Ionic Basis of the Receptor Potential in Primary Endings of Mammalian Muscle Spindles

C. C. HUNT, R. S. WILKINSON, and Y. FUKAMI

From the Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110

ABSTRACT The effect of changing the ionic composition of bathing fluid on the receptor potential of primary endings has been examined in isolated mammalian spindles whose capsule was removed in the sensory region. After impulse activity is blocked by tetrodotoxin, ramp-and-hold stretch evokes a characteristic pattern of potential change consisting of a greater dynamic depolarization during the ramp phase and a smaller static depolarization during the hold phase. After a high-velocity ramp there is a transient post-dynamic undershoot to below the static level. On release from hold stretch, the potential shows a postrelease undershoot relative to base line. The depolarization produced by stretch is rapidly decreased by the removal of Na^+ and Ca^{2+}. Addition of normal Ca^{2+} partly restores the response. Stretch appears to increase the conductance to Na^+ and Ca^{2+} in the sensory terminals. The postdynamic undershoot is diminished by raising external K^+ and blocked by tetraethylammonium (TEA). It apparently results from a voltage-dependent potassium conductance. The postrelease undershoot is decreased by raising external K^+, but is not blocked by TEA. It is presumably caused by a relative increase in potassium conductance on release. Substitution of isethionate for Cl^- or the addition of ouabain does not alter the postdynamic and postrelease undershoots.

INTRODUCTION

The generation of action potentials by primary endings in response to stretch results from nerve-terminal depolarization which spreads to an impulse-initiating site in the primary axon (Hunt and Ottoson, 1975). The depolarization of the sensory terminals presumably results from conductance changes produced by stretch deformation.

Studies on several mechanoreceptors indicate that the receptor potential is reduced in a Na^+-free solution, for example, the Pacinian corpuscle (Diamond et al., 1958), crustacean stretch receptor (Edwards et al., 1962; Obara, 1968), crab coxal receptors, (Roberts and Bush, 1971) and frog muscle spindle (Ottoson, 1964). Generally the receptor potential is reduced to ~20–25% of its control value. In the frog spindle the reduction occurs over a period of ~10 min in Na^+-free solution. The spindle capsule probably restricts diffusion between the bathing solution and the space surrounding the nerve terminals, thus leading to the slow onset of this effect.

The mammalian muscle spindle has a relatively large capsular space near the terminations of its sensory endings, and the capsule of isolated spindles may be removed without changing its response properties. In the present study we have used such decapsulated spindles to study the ionic basis of the receptor potential. After removing the capsule, changes in the ionic composition of the bathing fluid produce rapid effects on the receptor potential, indicating that there is ready exchange with the fluid external to the nerve terminals. It will be shown that the depolarization in response to stretch results from a conductance change to Na^+, and that Ca^{2+} can partially substitute for Na^+. Furthermore, the response to ramp-and-hold stretch is modified by changes in K^+ conductance, particularly in the postdynamic and postrelease periods.

METHODS

Adult cats were anesthetized with sodium pentobarbital, 35 mg/kg. Spindles were isolated from thin muscles in the dorsolateral portion of the tail by procedures which have been previously described (Hunt and Ottoson, 1975). The spindle capsule was removed by microdissection in the region of the capsular space, where sensory endings are located. The decapsulated spindle together with its isolated primary axon was then placed in the experimental chamber which initially contained a modified Locke's solution (ionic composition in mM: NaCl 124, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 2.4, KH_2PO_4 1.2, HEPES (N-2-hydroxyethyl piperazine-N'-ethane-sulfonic acid) buffer, 5.0; pH adjusted to 7.4 at 25°C). Tris (tris(hydroxymethyl) aminomethane), or glucosamine were used to substitute for Na^+ and Ca^{2+} in equimolar amounts. When the concentration of K^+ was raised, the $[K^+]_o$ $[Cl^-]_o$ product was kept constant by substituting methylsulfate or isethionate for Cl^-. Impulse activity was blocked by tetrodotoxin (TTX) (10^{-7} wt/vol). Addition of TTX at 10 times this concentration had no effect on the amplitudes of receptor potentials in response to stretch which were subthreshold for spike initiation.

The experimental chamber allowed simultaneous stretching of the spindle, recording from the axon, and rapid exchange of the bathing fluid. The ends of the spindle were tied to nylon rods driven by chart recorder pen motors (Brush, Gould Inc., Instrument Systems Div., Cleveland, Ohio); the motors were controlled by a computer programmed to give ramp-and-hold stretches. The drive to each motor was adjusted so that the spindle's sensory region remained at rest during stretch, to reduce motion artifact. The spindle was positioned near the bottom of the 2-cm-long × 3-mm-wide and 3-mm-deep flow channel; the top of the channel was then sealed with a cover slip. Test solutions entered at one end by gravity flow, and exited into a waste container from the other end. An electrically controlled valve (Hamilton Co., Reno, Nev.) located near the inflow tube allowed changing of the test solutions without interrupting the flow, and without introducing air into the sealed chamber.

At the bottom center of the channel, a 3-mm diameter × 3-mm-deep cylindrical well contained heavy fluorocarbon oil (Fluorolube S-30, Hooker Chemicals & Plastics Corp., Niagara Falls, N.Y.), such that the upper surface of the oil was continuous with the bottom surface of the channel on either side. The axon from the primary ending was drawn down into the oil on a pipette containing Locke's solution with agar and connected via an Ag-AgCl junction to one input of a 113 DC differential amplifier (Princeton Applied Research Corp., Princeton, N.J.). The other amplifier input was connected through a similar junction to the fluid in the flow channel.

