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# Evidence for $Ca^{2+}$ Control of the Transducer Mechanism in Crayfish Stretch Receptor

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*Summary.* Recording from the dendrite membrane indicated a resting potential of −51.6 mV, which was reduced by inhibition of the $Na^+/K^+$ pump. Voltage clamp at rest revealed a small inward current between −50 and −80 mV and a larger outward current at clamp potentials of −40 to +30 mV. Using ramp-changes of muscle tension as stimuli a time-variant tension-induced inward current (TIC) became apparent, the amplitude of which decreased towards larger depolarizing voltages until at +18 mV the current reversed the direction. The time course of the conductance changes corresponds to similar phases in the generator potential. The outward current only responded to fast reductions in tension, decreasing transiently. A contribution of the active $Na^+/K^+$ pump to the hyperpolarizing potential response is suggested by the effects of K-removal or Na-substitution by $Li^+$. In Na-free choline chloride media the generator potential and the TIC was depressed by 70–85%. Additional removal of $Ca^{2+}$ abolished the TIC. In contrast, lowering the $Ca^{2+}$ level in presence of $Na^+$ decreased the membrane resistance and markedly enhanced the TIC (maximally eightfold at $10^{-5}$ M $Ca^{2+}$) while 75–150 mM $Ca^{2+}$ or intracellular application of a Ca-ionophore had the reverse effect.

In contrast to other stretch receptors the fibers of the receptor muscle in the slowly adapting crayfish stretch receptor run continuously along the whole length of the muscle. In this way all fibers are equally strained by any length changes applied to the mechanical part of the system. The distal portions of the dendrites, which bifurcate in T-fashion between the muscle fibers, are only loosely attached to the latter (Florey & Florey, 1955). It is therefore not surprising that the tension developed by the receptor muscle rather than the actual length change has been shown to be the "stimulus" for the slowly adapting stretch receptor of the crayfish (Chaplain, Michaelis & Coenen, 1971), although another slowly adapting stretch receptor, the frog muscle spindle, definitely uses length changes of the intrafusal

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muscle fibers as an input (Coenen & Chaplain, 1973). The mechanical changes are the cause of the *generator* potential in the terminal region of the dendrites. The electrotonic spread along the larger dendritic branches then affects the membrane potential of the cell soma (Eyzaguirre & Kuffler, 1955*a*, *b*; Terzuolo & Washizu, 1962; Wendler, 1963; Nakajima & Onodera, 1969).

The ionic nature of the transducer mechanism of mechanoreceptors is now generally accepted (*compare* Husmark & Ottoson, 1971*a*, *b*; Loewenstein, 1971). Although regional differentiation of the membrane components has been clearly established for the crayfish stretch receptor (Eyzaguirre & Kuffler, 1955*a*; Loewenstein, Terzuolo & Washizu, 1963; Obara & Grundfest, 1968), the work has so far been limited to the receptor soma where the effects of ionic changes in the medium and the transmembrane current flow as the result of stretch have been studied (Edwards, Terzuolo & Washizu, 1963; Obara & Grundfest, 1968; Klie & Wellhöner, 1973). As the dendrites are the structures responsible for the mechano-electrical transduction process, the electrical changes induced in the dendrite membrane under mechanical stimulation have been reinvestigated.

It has previously been postulated that mechano-electrical transduction involves a rise in ion conductance as the result of a deformation of the sensory membrane (Loewenstein, 1971; Ottoson & Shepherd, 1971). Apart from the mechanical stimuli only $Ca^{2+}$ ions have been shown to exert a major modifying effect on the receptor potential and membrane resistance of two slowly adapting mechanoreceptors, the crayfish stretch receptor and the frog muscle spindle (Edwards *et al.*, 1963; Ottoson, 1965). Therefore, the attempt was made to reinvestigate the role of calcium and membrane deformation on the sodium and potassium permeability in the light of recent findings on receptor function. In this respect it was of particular interest that $Ca^{2+}$ ions, moving upon illumination from the disk membrane to the outer membrane of frog photoreceptors (Hendriks, Daemen & Bonting, 1974), have been postulated to be responsible for the light-induced decrease in sodium permeability of the rod outer membrane (Hagins, 1972).

## Materials and Methods

### *Receptor Preparation*

Slowly adapting stretch receptors have been isolated from the second and third abdominal segments of the American crayfish (*Orconectes virilis*) as described previously (Chaplain *et al.*, 1971). An inverted microscope was used to locate the dendrite tree and to correctly position the microelectrodes. Depending on the physiological state of

the animal in some preparations the connective tissue surrounding the cell body had to be softened with 0.5% pronase (Calbiochem) for 5 min at pH 7.5.

