repetitive Stimuli. To study the possible physiological relevance of facilitation, voltage command pulses similar to a train of action potentials were applied (Fig. 6). Each pulse depolarized the cell to +10 mV for 6–9 msec, with successive pulses separated by an interval equal to their duration. During the first 2–3 min of the recordings, Ca^{2+} -channel current amplitude increased with successive pulses during the train. With 6-msec pulses to +10 mV, the current amplitude during the seventh pulse was 10% greater than that during the first pulse. Increasing pulse duration and separation to 9 msec caused the current during the seventh pulse to be up to 20% greater than that during the first pulse. When the same protocols were applied more than 3–4 min after establishing the whole-cell configuration, the current amplitude decreased with successive depolarization pulses.

DISCUSSION

Adequacy of Ca^{2+} -Current Separation Procedures. Chromaffin cell membranes have numerous other ionic permeation mechanisms (6, 10) that could interfere with measurement of current through Ca^{2+} channels. We have relied primarily on pharmacological and ionic modifications of internal and external solutions to suppress all known conductance mechanisms except Ca^{2+} -channel permeation. Although these methods succeeded in blocking almost all signs of active current except Ca^{2+} -channel current, it is still conceivable that our measurements could be contaminated by residual current through other types of channels. For instance, if an outward current were decreased by a prepulse, net inward current during the test pulse would increase, and this condition might be mistaken for Ca^{2+} -channel facilitation. Two independent sets of observations have convinced us that the inward current facilitation faithfully reflects the behavior of Ca^{2+} channels. First, facilitation of inward tail current (see Fig. 1B) argues strongly that it must result from an increase in Ca^{2+} -channel current rather than from a decrease in any residual current. Since tail currents were measured at negative voltages, residual currents would most probably be inward. Thus, their reduction by a prepulse would decrease, rather than increase, inward tail current amplitude. Second, the dependence of inward current facilitation on test pulse voltage seems consistent only with facilitation being a genuine effect on Ca^{2+} channels. Facilitation as a function of test pulse voltage is always very nearly proportional to the unfacilitated Ca^{2+} current, as might be expected from enhancement of Ca^{2+} current. This I(V) relationship would be expected for a residual current only if activation of this current were dependent on Ca^{2+} entry, but this seems unlikely because Ba^{2+} currents facilitate even though Ba^{2+} normally does not substitute for Ca^{2+} in ion channel activation. Although this argument does not rule out effects of contamination by Ba^{2+} -activated residual current, this possibility is unprecedented and highly unlikely.

Comparison with Previous Studies. Most of our information on Ca^{2+} -channel gating has come from studies on neuronal cell bodies, muscle cells, and eggs. Facilitation, as observed in chromaffin cells, has not been documented in any of these other cell types. At present, it would be difficult to tell whether this is because Ca^{2+} channel facilitation occurs only in certain cell types (e.g., chromaffin cells) or because the proper experiments have not been done with the other cells. Most earlier studies that used two-pulse voltage commands found the opposite of what is described here: net inward currents were decreased by preceding depolarizations. This has been attributed either to Ca^{2+} -channel inactivation or to interference from residual currents through other channels (11, 12). Other studies have suggested the presence of facilitation, but they used indirect and, therefore, relatively inconclusive methods (13–16). One recent study of Ca^{2+} cur-

rent in EGTA-injected molluscan somata designed to minimize interference from other ionic currents does provide direct evidence for a weak form of facilitation (17). However, using the identical techniques and protocols used here on chromaffin cells, we did not observe any facilitations of Ca^{2+} -channel currents in GH₄ pituitary tumor cells (unpublished observation). It is clear that Ca^{2+} channels can be quite diverse in their gating properties (12).

Although our study essentially confirms the observations of Fenwick *et al.* (6) regarding Ca^{2+} -channel facilitation in chromaffin cells, there is one apparent discrepancy. Fenwick *et al.* reported a decrease in P_2 current by P_1 depolarization when tested at P_2 voltage more positive than +20 mV, while we observed only increases in P_2 currents at all levels of P_2 following P_1 to +80 mV. Although we cannot account for this difference, it does not seem surprising, because we selected experimental conditions that maximized facilitation and, therefore, might have minimized any opposing process.

