Japanese Journal of Physiology, 32, 69-81, 1982

Rapid Pressure Changes and Surface Displacements in
the Squid Giant Axon Associated with Production
of Action Potentials

Ichiji TASAKI and Kunihiko IWASA

Laboratory of Neurobiology, National Institute of Mental Health, Bethesda,
Maryland 20205, U.S.A. and Marine Biological Laboratory,
Woods Hole, Massachusetts 02543, U.S.A.

Abstract By using both optical and mechano-electric detectors, we have shown that the squid giant axon swells when an action potential is gen-erated. The maximum swelling is reached at the peak of the action potential. The undershoot of the membrane potential is associated with a marked shrinkage of the axon. We have also demonstrated these mechanical changes in axons from which a major portion of the axoplasm has been removed. We have examined the effects of changing the tonicity of the external medium and of applying several chemical re-
agents.
Key Words: nerve excitation, pressure changes, surface displacements, axolemma-ectoplasm complex.

More than half a century ago, LOEB (1906), EMBER (1920), and BETHE (1920) proposed that the superficial layer of the nerve fiber undergoes rapid structural changes during excitation. They argued that the process of excitation involves exchanges between Ca ions and univalent cations in the superficial layer and that such cation-exchanges bring about swelling and shrinkage of the structure. More recently, TEORELL (1962) predicted swelling of the nerve fiber during excitation based on his investigation of a nerve analog which involves water fluxes. How-ever, these predictions about mechanical changes in the nerve fiber had been ignored in the recent literature of electrophysiology, because no reliable experi-mental support had been reported (SANDLIN et al., 1968; COHEN, 1973). Recently, however, we found that the production of an action potential is indeed accompanied by rapid mechanical changes in the nerve fiber (TASAKI and IWASA, 1980; IWASA and TASAKI, 1980; IWASA et al., 1980). In our preliminary report, we emphasized that the first phase of the observed mechanical signal is in fact swelling of the nerve fiber, and not a decrease of the fibre diameter as reported by HILL et al. (1977). We have also pointed out that the old reports about me-

Received for publication June 24, 1981

69

70 I. TASAKI and K. IWASA

chanical responses of nerve fibers (HILL, 1950; BRYANT and TOBIAS, 1955) deal only with slow, cumulative effects of prolonged repetitive stimulation.
In this paper, we describe general properties of the mechanical changes in the squid giant axon measured by using both a mechano-electric transducer and an optical sensor. We show that the production of an action potential is accompanied by an outward movement of the nerve membrane surface generated by a rise in pressure within the axon. By modifying the connective tissue surrounding the axon, and also by removing the inner part of the axoplasm, we demonstrate that the mechanical changes are generated in a region which consists of axolemma and ectoplasm. The mechanical responses observed are sensitive to changes in the Ca ion concentration and in the tonicity of the external medium. The importance of water molecules in the process of nerve excitation is emphasized.

MATERIALS AND METHODS

Giant axons were taken from the squid, Loligo pealei, available at the Marine Biological Laboratory, Woods Hole, Mass. An approximately 5 mm long portion of the axon was meticulously cleaned by removing all small fibers and adherent tissues under a dissecting microscope provided with dark-field illumina-tion. The giant axon was then introduced into a nerve chamber with a convex bottom and was kept immobilized by applying mild tension (see Fig. 1). The axon was excited by electric current pulses of 10 to 30 sec in duration applied with a pair of platinum electrodes on one side of the cleaned portion. The effec-tive cathode of the stimulating circuit was located at about 5 mm from the me-chanical recording site. The action potential of the axon was recorded with a pair of platinum electrodes located on the other side at about 5 mm from the mechanical recording site.

Fig. 1. Experimental set-ups employed for measuring mechanical responses of the squid giant axon. A: schematic diagram of the set-up for recording pressure changes. A piezo-ceramic bender was used for converting pressure changes into voltage changes.
El and E2 represent extracellular platinum electrodes. B: diagram of the set-up for recording surface displacements of the axon. The light from a source, L, was trans-
mitted to a gold leaf, G, placed on the axon. The light scattered and reflected by the gold leaf was carried to a photodetector, D.