The receptor was immersed in van Harreveldt's (1936) solution containing (in mM) 205 NaCl, 5.4 KCl, 13.5 $CaCl_2$, 2.6 $MgCl_2$ and 2.3 $NaHCO_3$, pH 7.5. In the low-sodium experiments $Na^+$ ions were replaced by choline, while in the low-calcium media $CaCl_2$ was replaced by $MgCl_2$. By inhibiting the axonal spike discharge with $2 \times 10^{-7}$ g/ml tetrodotoxin in the solution (Loewenstein *et al.*, 1963) contributions from impulses travelling backwards into the soma-dendrite region have been excluded. All experiments have been carried out at 16 °C.

### *Experimental Conditions*

The mechanical apparatus and the electronic set-up for voltage clamping were the same as described in previous publications (Chaplain *et al.*, 1971; Kugler & Chaplain, 1974). Therefore, only the technical points essential for the understanding of the present investigation will be repeated.

The ends of the receptor muscle were again fixed onto two pins, one connected to a semiconductor strain gauge foil (WWH 141, Meßelektronik, Dresden), the other to a vibrator. The length changes were detected with a variable-inductance transducer. The vibrator was servocontrolled, the feedback was taken from the tension transducer. All experiments have been controlled by an on-line laboratory computer KRS 4200 (Robotron) interfaced to the data processing system recording the tension, potential and current changes. The interface (SIM 101, Meßelektronik, Dresden) ensures that the digitalized values of the amplified signals are transferred to the core store of the computer. Commands were stored in the computer memory for automatic sequential execution to initiate the tension ramp (always with a velocity of 0.3 mg/msec as controlled from the output of a D-A converter) and either the potential recording or the voltage clamping of the dendrite membrane. The accumulated data, clamp currents and voltage traces (together with the reference tension and the holding potentials), were punched on paper tape in a condensed binary format.

The dendrite membrane, about 80–120 μm away from the dendrite base, was penetrated with a double-barrelled microelectrode, with the tips of 0.3–0.5 μ diameter separated about 20 μm. The current electrodes were filled with a mixture of 0.6 M $K_2SO_4$ – 0.8 M KCl and 50 mM $MgCl_2$ and the potential electrodes with 0.6 M $K_2SO_4$ – 0.8 M $K_3$-citrate – 0.1 M KCl. For the recording nonpolarizable silver pellets (sintered AgCl/AgCl – Pt Black, Annex Instruments, Santa Ana, Calif.) have been used. Capacitative coupling between the two microelectrodes was eliminated by coating the electrode wall to within 500 μm of the tip with silver conducting paint (Silver print 21-1,G.C. Electronics, Rockford, Ill.) insulated by a thin covering layer of polystyrene, shielding the electrodes with some 008 gauge silver wire. To minimize this effect even further a cross-neutralization amplifier (Fairchild 702) with a source follower input (FET 3821, Texas Instruments) was employed.

For the voltage clamping Philbrick-Nexus operational amplifiers were used; the input amplifier being of the type QFT-2 (input resistance $5 \times 10^{11}$ Ω, offset current 22 pA) and the end amplifier the model MLF 100. The current electrode could be connected alternatingly either to the output of the clamp amplifier or for parallel recording of the voltage to a second FET preamplifier. A Philbrick-Nexus 1011 current-voltage converter was used for current recording. The relatively slow changes in muscle length associated with the force ramps (of constant rise-time of 0.3 mg/msec up to the final amplitude) did not displace the intracellular microelectrodes. For the voltage clamp rectangular pulses were used with the actual clamp being complete within 100 μsec. As the current transients were still increasing in magnitude for 5–7 msec after the end

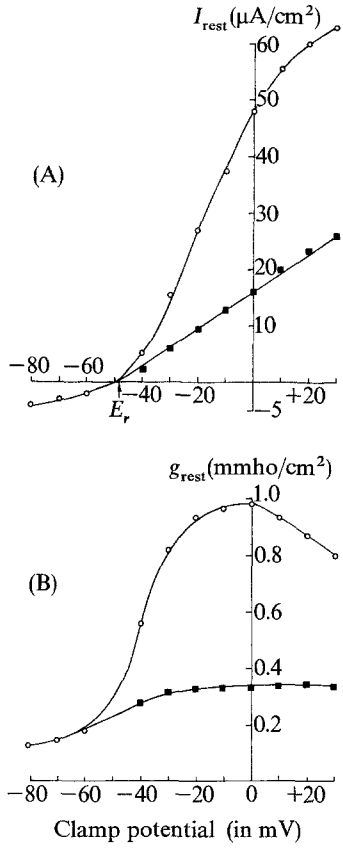


Fig. 1. Current and conductance changes of the resting dendrite membrane under voltage clamp. (*A*) The amplitude of the resting current ($I_{rest}$) *vs.* the clamping voltage ($V$) is plotted at 0.1 mg tension (○). Also included in the illustration are changes in outward current at the end of a force ramp in which the muscle tension was reduced within 4 msec from 2.1 mg to 0.9 mg (■). (*B*) The corresponding cord conductance ($g$) under resting conditions or upon fast reduction of muscle tension, calculated from the currents using the relation $g = I/(V - E_r)$, is plotted as a function of the clamping potential. The same symbols as in *A* have been used