Mechanism of Facilitation. Our results support an earlier conclusion (6) that facilitation reflects an intrinsic reaction of Ca^{2+} channels to changes in membrane potential because large P_1 depolarizations produce facilitation even though divalent ion influx must be suppressed at very positive P_1 . In addition, since Ba^{2+} rarely substitutes for Ca^{2+} in intracellular actions, facilitation of Ba^{2+} currents provides further evidence against a role of divalent ion entry *per se* in facilitation.

Since facilitated Ca^{2+} -channel current activates with virtually the same voltage dependence as the control current, it seems most likely that facilitation reflects enhanced activation of a single class of Ca^{2+} channels rather than differential activation of a second class of Ca^{2+} channels. Activation of Ca^{2+} channels in chromaffin cells has been modeled by a sequential three-state model, including two closed states and one open state (closed–closed–open) (6). While such a model could predict some forms of two-pulse facilitation, other prominent aspects of channel behavior, such as the inactivating time course of facilitated current, are not predicted by this type of model. Clearly, a model with more than three states will be necessary to entirely explain gating of Ca^{2+} channels in chromaffin cells.

Possible Physiological Significance. Since Ca^{2+} influx leads to exocytotic secretion of catecholamines in chromaffin cells (18), facilitation of the Ca^{2+} currents could enhance secretion during repetitive action-potential firing. Various studies have indicated that release of transmitter is proportional to the second or higher power of the Ca^{2+} current amplitude (19). The 20% increase in the Ca^{2+} -channel current observed

during a train of stimuli in this study (Fig. 6) could increase secretion by 40% if secretion is related to the second power of the Ca^{2+} -channel current amplitude (4) or by 100% if it is related to the fourth power of the current amplitude (20). Although Ca^{2+} -channel facilitation does not appear to be involved in facilitation of transmitter release at the squid synapse (4), it could enhance release of catecholamines from chromaffin cells during a train of action potentials, and it may be one of the underlying mechanisms of synaptic facilitation in some synapses.

We thank Drs. R. W. Aldrich, G. J. Augustine, and C. F. Stevens for valuable discussions. This work was supported by National Institutes of Health Grants NS-16671 and RR-05358 to S.J.S. and NS-069515 to J.R.

1. Zucker, R. S. (1982) in *Conditioning*, ed. Woody, C. D. (Plenum, New York), pp. 249–264.
2. Katz, B. & Miledi, R. (1968) *J. Physiol. (London)* **195**, 481–492.
3. Rahamimoff, R. (1968) *J. Physiol. (London)* **195**, 471–480.
4. Charlton, M. P., Smith, S. J. & Zucker, R. S. (1982) *J. Physiol. (London)* **323**, 173–193.
5. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391**, 85–100.
6. Fenwick, E. M., Marty, A. & Neher, E. (1982) *J. Physiol. (London)* **331**, 599–635.
7. Kilpatrick, D. L., Ledbetter, F. H., Carson, K. A., Kirshner, A. G., Slepatis, R. & Kirshner, N. (1980) *J. Neurochem.* **35**, 679–692.
8. Wilson, S. P. & Viveros, H. (1981) *Exp. Cell Res.* **133**, 159–169.
9. Byerly, L. & Hagiwara, S. (1982) *J. Physiol. (London)* **322**, 503–528.
10. Fenwick, E. M., Marty, A. & Neher, E. (1982) *J. Physiol. (London)* **331**, 577–597.
11. Hagiwara, S. & Byerly, L. (1981) *Annu. Rev. Neurosci.* **4**, 69–125.
12. Tsien, R. W. (1983) *Annu. Rev. Physiol.* **45**, 341–358.
13. Lux, H. D. & Eckert, R. (1974) *Nature (London)* **250**, 574–576.
14. Heyer, C. B. & Lux, H. D. (1976) *J. Physiol. (London)* **262**, 319–348.
15. Eckert, R., Tillotson, D. & Ridgway, E. B. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1748–1752.
16. Lux, H. D. & Heyer, C. B. (1977) *Neuroscience* **2**, 585–592.
17. Eckert, R. & Ewald, D. (1983) *J. Physiol. (London)* **345**, 549–565.
18. Douglas, W. W. (1968) *Br. J. Pharmacol. Chemother.* **30**, 612–619.
19. Reichardt, L. F. & Kelly, R. K. (1983) *Annu. Rev. Biochem.* **52**, 871–926.
20. Dodge, F. A. & Rahamimoff, R. (1968) *J. Physiol. (London)* **193**, 419–432.