Responses from the nerve, together with a stretch displacement signal derived from the pen motor feedback transducers, were recorded on FM analog magnetic tape (3960

instrumentation recorder. Hewlett-Packard Co., Palo Alto, Calif.) and analyzed offline with the aid of a computer (980B, Texas Instruments Inc., Houston, Tex.). Records of 1.5-s duration (just before, during, and just after ramp-and-hold stretches of 1-s duration) were digitized from the analog tape and stored on a magnetic disc (sampling frequency, 2,000 s^{-1}; resolution, 1 part in 4,096). Each digitized record contained 3,000 data points together with identification, stimulus parameters, and the gain of the recording. Records were edited, and were discarded if base-line drift or other artifacts were present. The base-line potential level was calculated by averaging for 50 ms before the application of ramp-and-hold stretch. This level, relative to zero volts, was determined by the difference in junction potentials between the two Ag-AgCl electrodes and DC imbalance in the recording electronics, as well as the DC component of the measured receptor potential. Base-line levels were stable provided recording conditions were not changed. Hence, changes in the base-line level reflected changes in base-line receptor potential, as occurred, for example, with changes in resting length. The amplitude of the receptor potential varied in different preparations from several hundred microvolts to several millivolts depending on the amount of shunting around the nerve. For this reason absolute calibrations are omitted from some figures. Several characteristic points from each digitized record were selected by means of a movable cursor which was displayed along with the record on a cathode ray tube. These points were stored separately for subsequent analysis, described below.

In some experiments tension was measured by a semiconductor strain transducer (Akers AE 802, Akers Electronics, Horten, Norway) to which one end of the spindle was tied. Stretch was then delivered to the other end by a single pen motor, and the flow channel was left open to accommodate the transducer. Amplified signals from a Wheatstone bridge exciting the transducer were recorded, digitized, and analyzed in a manner similar to that described for receptor potentials.

Measurements were performed at room temperature (ca. 25°).

RESULTS

The receptor potential of the primary ending, in response to ramp-and-hold stretch, consists of a characteristic sequence of potential changes which depend upon stretch velocity and amplitude (Hunt and Ottoson, 1975). In the present study, amplitudes of several hundred microns were used with moderate to high ramp velocities. A typical response is shown in Fig. 1 illustrating the parameters that were routinely measured by the computer from digitized records. These included (1) the base-line level which varied with initial length of the preparation; (2) the peak of the initial component of the dynamic response; (3) the peak of the later dynamic component at the end of the ramp; (4) the lowest level reached during the postdynamic undershoot (the postdynamic minimum); (5) the highest level during the static response (the static maximum); (6) the level at the end of hold stretch (the end static); and (7) the lowest value reached during the release undershoot (postrelease minimum). The initial component of the dynamic response is responsible for the initial burst and the later dynamic component for the subsequent dynamic response of the impulse discharge. The postdynamic undershoot appears to determine, at least in part, the brief pause in discharge which may follow the dynamic response to a high velocity ramp. The decline in receptor potential after the static maximum during hold stretch accounts for the adaptive fall in static frequency. The postrelease undershoot

seems mainly responsible for the pause or decrease in baseline discharge on release. These features have been defined and described in further detail by Hunt and Ottoson (1975, 1976).

I. Ionic Mechanisms Producing Depolarization in Response to Stretch

EFFECTS OF Na⁺- AND Ca²⁺-FREE SOLUTIONS   On changing from normal Locke's solution to a solution lacking both Na^+ and Ca^{2+}, the receptor potential response to ramp-and-hold stretch declined within 15–30 s to about one-fifth of its control amplitude. This was followed by a much slower reduction over the ensuing 20–30 min until the response was essentially abolished. The time-course of the effect is shown in Fig. 2; in this experiment Na^+ and Ca^{2+} were replaced
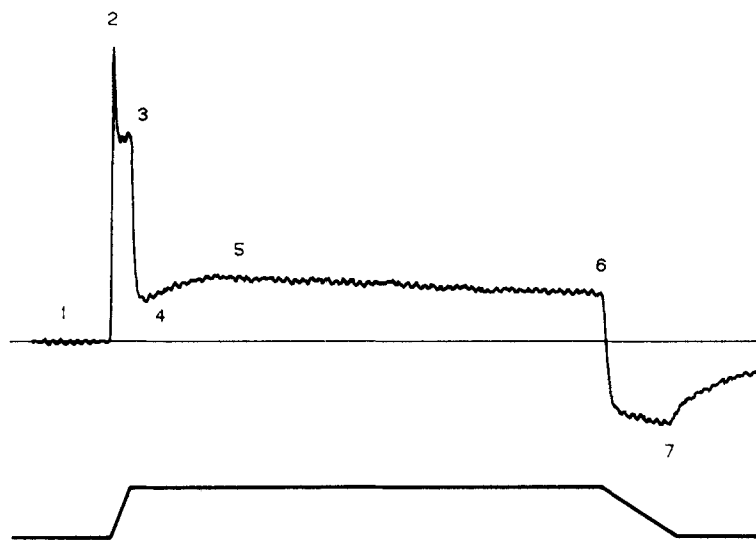
FIGURE 1.   Receptor potential of a primary ending of an isolated, decapsulated spindle, recorded in flow chamber. Upper trace: potential recorded from primary axon. Lower trace: length change. (1) Base line; (2) peak of initial dynamic component; (3) peak of late dynamic; (4) postdynamic minimum; (5) static maximum; (6) end static level; and (7) postrelease minimum. Sweep duration: 1.5 s.

by Tris and 1.8 mM EGTA was added. The average dynamic amplitude of the receptor potential, evoked by ramp-and-hold stretch every 5 s, showed a rapid initial decline. A slower fall then occurred, as may be noted after the two 5-min gaps in the record. On reintroducing normal Locke's solution, there was a rapid restoration of the response. Similar effects were produced when choline or glucosamine were used to substitute for Na^+ and Ca^{2+}. Fig. 3 shows that the dynamic and static components were similarly reduced. Both the potential at the peak of the late dynamic response (dynamic peak 2) and the static maximum rapidly declined on substituting glucosamine for Na^+ and Ca^{2+} and were promptly restored on reintroducing normal Locke's solution. The effects of removing and restoring Na^+ and Ca^{2+} were readily repeatable (Fig. 3).