of the tension increase, the first recording of the peak transient current was taken 8 msec after the end of the mechanical stimulus. A true steady-state value of the tension-potentiated current could be obtained after 2 sec. In the illustrations referring to the tension-potentiated inward current the membrane current at resting tension (Fig. 1) has been subtracted. As these currents are associated with identical potential changes, the capacitative currents should cancel out. The current densities have been calculated by dividing the recorded currents by the area of the tip of the recording electrode.

In those experiments in which either $Na^+$ ions or the calcium ionophore A 23187 was iontophoretically injected, a further microelectrode of 1–1.5 μ diameter was inserted into the base of the particular dendrite branch under investigation.

## Results

All results have been recorded from a membrane area about 100 μm away from the base of one of the three large dendrites branching off from the receptor soma. It is assumed that as the result of the electrotonic spread, the large surface area of the dendrite tree is in equilibrium with the terminal ends of the dendrites where mechano-electrical transduction takes place.

### *Transmembrane Potential and Current Flow Across the Dendrite Membrane Under Resting Conditions*

The normal resting potential (recorded as the potential difference between the intracellular electrode impaled into dendrite branch and a recording microelectrode in the solution) was $51.6 \pm 0.17$ mV (mean ±SE, 34 experiments). Upon addition of 1 mM ouabain to the medium or on cooling the preparation to 2 °C the resting potential decreased to −34 mV. Hence, the active $Na^+/K^+$ pump may contribute to the resting potential of the dendrites.

When a patch of the dendrite membrane is voltage-clamped at rest (corresponding per definition to 0.1 mg tension of the receptor muscle), there exists an outward current in response to depolarizing voltages (Fig. 1*A*) and a much smaller inward current at clamp potentials between −50 and −80 mV. The resting conductance increases with the clamp potential up to 0.98 mmho/cm$^2$ at zero potential and falls again at larger depolarizing voltages (Fig. 1*B*).

### *The Transducer Characteristics for Changes in Muscle Tension*

In view of the observation that potential changes of the slowly adapting stretch receptor are more closely related to changes in tension than in length of the receptor muscle (Chaplain *et al.*, 1971), various degrees of muscle tension have been set up experimentally as a receptor stimulus. In this way differences in muscle length between different receptor preparations proved no longer critical.

When a force ramp is applied, the length of the receptor muscle adjusts itself with a slight delay. Increases in tension correspond in this way to a stretch of the receptor muscle, while fast reductions in tension are equal to a release of muscle length.

In Fig. 2*A* the response of the generator potential is shown for a force ramp, increasing the muscle tension from 0.1 to 2.1 mg. The generator potential increases at first and then decays again to a steady value. The

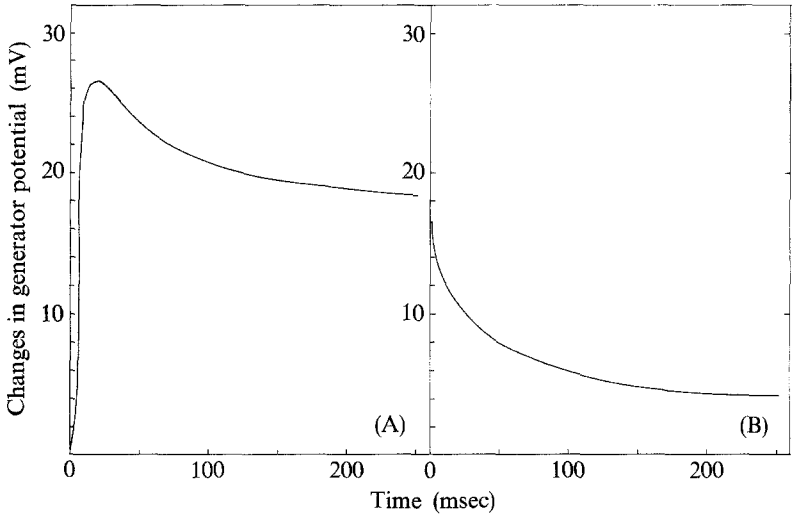


Fig. 2. Changes in membrane potential under force input. (*A*) The muscle tension was increased by a force ramp with a rise-time of 0.3 mg/msec from 0.1 to 2.1 mg tension. (*B*) Time course of the potential changes on reducing the muscle tension from 2.1 to 1.1 mg within 3.4 msec

ratio between the peak and final value of the generator potential has been found to decrease towards larger tension amplitudes as the membrane potential becomes more positive (*see* Fig. 3). For example, the ratio is 2.1 when the tension was increased from 0.1 to 1 mg, but only 1.3 for a 5-mg tension input. The pattern shown in Fig. 2*A* which can be approximated by a proportional-plus-derivative element is also characteristic for the potential changes recorded in the receptor soma under constant tension (Wendler, 1963; Nakajima & Onodera, 1969).