EFFECTS OF VARYING ca^{2+} In Na^+-free solutions the amplitude of the receptor potential varied with the concentration of Ca^{2+} in the bathing solution. Thus, in Fig. 4, a bathing solution deficient in Na^+, but containing 1.8 mM Ca^{2+}, caused a reduction in receptor potential response to about one-third of
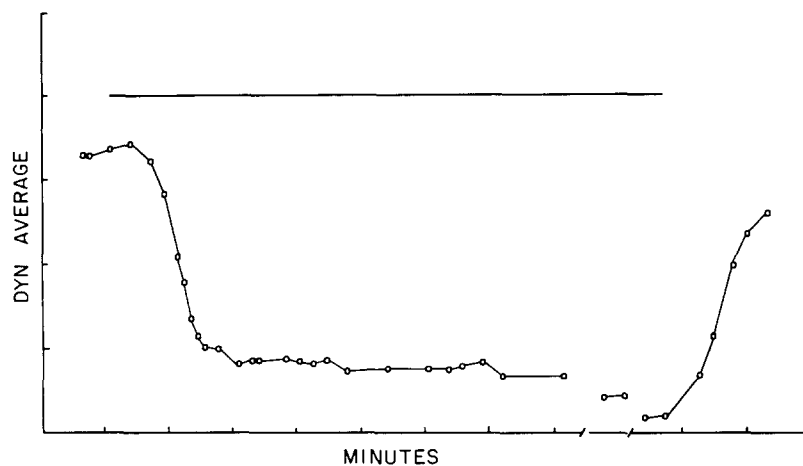

FIGURE 2. Effect of Na^+- and Ca^{2+}-free solution on the average dynamic (DYN) amplitude (initial and late dynamic peak amplitudes/2) of the receptor potential response to ramp-and-hold stretch. Each mark on the abscissa annotates 1 min. Gaps in record are of 5 min each. During bar, normal Locke solution was replaced by one containing no Na^+, no Ca^{2+}, and 1.3 mM EGTA. Tris substituted for Na^+ and Ca^{2+}.
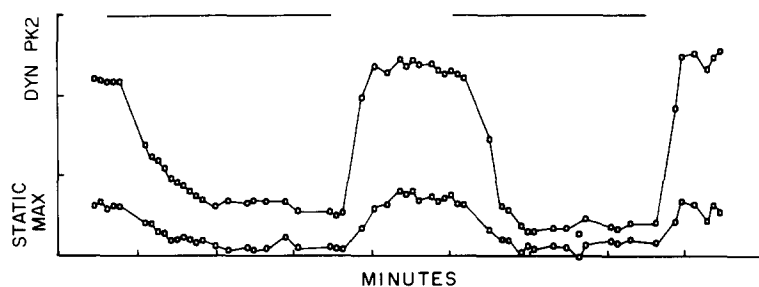

FIGURE 3. Effect of Na^+- and Ca^{2+}-free solution on the late dynamic peak (DYN PK 2) and static maximum (MAX) of the receptor potential response to ramp-and-hold stretch. During bars, normal Locke's solution was replaced by one containing no Na^+, no Ca^{2+}, and 1.3 mM EGTA. Glucosamine substituted for Na^+ and Ca^{2+}.

the control. Removal of Ca^{2+} caused a further reduction; the receptor potential became very small after a period of ~8 min. Reintroduction of the bathing solution containing Ca^{2+} but no Na^+ caused a partial restoration of the response. The amplitude of the response was further increased when $[Ca^{2+}]_o$ was raised to 8–10 mM (not shown). In contrast, when the bathing solution contained normal

Na$^+$, varying $[Ca^{2+}]_o$ from 0 (with EGTA) to 10 mM produced no significant change in the amplitude of the receptor potential.

In the absence of Na$^+$, but in the presence of normal $[Ca^{2+}]_o$, addition of D600 produced a marked and abrupt reduction in the receptor potential, an effect which rapidly reversed on removing the agent. D600, a derivative of verapamil, reduces or blocks a voltage-dependent Ca^{2+} conductance in nerve (Baker and Glitsch, 1975). When the bathing solution contained normal [Na$^+$], the same concentration of D600 had no effect on receptor potential amplitude. Except for the removal of Mg^{2+} from the bathing solution, which had no discernible effect, the effects of divalent cations other than Ca^{2+} were not studied.

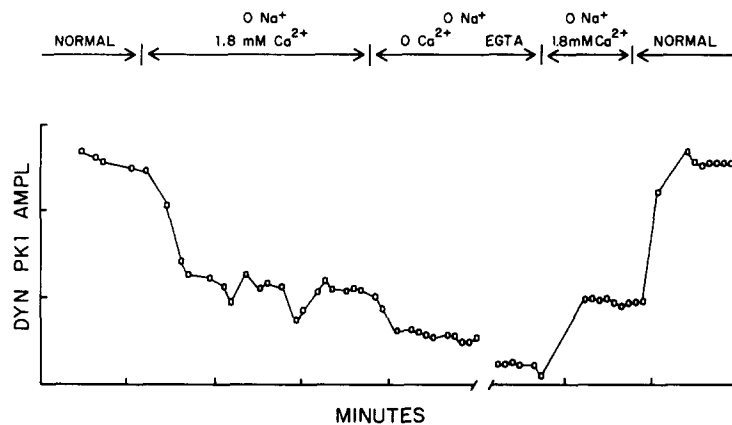EFFECT OF LITHIUM   Li$^+$ is known to substitute for Na$^+$ in the Na$^+$