When the muscle tension was decreased from a steady value of 2.1 mg to 1.1 mg the time course of the potential changes is that of a first-order delay (Fig. 2*B*). For larger reductions in muscle tension the observed hyperpolarization was even more marked (*compare* Eyzaguirre & Kuffler, 1955*a*).

Similar differences in the potential response to fast increases or reductions in the intensity of the mechanical stimulus have previously been reported for the frog muscle spindle (Husmark & Ottoson, 1970; Coenen & Chaplain, 1973).

The steady-state values of the generator potential in response to tension increases and reductions have been recorded over the whole range of tensions which can be reversibly set up in a receptor muscle (Fig. 3). For this purpose the tension was first increased in 0.5-mg steps up to 5.5 mg, lowered to

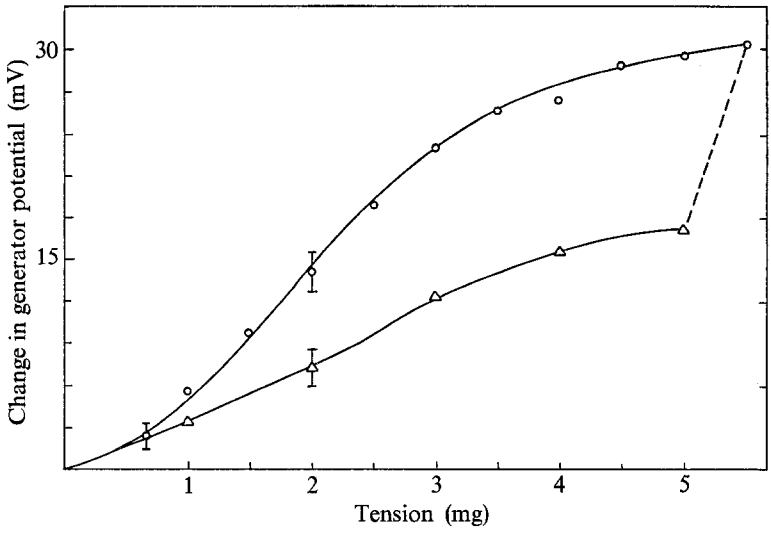


Fig. 3. Stimulus-potential relation under steady muscle tensions. The muscle tension was gradually increased by force ramps (○) lasting 200 msec which gives directly a quasi-steady tension value. Once a 5.5-mg steady muscle tension was obtained the tensions were successively lowered again (△) using release-ramps of 150-msec duration. In these experiments the potential changes have been recorded with a single impaling microelectrode. Error bars averaged from 12 experiments are given in the Figure

5 mg, and then reduced in 1-mg steps. Only for the range of very small potential changes between 0.75 – 1 mg tension was the amplitude of the force steps limited to 0.25 mg. In this way a nonlinear relation between mechanical stimulus and generator potential is obtained which cannot be attributed to any visco-elastic effects of the receptor muscle. Such a non-linear stimulus-potential relation can also be derived for other mechano-receptors such as the Pacinian corpuscle (Gray & Sato, 1953; Loewenstein, 1961) and the frog and the cat muscle spindle (Katz, 1950; Hasan & Houk, 1972).

### *Current and Conductance Changes Induced by Increases in Muscle Tension*

When the dendrite potential was clamped at the resting level and then the tension of the receptor muscle increased from 0.1 to 2.1 mg by a force ramp, a tension-induced inward current became apparent (Fig. 4). With the onset of the mechanical stimulus the tension-potentiated inward current increases rapidly through a transient phase and subsides to a steady value. The time course of the conductance changes corresponded to similar phases in the generator potential. The time-variant behavior of the tension-potentiated inward current (with the inward current at resting tension subtracted)