FIGURE 4.   Effect of Na$^+$-free solution, with and without 1.8 mM Ca^{2+}, on the initial dynamic peak (DYN PK 1) in response to ramp-and-hold stretch. Composition of bathing solution shown above horizontal bars. Gap in record 6 min. Tris substituted for Na$^+$.

conductance associated with the nerve impulse. In squid axon Li$^+$ appears to be slightly more permeant than Na$^+$ (Chandler and Meves, 1965), whereas in frog node Na$^+$ and Li$^+$ appear to be equally permeant (Hille, 1972). It was therefore of interest to determine whether or not Li$^+$ could substitute for Na$^+$ in the receptor potential response. When all Na$^+$ in the bathing solution was replaced by Li$^+$, the receptor potential showed an increase of ~20% in both static and dynamic components (Fig. 5). This was readily reversible, and the postrelease undershoot was not affected. Thus, Li$^+$ appears to be more permeant than Na$^+$ during the conductance change produced by stretch. The long-term effects of bathing the spindle in Li$^+$ were not studied.

These effects of Li$^+$ on the primary ending differ from those on the crustacean stretch receptor (Obara and Grundfest, 1968) where Li$^+$ reduces the amplitude of the receptor potential, abolishes hyperpolarization on release, and depolarizes the cell.

THE BASIS FOR THE SLOW REDUCTION OF THE RECEPTOR POTENTIAL IN Na$^+$ AND Ca^{2+}-FREE SOLUTIONS   Removal of the capsule allows rapid changes in the

fluid bathing the nerve terminals, as evidenced by the prompt effects of changing ionic composition on the receptor potential. The question arises as to why the response is initially reduced only to about one-fifth of its control level in the absence of Na^+ and Ca^{2+} and what the basis is for the subsequent slow reduction. Among the factors that could play a role are the following: (*a*) the space external to some terminals might exchange rapidly but not that to others; (*b*) there might be a persistence of Na^+ or Ca^{2+} in an extracellular compartment; (*c*) the cations used to substitute for Na^+ and Ca^{2+} might be sufficiently permeant to account for the initial residual response.
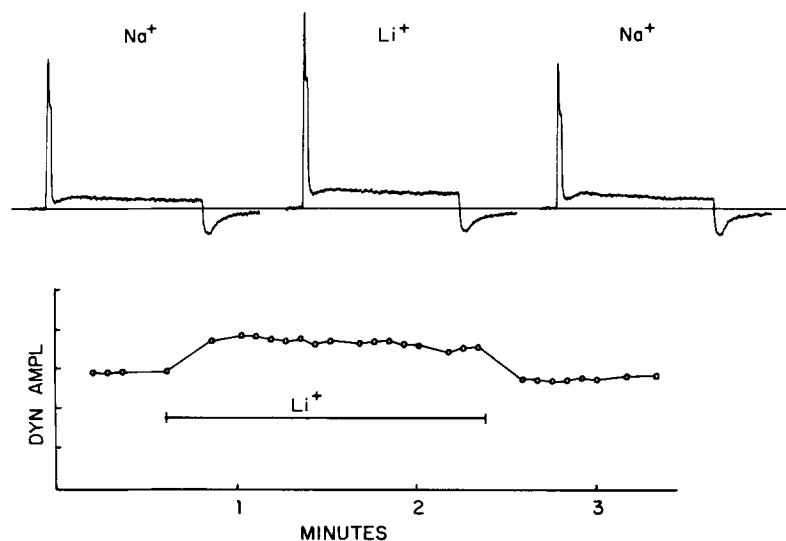
FIGURE 5. Effect of substituting Li^+ for Na^+ on the receptor potential response to ramp-and-hold stretch. Upper records show typical responses in normal Na^+ solution, in Li^+ solution, and on return to normal Na^+ solution (all containing TTX 10^{-7}). Lower record shows the change in amplitude of the initial dynamic peak (DYN) as solution was changed from normal Na^+, to Li^+, and back to normal.

Inasmuch as capsular cells normally provide a marked barrier to diffusion, it is possible that removal of the capsule in the region of the sensory endings exposes some terminals, permitting rapid exchange of their extracellular space with the bathing fluid, but that others remain encapsulated by inner capsule cells and exchange is slow. The residual, slowly declining receptor potential could, in this case, be produced by the still encapsulated terminals. This possibility has been investigated by electron microscopy using lanthanum nitrate as an extracellular marker. Fig. 6 shows that lanthanum failed to penetrate the intact capsule; however, when the capsule of the spindle in the sensory region was removed, there was intense staining of the cleft between all the sensory terminals of the primary ending and the intrafusal fibers. These findings indicate that all the terminals of the primary ending in the decapsulated spindle
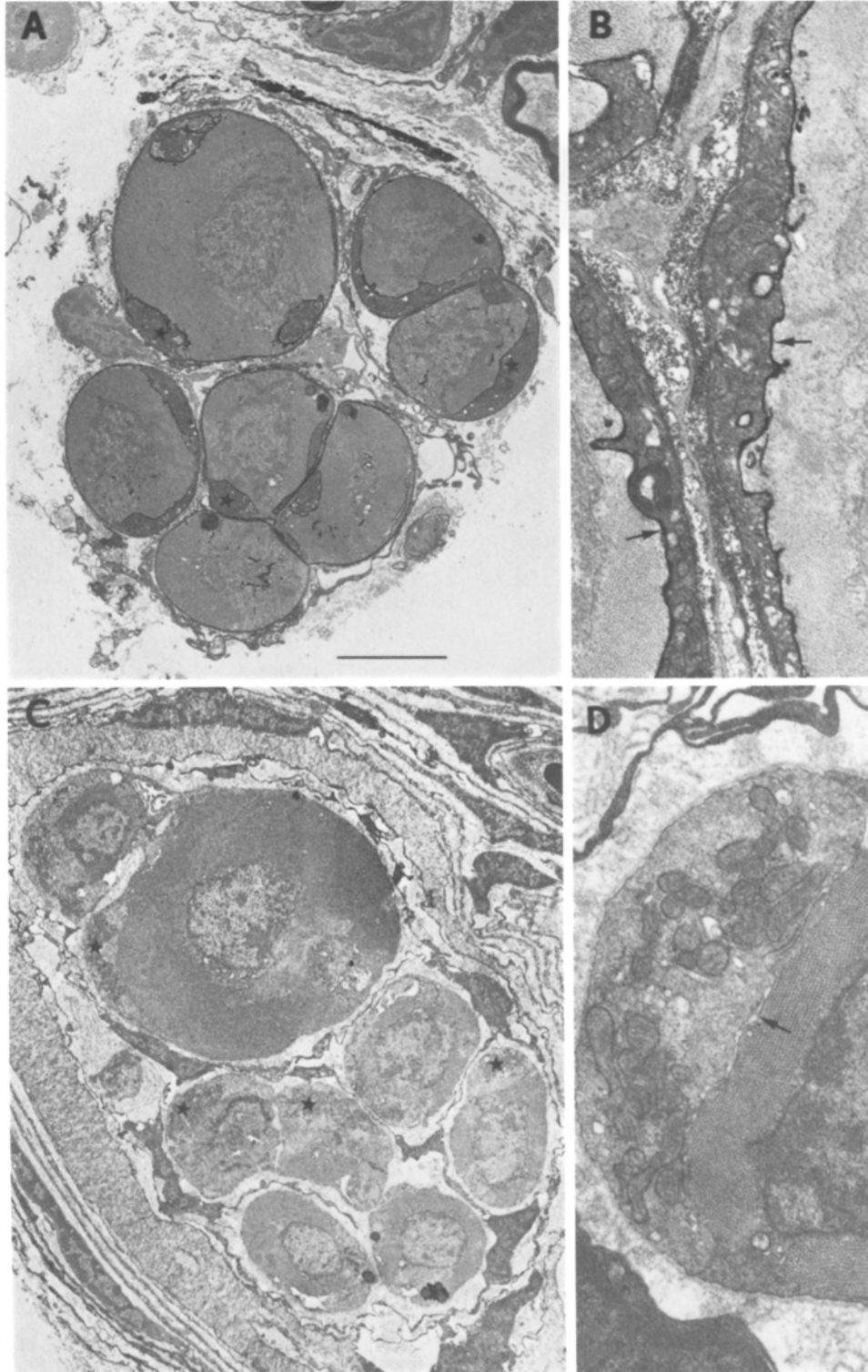
FIGURE 6. Electron micrographs of tandem spindle exposed to lanthanum nitrate during fixation. (A, B) Cross section through region of primary terminals in sensory region which had been decapsulated. (C, D) Cross section through region of primary terminals in sensory region whose capsule had been left intact. Calibration: (A, C) 5 μm; (B, D) 1 μm. Asterisks indicate sensory endings. Arrows indicate cleft between sensory ending and intrafusal fiber. Note higher electron density of sensory endings in decapsulated sensory region.

were readily accessible to the bathing solution. The fact that lanthanum nitrate appears concentrated in the clefts might result either from binding of lanthanum to some elements within the clefts or from limited diffusion out of the clefts.

Since the 200-A cleft between the membrane of the sensory terminal and the membrane of the intrafusal fiber has a longitudinal extent of only about 5 μm, one would expect Na^+ to diffuse rapidly from the center of the cleft to the outside solution unless its egress were restricted (see Kuffler and Nicholls, 1966, for discussion of the similar question of diffusion in the cleft between nerve cells and glia).

The possibility that the residual response might depend on the persistence of Ca^{2+} in the space immediately external to the terminals was tested by the application of D600. Although the response in a Na^+-free solution containing Ca^{2+} is abolished by D600 (see above), the response remaining after the initial rapid fall in a Na^+- and Ca^{2+}-free solution was unaffected by D600. This suggests that the residual response is not due to a persistence of Ca^{2+} alone in the extracellular space.

Whether or not the cations used to substitute for Na^+ are sufficiently permeant to account for the residual response is not known, but this seems unlikely. At nodes of Ranvier, choline and Tris appear to be impermeant during the regenerative conductance change associated with the action potential (Hille, 1971). We tried several other cations of larger molecular weight as substitutes for Na^+. However, neither glucosamine, tetraethylammonium, nor spermidine eliminated the response which remains after the initial rapid reduction after exposure to Na^+- and Ca^{2+}-free solutions.

II. *The Role of Potassium Conductance*

The foregoing experiments suggest that depolarization of the primary ending in response to stretch results from a conductance change, principally to Na^+, in the terminal membrane. We will now describe experiments which suggest that certain features of the impulse response, as well as the receptor potential produced by ramp-and-hold stretch, result from changes in K^+ conductance. Impulse frequency immediately after the dynamic response, and also on release, falls below the level anticipated from the stretch stimulus. This is reflected in the postdynamic fall in the receptor potential, when the ending becomes more polarized than in the subsequent static phase, and in the postrelease undershoot, when the ending becomes hyperpolarized relative to the base-line level. Such changes play an important role in determining the response pattern to ramp-and-hold stretch and may also contribute to the ability of the receptor to respond to repetitive stimuli.