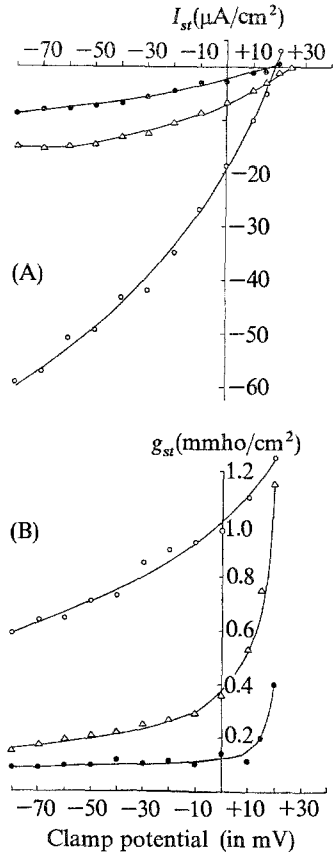


Fig. 4. Tension-induced current and conductance changes on the dendrite membrane. (*A*) The additional tension-potentiated current measured from the resting current ($I_{st}$) is plotted *vs.* the clamping potential. Two time intervals during the response are shown, the peak transients (○) and the steady state (●). The reversal potential $E_r$ remained with +18 mV, the same for both phases. The peak transient of the tension-induced inward current was reduced when the ionophore A 23187 was applied intracellularly by iontophoresis (△). (*B*) The corresponding changes in cord conductance, $g_{st}=I_{st}/(V-E_r)$, have been calculated, using the same symbols as in *A* for illustration

remained unaltered over the whole range of clamping voltages. The curves in Fig. 4 for the peak and final tension-induced inward current differ, on the average, by a factor of about 7. The steady phase of this inward current is maintained as long as the steady tension. When the dendrite membrane was clamped in the hyperpolarizing direction the amplitude of the tension-induced current increased, but decreased progressively towards larger depolarizing voltages. The reversal potential of the tension-potentiated inward current was +18 mV (SD ± 1.2 mV, 11 preparations). Using this value for $E_{str}$ the conductance was calculated as a function of the clamp

potential. The tension-induced conductance change becomes larger as the membrane potential is shifted to more positive values, an effect which is particularly marked under steady-state conditions (Fig. 3). The amplitude of the peak current (as well as the final value after 2 sec) increases with the magnitude of the force step, while the reversal potential of the tension-potentiated inward current remained unaltered. When the dendrite membrane was clamped near the resting potential, the respective "instantaneous" conductance values were 0.72 mmho/cm$^2$ at 2.1 mg tension, 2.6 mmho/cm$^2$ at 3.1 mg and 4.3 mmho/cm$^2$ at 5.1 mg with the ratio between peak transient current and steady value decreasing from 7.5 to 4.7 towards the greater tension amplitudes.

Evidence for a stretch-induced inward current has previously been obtained when the soma membrane of the slowly adapting stretch receptor was clamped near the resting potential (Klie & Wellhöner, 1973). Here the peak amplitude of the inward current following a 40% stretch was 8.1 times the value recorded after 3 sec. Further, a reversal potential of + 25 mV (SD ± 14 mV) has been deduced by these authors.

Similar conductance changes induced by light flashes and a positive reversal potential have been reported also for photoreceptors from *Balanus* (Brown, Hagiwara, Koike & Meech, 1970) and *Limulus* (Millecchia & Mauro, 1969).

In contrast to the inward current the outward current crossing the dendrite membrane under voltage-clamp conditions remained unaffected when the muscle tension was increased. However, it is of considerable interest that the outward current was reduced transiently when the muscle tension was lowered by a fast ramp input (Fig. 1). The outward current returned to its normal level recorded at resting tension (or for that matter at any increased muscle tension) within a period of 150 msec. The transient conductance decrease was particularly effective over a range of voltages covered by the generator potential changes in the normal working range of the stretch receptor (Figs. 1 and 2). The fact that a reduction in the tension and hence in the length of the receptor muscle brings about a strong inhibition of the outward current flow (in addition to the reduction in inward current) may well bear an important relation to the physiological function of the stretch receptor. In this way all electrical activity will be depressed within a minimum of time.

### *Low Sodium and High Potassium Media*

When sodium was replaced by choline and the receptor equilibrated in this solution for 5 min the receptor potential increased by about 8 mV.

There was a 20% decrease in the peak amplitude of the generator potential and, on the average, a 36% reduction in the steady value. In contrast, both the peak and final values of the tension-potentiated current were depressed rather strongly, being 13–16% of the normal values. At the same time the reversal potential was only +9 mV instead of the +18 mV in van Harreveldt's (1936) solution (Fig. 4). The conductance changes deduced from the peak current transients in Na-free solution for a 2 mg force ramp now closely resembled in magnitude those of the steady-state conductances in the normal medium (Fig. 4). When the $Na^+$ concentration was successively lowered it could be shown that the removal of $Na^+$ ions from the medium caused a graded response. For example, at an external $Na^+$ level of 100 mM the tension-potentiated peak current transient was 76% of that in the normal medium, the reversal potential being +14 mV. Reductions in the external $Na^+$ concentration had essentially no effects on the outward current.