EFFECT OF CHANGING INITIAL LENGTH   When the resting length was increased, the base line showed a maintained depolarization. The dynamic and static phases of the receptor potential, in response to the same ramp-and-hold stretch, showed minor changes when initial length was varied, whereas the effects on the postdynamic and postrelease phenomena were striking (Fig. 7). At the shortest initial length in Fig. 7 (lowest record), the potential falls smoothly from the dynamic level at the end of the ramp to the static phase; on release

there is a small hyperpolarization followed by a slow return to base line (only the very early portion of this return is seen in Fig. 7). As base-line depolarization is increased by raising initial length (middle and upper records), the postdynamic undershoot becomes progressively more prominent. Upon release from ramp-and-hold stretch at different initial lengths, the potential tended to approach the same absolute level. Such behavior would be expected if the membrane voltage were being driven toward an equilibrium potential more polarized than the base-line level during the release undershoot. In some preparations, as initial length increased, the postrelease hyperpolarization relative to base line not only became greater but its time-course was changed. At low initial lengths the potential returned to base line slowly whereas at higher initial lengths there was an initial rapid and then a slower return to baseline.
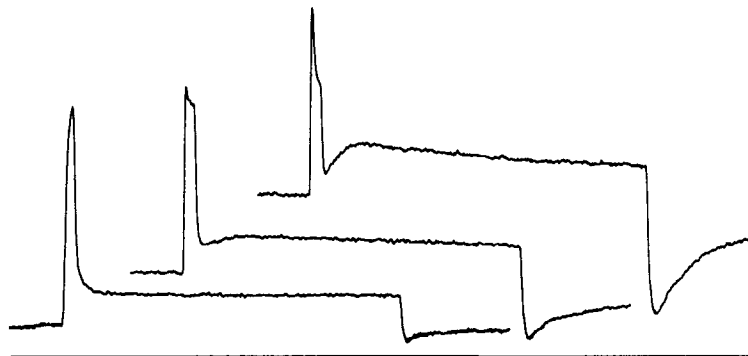
FIGURE 7. Receptor potential response to ramp-and-hold stretch at three different initial lengths. Lowest trace: low initial length. Middle trace: initial length increased. Upper trace: further increase in initial length. Only the early phase of the return toward base line after release is shown; the potential reaches base line after an additional 1–1.5 s. Trace duration: 1.5 s. Stretch duration: 1 s. All records are plotted on the same vertical voltage axis.

Fig. 8 shows the relation between the minimal values reached during the postdynamic and postrelease undershoots, and the base-line level when the initial length was varied. If the receptor potential during the postrelease minimum were produced only by the conductance to one ionic species, say K^+, its value should be independent of base-line level. The small slope shown in Fig. 8 is consistent with the potential at the postrelease minimum being determined largely by conductance to an ion with some contribution from a sodium conductance which increases as initial length is increased. The relation between postdynamic minimum and base-line level has a steeper slope as might be expected from a proportionately greater contribution of sodium conductance to the potential during this phase of the response.

EFFECT OF CHANGING EXTERNAL CL^- AND K^+ If, during the postdynamic and postrelease minima, the membrane is being driven towards an equilibrium potential more polarized than the base-line level, the underlying conductance change could be to either K^+ or Cl^-.

If changes in potassium conductance are responsible, varying the equilibrium potential for K^+ should have a greater effect on the postdynamic and postrelease minima than on base line and other features of the response. The effect of changing $[K^+]_o$ from 5 to 10 and back to 5 mM, while maintaining a constant $[K^+]_o$ $[Cl^-]_o$ product, is illustrated in Fig. 9. When $[K^+]_o$ was raised from 5 to 10 mM, the base line shifted and the amplitude of the response to ramp-and-hold stretch was reduced. The shifts in the postdynamic and postrelease minima
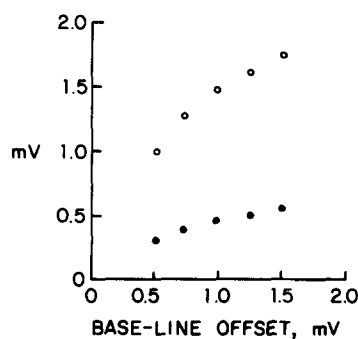

FIGURE 8. Effect of base-line level shift, produced by changing initial length, on postdynamic and postrelease minimal potential levels. (O) Relation between postdynamic minimum and base line. (●) Relation between postrelease minimum and base line.
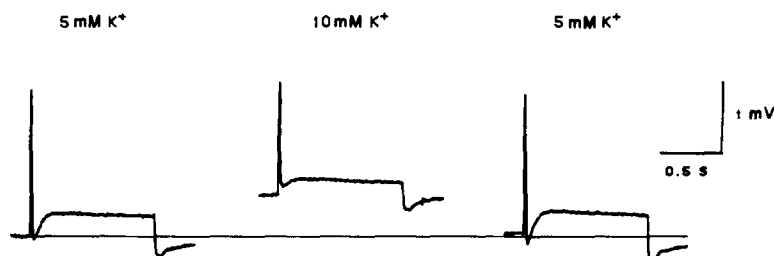

FIGURE 9. Effect of changing the concentration of K^+ in the bathing solution on the receptor potential response to ramp-and-hold stretch. Initial length held constant. All records are plotted on the same vertical voltage axis. $[K^+]_o$ $[Cl^-]_o$ product held constant.

were greater than that of base line, which is consistent with the potential being determined to a greater extent by G_K during these undershoots. On return to normal solution the changes were rapidly reversed.

Replacing Cl^- in the bathing solution with isethionate had no significant effect on the postdynamic and postrelease minima. Occasionally, however, a slight increase in overall receptor potential amplitude was observed in the absence of Cl^-, possible because of reduced leakage current.

EFFECTS OF TETRAETHYLAMMONIUM (TEA) TEA in concentrations of 7–21 mM caused a small increase in the amplitude of both the dynamic and static components of the receptor potential response to ramp-and-hold stretch. The

base-line level sometimes showed a small shift in the depolarizing direction. The most striking effect was a reduction or abolition of the postdynamic undershoot (Fig. 10). The effective extracellular recording site is separated from the receptor membrane by a branched myelinated axon containing nodes of Ranvier. Hence, the recorded receptor potential will depend on the length constant of the primary axon and its branches. A decrease in potassium conductance at the nodes of Ranvier by TEA could increase the amplitude of the recorded receptor potential by effectively increasing the length constant. The reduction in the postdynamic minimum may be attributable to block of a voltage-dependent potassium conductance by TEA.