A substitution of the $Na^+$ ions by $Li^+$ or $K^+$, or removal of $K^+$ from the medium, resulted in a depolarization of the resting potential to between −30 mV and −4 mV; this also slowed the time course and reduced the extent of the hyperpolarizing response (for the $Li^+$ effect *compare* Obara & Grundfest, 1968). In Na-free solution there was still a detectable tension-potentiated inward current which was about one-tenth that in the normal medium. However, when the $Ca^{2+}$ in the medium was additionally replaced by $Mg^{2+}$ the inward current was completely depressed. All these changes could be partially reversed by iontophoretic injection of $Na^+$ ions into the base of the dendrite. The results of changes in the ion composition of the medium are consistent with the view that $Na^+$ ions are the major ion species required for the generator potential and as a current carrier for the tension-potentiated inward current (*compare also* Edwards *et al.*, 1963; Ottoson, 1964; Obara & Grundfest, 1968).

Similar effects of sodium substitution on the light-induced potential and current changes have been reported for photoreceptors from *Limulus* (Millecchia & Mauro, 1969), *Balanus* (Brown *et al.*, 1970) and the honeybee (Fulpius & Baumann, 1969). Marked effects of $Na^+$ removal on the static response of the frog muscle spindle and to a lesser extent on the dynamic phase, and of the external $K^+$ level on the adaptive decline of the receptor potential have also been noted by Husmark and Ottoson (1971*b*).

### *Effects of Calcium on the Potential and Current Changes*

When the $Ca^{2+}$ concentration in van Harreveldt's solution was varied from the normal level of 13.5 mM, the resting potential (*RP*) decreased

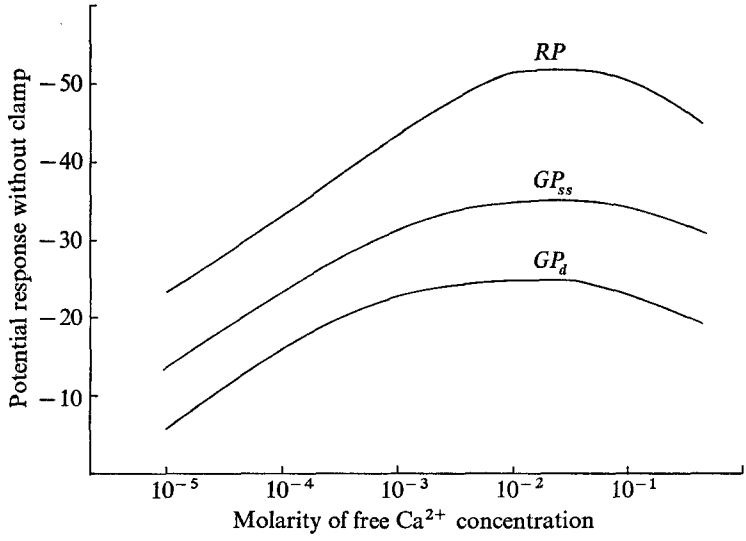


Fig. 5. Variations in resting and generator potential as a function of the external $Ca^{2+}$ concentration. The membrane potential (*RP*), as well as the dynamic phase ($GP_d$) and the static phase ($GP_{ss}$) of the generator potential for a 2-mg tension ramp, have been recorded at the $Ca^{2+}$ levels indicated in the Figure. The curves have been computer-averaged from 114 experiments

towards lower $Ca^{2+}$ levels and also when the $Ca^{2+}$ concentration was increased above 75 mM (Fig. 5). The same effect is also shown by the generator potential both under steady-state conditions ($GP_{ss}$) and for the maximal dynamic changes ($GP_d$) recorded immediately at the end of the force ramp (*compare* Fig. 2). A similar effect of the external $Ca^{2+}$ level on the generator potential recorded from the cell soma has previously been reported for the crayfish stretch receptor (Edwards *et al.*, 1963).

When 1 mM ouabain was present in the medium the potential changes shown in Fig. 5 for reductions in the $Ca^{2+}$ level were still apparent. However, the resting potential, which in presence of 1 mM ouabain was lowered to − 34 mV, could not be reduced any further on increasing the $Ca^{2+}$ concentration from 75–150 mM. This was also true when the preparation was cooled to 2 °C. Hence, the effect of high $Ca^{2+}$ ions may be due to an inhibition of the active ion pump (Dunham & Glynn, 1961; Carpenter & Alving, 1968). The active $Na^+/K^+$ pump, which transports against an electrochemical gradient of $K^+$ ions into the cell coupled to an active efflux of $Na^+$ ions, appears to be involved in the maintenance of the resting membrane potential of the dendrites (*see above*). Further, the outward current at resting tension was increased in amplitude by 10–35% as the $Ca^{2+}$ level in the medium was lowered to 1 mM or even $10^{-5}$ M.

When the effect of calcium on the tension-potentiated currents was investigated, the current transients at the clamped resting potential increased about 2.2-fold at 1 mM $Ca^{2+}$ and 7.8-fold at $10^{-5}$ M $Ca^{2+}$. At the same time there was a slight negative shift of the reversal potential, which was +16 mV at 1 mM $Ca^{2+}$ and +12 mV at $10^{-5}$ M $Ca^{2+}$. The evaluated conductance values for holding potentials of −10 to +20 mV were increased between 1.6- and 4.2-fold above the values in the normal solution (Fig. 4).

An increase in the $Ca^{2+}$ level above 75 mM had the opposite effect. Not only did the resting potential become less negative and the changes in generator potential for a given increase in muscle tension actually decrease (Fig. 5), but the tension-potentiated peak inward current was now reduced on average by about 15–24%.

An evaluation of the effective membrane resistance from the slopes of the current-voltage relations at membrane potentials more negative than the resting potential gave the following values (in MΩ): 0.45 at $10^{-5}$ M $Ca^{2+}$, 1.18 at 13.5 mM $Ca^{2+}$, and 3.34 at 100 mM $Ca^{2+}$.

For the crayfish stretch receptor a decrease of the membrane resistance in the cell soma has previously been noted upon removal of $Ca^{2+}$ from the medium (Edwards *et al.*, 1963). These changes in membrane resistance seem to be genuine $Ca^{2+}$ effects as Yamagishi and Grundfest (1971) observed an increase in membrane resistance together with a depolarization by $Ca^{2+}$ ions in the medium, although $Na^+$, $K^+$ and $Cl^-$ ions had been absent from the solution. The latter authors therefore deduced that in the crayfish stretch receptor $Ca^{2+}$ ions decrease the conductance of the membrane. A $Ca^{2+}$-mediated suppression of the sodium permeability has also been postulated for the photoreceptor systems of the barnacle (Brown *et al.*, 1970), the honeybee (Fulpius & Baumann, 1969) and the frog rod outer membrane (Hagins, 1972).

*Reduction of the Inward Current by a Calcium Ionophore*

To test whether the liberation of $Ca^{2+}$ from intracellular binding sites was involved in the suppression of the inward (or sodium) current, in a way similar to that in rod outer segments (Hendriks *et al.*, 1974), the ionophore A 23187 was injected into the dendrite. The low-molecular weight ionophore A 23187 (Eli Lilly) has been shown to increase effectively the permeability of biological membranes to $Ca^{2+}$ ions (*cf.* Case, Vanderkooi & Scarpa, 1974). In this way the ionophore transports $Ca^{2+}$ ions into mitochondria, releases $Ca^{2+}$ from the sarcoplasmic reticulum and mimics the $Ca^{2+}$-mediated hormonal induction of enzyme secretion (Eimerl, Savion, Heichal & Selinger, 1974).

A concentration of approximately 10 μM A 23187 was applied by microiontophoresis into the base of one of the large dendrites. After an equilibration period of 5 min the proximal membrane region of this dendrite was voltage clamped. For the same tension change of the receptor muscle investigated in Figs. 2 and 4 the inward current at both hyperpolarizing and depolarizing voltages is now considerably decreased (*see* triangles in Fig. 4). A shift in the reversal potential to + 25 mV was noted. The general pattern of the conductance changes remains similar to that of the normal tension-induced inward current, with conductance values at the highest depolarizing voltages still approaching those of the peak current transients in the absence of the ionophore.

Case and co-workers (1974) report that the ionophore-mediated movement of $Ca^{2+}$ across bilayer membranes is essentially an electroneutral process. The possibility that a cationic form of A 23187 would carry some current across the dendrite membrane appears unlikely as the outward current after injection of the ionophore was even depressed by about 10–15%.

The calcium ionophore apparently exerts an indirect effect on the tension-potentiated inward sodium current. The small inward (calcium) current remaining in a sodium-free medium remained essentially unaffected by the intracellular application of the ionophore.

No systematic studies on the effect of extracellular additions of the ionophore have been carried out as this affected the mechanical properties of the receptor muscle.

## Discussion

The electrophysiological experiments on the dendrite membrane of the slowly adapting crayfish stretch receptor have provided a number of important results:

1. There exists a nonlinear relation between the intensity of the mechanical stimulus and the generator potential.

2. When the tension of the receptor muscle is increased by a force ramp the generator potential increases to a maximum and then subsides to a steady value, with the potential changes closely similar to that of a proportional-plus-derivate element.

3. For fast reductions in muscle tension the hyperpolarizing response of the generator potential can be approximated by an exponential lag system.

4. Under voltage clamp a ramplike increase of muscle tension induces an inward current across the dendrite membrane with the time course of

the conductance changes similar to that of the generator potential under unclamped conditions.