The effect of TEA on the postrelease undershoot is complex. The initial undershoot on release was not blocked and, at times, even increased in amplitude in the presence of TEA; however, the subsequent long-lasting
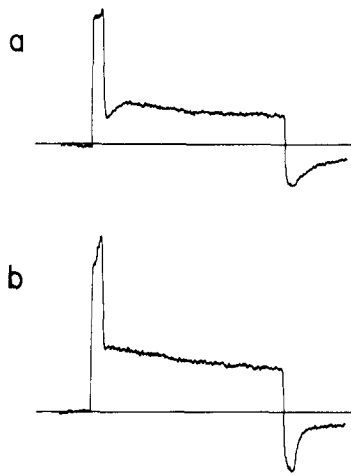

FIGURE 10.   Receptor potential in response to identical ramp-and-hold stretch in (a) normal solution and (b) solution containing 10 mM TEA. Stretch duration 1 s.

hyperpolarization was reduced or abolished. In normal solution the return to base line after release was slow, often taking several seconds. In TEA-containing solution the potential returned more rapidly towards base line after the postrelease minimum. The question remains as to why the undershoot during release persists. Two explanations are possible: (a) there may be an increase in G_K on release which is not blocked by TEA or (b) G_{Na} might be reduced relative to its base-line level so that a resting potassium conductance in the terminals could have a proportionately greater effect (see Discussion).

EFFECT OF OUABAIN AND Ca^{2+} Another possible mechanism that could play a role in postrelease hyperpolarization is an electrogenic sodium pump, activated by an influx of Na^+ during stretch. To test this possibility ouabain (1 × 10^{-5} wt/vol) was added to the bathing fluid. There was no effect on the base-line level or postrelease undershoot over a period of several minutes. We have not examined the effect of ouabain over a longer period of time.

The lack of a ouabain effect suggests that electrogenic pumping does not contribute significantly to the postrelease hyperpolarization. It does not rule out the possibility that impulse activity produced by stretch might add a hyperpolarization due to electrogenic pumping which could contribute to the pause or reduction of discharge during the postrelease period.

In some neurons, increases in K^+ conductance can result from a rise in internal Ca^{2+} concentration, consequent to entry of Ca^{2+} into the cell (Meech, 1972, Jansen and Nicholls, 1973). Thus, the postrelease hyperpolarization might be due to an increase in G_K secondary to an entry of Ca^{2+} into the terminals during stretch. However, removal of Ca^{2+} from the bathing solution, with the addition of 1.3 mM EGTA, did not abolish the postrelease undershoot. Nor was it significantly changed by varying $[Ca^{2+}]_o$ between 0 and 10 mM. These findings indicate that the postrelease phenomenon probably does not result from a change in potassium conductance produced by Ca^{2+} entry during stretch.

THE CONTRIBUTION OF TENSION CHANGES TO THE OBSERVED EFFECTS   In order to determine whether the bathing solutions used in the above experiments might have altered the mechanical properties of the spindle, in particular the intrafusal fibers, tension was recorded from one pole of the spindle and stretch applied to the other. With the exception of 20 mM $[K^+]_o$, which caused a reversible 14% increase in the tension response to ramp-and-hold stretch, all the solutions used were without significant effect on the tension response. This indicates that the observed effects were not due to changes in mechanical properties of the intrafusal fibers.

DISCUSSION

Except for an earlier study showing that the receptor potential of the Pacinian corpuscle is reduced when perfused with a Na^+-deficient solution (Diamond et al., 1958) the present results provide the first evidence about conductance changes in mammalian mechanoreceptors. In response to stretch the receptor membrane of the primary ending of the muscle spindle shows an increased conductance to Na^+, resulting in depolarization of the ending. It is not known whether this conductance change also involves K^+, in that the equilibrium potential for the stretch-evoked conductance change has not been determined. The evidence indicates that the membrane also becomes more permeable to Ca^{2+} during stretch. In the absence of Na^+, Ca^{2+} can partially substitute in producing the receptor potential, but in the presence of normal Na^+, variations in external Ca^{2+} produce no detectable effect on receptor potential amplitude. Normally, therefore, inward current through the receptor membrane must be carried largely by Na^+, but enough influx of Ca^{2+} might occur to produce significant changes in the concentration of Ca^{2+} within the nerve terminals. No agent is known which specifically blocks the Na^+ receptor current, although D600 appears to block Ca^{2+} current in the absence of Na^+. At present there is insufficient information to indicate whether one or more membrane channels are involved in the conductance changes producing the depolarization in response to stretch.

The conductance change produced by deformation of the terminals must

depend on the rate of change as well as amplitude of stretch. The present results provide no information concerning the basis of the dynamic sensitivity of the primary ending, i.e., whether it is due to mechanical properties of the intrafusal fibers, coupling between the latter and the receptor membrane, or is intrinsic to the nerve terminals themselves. Evidence from reptilian spindles suggests that overt mechanical changes, such as might be produced by greater viscosity in the polar as compared to the sensory region of the intrafusal muscle fibers, cannot alone account for the dynamic behavior (Fukami and Hunt, 1977). This, however, does not rule out the possibility that other mechanical factors might be involved. The ionic conductance changes producing the dynamic and static phases of the receptor potential appear to be qualitatively similar as judged by the effects of substituting for Na^+ and (or) Ca^{2+} by a number of organic cations and by Li^+. The dynamic and static responses appear to change in a parallel manner.