5. The outward current apparent in the resting dendrite membrane for depolarizing voltages (which remains unaffected by increases in muscle tension) is transiently reduced upon lowering the tension load on the receptor muscle.

Variations in the ion composition of the medium indicate that $Na^+$ ions are the major ion species required for the development of the generator potential and as a current carrier for the tension-potentiated inward current. The finding that the reversal potential of the tension-induced inward current in Na-free solution is only shifted by 9 mV (instead of a 58-mV shift predicted for a 10-fold change in external sodium if this ion species were the sole current carrier) together with the demonstration of a small Ca-dependent tension-potentiated inward current, indicates the existence of a Ca-current, similar to crustacean muscle (Werman & Grundfest, 1961), Purkinje fibers (Reuter, 1967) and *Aplysia* neurons (Geduldig & Junge, 1968).

As indicated by the effect of ouabain, cooling and substitution of external $Na^+$ for $Li^+$ ions [all known to inhibit the active ion pump in the crayfish stretch receptor (Sokolove & Cooke, 1971)], the $Na^+/K^+$ pump seems to insure that the resting potential of the crayfish stretch receptor is more negative than the potassium equilibrium potential (or the equilibrium potential of any other dominant ion species). Following changes in the tension (or length) of the receptor muscle, the mechanical distortion of the terminal dendrite membrane leads to dynamic changes in sodium conductance. As a result the membrane potential becomes more positive than the potassium equilibrium potential which accounts for the maxima of the dynamic phase in the generator potential (Fig. 2) and the inward current transients (Fig. 4). The slow activation of the outward current towards more positive membrane potentials (Fig. 1) results in an increase of the respective ion conductance (most probably for intracellular $K^+$ ions) with the dendrite membrane potential shifted again into the negative direction (Fig. 2). The continuing inward flow of $Na^+$ ions (which is still high as long as the muscle tension is maintained, Fig. 4) then activates the $Na^+/K^+$ pump which leads to a further hyperpolarization of the dendrite membrane. Consistent with the postulate of a Na-dependent stimulation of the ion pump is the observation that intracellular iontophoretic injection of $Na^+$ ions partially reverse the effects of a low-sodium (high $Li^+$ or high $K^+$) medium. In this way the ion pump enhances the phenomenon of transducer adaption

apparent in the decline of the generator potential (*compare also* Nakajima & Onodera, 1967).

Whenever the muscle tension is lowered again, which during normal receptor operation would be the case for a release of the previously extended muscle, the potassium conductance undergoes a transient reduction (Fig. 1). In consequence there is a hyperpolarization of the membrane potential (Fig. 2 and Eyzaguirre & Kuffler, 1955*a*). The additional involvement of the active ion pump is suggested from two findings. First, the membrane potential decreases actually below the potassium equilibrium potential, an effect which is reversed by ouabain or upon cooling the receptor, and further, substitution of external $Na^+$ by $Li^+$ (which fails to activate the ion pump) or removal of $K^+$ ions greatly reduces the hyperpolarizing response on release of muscle stretch (*see also* Obara & Grundfest, 1968). The fact that the conductance of the outward current decreases towards more negative potentials (which are generated initially as the result of a transient potassium inactivation and in a delayed fashion by the operation of the ion pump) results in an exponential decrease of the generator potential (Fig. 2).

Although the described changes in sodium and potassium conductance account for the observed potential and current changes inherent in the general mechanism of *mechano-electrical transduction*, these reactions would have to be under direct control by calcium to explain the effects of $Ca^{2+}$ ions in the medium and by an intracellular calcium ionophore (Figs. 4 and 5). The working hypothesis is that initially a *mechano-chemical transduction* process takes place governing the extent of $Ca^{2+}$ binding to the membrane (the effect of an intracellular ionophore suggests that this may be centered on the lipid component) which, in the subsequent *chemo-electrical transduction* step, controls the ion permeability of the dendrite membrane to monovalent ions.

Previous experiments on perfused squid axons have shown that in presence of $Na^+$ ions external calcium can stabilize (or re-induce) the resting state of the membrane which can be depolarized by the addition of $K^+$ ions (Tasaki, 1968; Inoue, Kobatake & Tasaki, 1973). Consistent with this inhibitory action of $Ca^{2+}$ is the marked increase in sodium and potassium conductance of squid axons in calcium-free solution (Frankenhaeuser & Hodgkin, 1957) and the Ca-dependent reduction of both ion conductances in myelinated nerve (Moore, 1971).

Similar modifying effects of $Ca^{2+}$ ions on the dendrite membrane, for example by suppressing the sodium permeability, would be consistent with earlier proposals for the crayfish stretch receptor (Yamagischi & Grundfest,

1971) and various photoreceptor systems (Fulpius & Baumann, 1969; Brown *et al.*, 1970; Hagins, 1972).

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