The basis for the initial rapid and subsequent slow reduction of the receptor potential in Na^+- and Ca^{2+}-free bathing solution remains unexplained. All the terminals of the primary ending appear to be readily accessible to the bathing solution when the capsule is removed. The residual response after the initial rapid decline appears most likely due to persistence of Na^+ in the cleft between the sensory terminal and intrafusal fiber. However, the dimensions of the 200-A cleft are such that diffusion time of small ions through this space could not be responsible unless access to the cleft were restricted. An alternative possibility is that the organic cations used to substitute for Na^+ are sufficiently permeant to produce the residual response.

Superimposed on the depolarization produced by ramp-and-hold stretch are variations in potential which appear to depend on potassium conductance changes. These are the postdynamic and postrelease undershoots. Both have important consequences in the impulse response pattern, the postdynamic undershoot causing a pause or drop in impulse frequency after a high velocity ramp, and the postrelease undershoot causing a reduction or cessation in baseline discharge on release. When impulse activity is present, it is possible that an electrogenic sodium pump at the impulse-initiating site might also contribute to these frequency changes. The postdynamic undershoot may be attributed to a voltage-dependent potassium conductance which is turned on by depolarization during the dynamic phase of the receptor potential, and slowly turned off when depolarization is reduced at the end of ramp stretch. The effects of varying initial length, of changing the concentration of K^+ in the bathing solution, and of TEA are consistent with this interpretation.

The basis for the apparent increase in potassium conductance on release is different. TEA does not block the hyperpolarization on release, although it modifies its time-course. Therefore, postrelease hyperpolarization cannot be attributed to the same type of voltage-dependent increase in G_K that underlies the postdynamic undershoot, although it might have a voltage-dependent component. The postrelease hyperpolarization appears to be due, at least in part, to a reduction in G_{Na}, relative to base line, producing an increase in the G_K/G_{Na} ratio. This results, to some extent, from mechanical changes. During

release tension in the isolated spindle drops below the base-line level[1] so that stretch deformation of the endings is reduced. It is also possible that the sodium conductance change which depends on velocity during stretch is reduced, relative to base line, during the negative velocity of release.

The primary ending consists of a myelinated axon which branches repeatedly, with nodes of Ranvier at branch points. The branches are myelinated nearly to the intrafusal muscle fibers where they give rise to the unmyelinated annular terminals. We assume the stretch-induced conductance changes which lead to depolarization occur in the terminal membrane, probably in the membrane which faces the intrafusal fiber. The site of the voltage-dependent potassium conductance change could be in the nodes of Ranvier of the myelinated branches of the primary axon or, possibly, in the receptor membrane itself. There is as yet no basis for deciding between these possibilities. Potassium conductance changes may be important in the responses of the primary ending to repetitive phasic length changes. For example, the receptor potential to sinusoidal stretch at frequencies of 50–100 Hz, shows a rapid repolarization after each cycle of depolarization (Hunt and Ottoson, 1977).

REFERENCES

BAKER, P. F., and H. G. GLITSCH. 1975. Voltage-dependent changes in the permeability of nerve membranes to calcium and other ions. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **270:**389–409.

CHANDLER, W. K., and H. MEVES. 1965. Voltage clamp experiments on internally perfused giant axons. *J. Physiol. (Lond.).* **180:**788–820.

DIAMOND, J., J. A. B. GRAY, and D. R. INMAN. 1958. The relation between receptor potential and the concentration of sodium ions. *J. Physiol. (Lond.).* **142:**382–394.

EDWARDS, C., C. A. TERZUOLO, and Y. WASHIZU. 1963. The effect of changes of the ionic environment upon an isolated crustacean sensory neuron. *J. Neurophysiol.* **26:**948–958.

FUKAMI, Y., and C. C. HUNT. 1977. Structures in sensory region of snake spindles and their displacement during stretch. *J. Neurophysiol.* **40:**1121–1131.

HILLE, B. 1971. The permeability of the sodium channel to organic cations in myelinated nerve. *J. Gen. Physiol.* **58:**599–619.

HILLE, B. 1972. The permeability of the sodium channel to metal cations in myelinated nerve. *J. Gen. Physiol.* **59:**637–658.

HUNT, C. C., and D. OTTOSON. 1975. Impulse activity and receptor potential of primary and secondary endings of isolated mammalian muscle spindles. *J. Physiol. (Lond.).* **252:**259–281.

[1] Hunt, C. C., and R. S. Wilkinson. Unpublished observation.

HUNT. C. C., and D. OTTOSON. 1976. Initial burst of primary endings of isolated mammalian muscle spindles. *J. Neurophysiol.* **39:**324-330.

HUNT, C. C., and D. OTTOSON. 1977. Responses of primary and secondary endings of mammalian muscle spindles to sinusoidal length changes. *J. Neurophysiol.* **40:**1113-1120.

JANSEN. J. K. S., and J. G. NICHOLLS. 1973. Conductance changes, an electrogenic pump and the hyperpolarization of leech neurones following impulses. *J. Physiol. (Lond.).* **229:**635-655.

KUFFLER. S. W., and J. G. NICHOLLS. 1966. The physiology of neuroglial cells. Ergeb. *Physiol. Biol. Chem. Exp. Pharmakol.* **57:**1-90.

MEECH, R. W. 1972. Intracellular calcium injection causes increased potassium conductance in Aplysia nerve cells. *Comp. Biochem. Physiol.* **42A:**493-499.

OBARA. S. 1968. Effects of some organic cations on generator potential of crayfish stretch receptor. *J. Gen. Physiol.* **52:**363-386.

OBARA. S., and H. GRUNDFEST. 1968. Effects of lithium on different membrane components of crayfish stretch receptor neurons. *J. Gen. Physiol.* **51:**635-654.

OTTOSON. D. 1964. The effect of sodium deficiency on the response of the isolated muscle spindle. *J. Physiol. (Lond).* **171:**109-118.

ROBERTS. A., and B. M. H. BUSH. 1971. Coxal muscle receptors in the crab: the receptor current and some properties of the receptor nerve fibres. *J. Exp. Biol.* **54:**515-524.