Japanese Journal of Physiology

RAPID MECHANICAL CHANGES IN SQUID AXON 71

For measurements of rapid changes in the pressure developed by the giant
axon, bimorph piezoelectric tansducers (piezo-ceramic benders, Gulton Industries,
New Jersey) were employed. The piezo-electric elements of the bender are made
of lead zirconium titanate. The bender used (RO505 Element, G1195 material)
was about 1.5 mm in width, 12 mm in length and approximately 0.5 mm in thick-
ness ; it had a capacity of 0.001 ƒÊF and a very high resistance. Using two layers
of hard rubber, the bender was held rigidly in the middle. Pieces of fine platinum
wire, usually 50 ƒÊm in diameter, were soldered to the nickel-plated electrodes of
the bender. To transmit changes in the pressure of the axons to the bender, a
plastic rod, 0.5 mm in diameter and 10 mm in length, was attached perpendicu-
larly near the free end of the bender (see Fig. 1A). The voltage developed by
the bender was measured by the use of a voltage-follower constructed with an
operational amplifier (515, Analog Devices). The sensitivity of the bender was
between 0.5 and 1.0 V/g. The output of the voltage follower was connected to
a signal averager (Model SW71-B, Nicolet Instrument Corp., Madison, Wisc.)
after amplification by a factor of 1,000 by using a low-noise preamplifier (Model
122, Tektronix, Portland, Oreg.). The amplitude of the random noise in the
frequency range between 8 and 10,000 Hz was usually of the order of 30 mV,
peak-to-peak, at the output of the preamplifier, and is dependent on the acoustical
noise level of the room.

To measure small displacements of the axon surface, an optical sensor probe
(Fotonic sensor KDP 032R, Mechanical Technology, Inc., Latham, N.Y.) was
employed. This device consists of two bundles of optical fibers mixed evenly at
one, i.e., sensing, end (see Fig. 1B). One of the two bundles was used for carrying
white light from a source, a 100-W Osram quartz iodine lamp, to the sensing
end. The other bundle was utilized to transmit light from the sensing end to a
photodetector (Pin-l0, United Detector Technology, Inc., Culver City, Cal.).
In order to increase the intensity of light reflected or scattered at the axon surface,
a small piece of gold leaf (100-300 ƒÊm in diameter), made from gold dust flattened
by using a mortar and pestle, was placed on the axon. The diameter of the sensing
end of the sensor was roughly 0.5 mm. The output of the photodetector was
led to the signal averager through a Tektronix preamplifier operated at a gain of
1,000 in the frequency range of 8 Hz to 10 kHz. With this device, the intensity
of the photocurrent detected strongly depends on the distance between the gold
leaf on the axon surface and the sensing end of the sensor. An example of cali-
bration curves is given in an earlier report (TASAKI et al., 1980).
The artificial seawater used was made according to the MBL formula, con-
taining 423 mm NaCl, 9 mm KCl, 9.27 mm CaCl2, 22.94 mm MgCl2, 25.5 mm
MgSO4, and 10 mm Tris-HCl buffer (pH 8.1). The potassium salt solution used
for internal perfusion of axons was prepared by mixing 36 ml of a 600 mm KF
solution, 20 ml of a 12 % glycerol, and 4 ml of a 600 mEq/liter potassium phos-
phate buffer (pH 7.3). Trypsin (type III), colchicine and ouabain were purchased

Vol.32, No.1, 1982

72 I. TASAKI and K. IWASA

from Sigma Chemicals, St. Louis, Mo. Pronase and cytochalasin D were obtained
from Calbiochem, La Jolla, Cal. Phalloidine was purchased from Boehringer
Mannheim.

RESULTS

1. Rise and fall of pressure during action potential
The experimental set-up used for measuring pressure changes in a squid giant
axon during action potentials is illustrated schematically in diagram A in Fig. 1.
A cleaned portion of the axon in the nerve chamber was brought directly beneath
the tip of the stylus attached to a piezo-ceramic bender. Next, the bender was
slowly lowered until the tip was brought into gentle contact with the axon surface.
Taking action potentials arriving at one end of the axon as an index, brief pulses
of stimulating current were delivered to the axon with a pair of electrodes near
the other end of the axon. Associated with a propagated action potential, a
mechanical response of relatively small amplitude could be observed under these
conditions (see the top record in Fig. 2A).
As the piezo-ceramic bender was lowered further, there was a gradual increase
in the amplitude of the mechanical response observed. In axons with a diameter
of about 400 ƒÊm, the amplitude of the response was found to reach a more or less
constant level when the stylus tip was between 100 and 200 ƒÊm below the point
of initial contact (Fig. 2A). A further lowering of the stylus tip could result in
irreversible injury of the axon.
As has been pointed out in our preliminary report (IWASA and TASAKI, 1980),
the mechanical response of the axon detected with a piezo-ceramic bender was
always diphasic. The first phase of the response represents a rise in the pressure

Fig. 2. A: rapid pressure changes associated with the action potential of the squid giant
axon. The piezo-ceramic bender was lowered stepwise by the distance indicated from
the point of contact. Upward deflections of the signal-averager trace correspond to a
rise in pressure. Temperature, 23•Ž. B: comparison of surface displacements (a)
associated with the action potential with pressure changes (b). Record c represents the
extracellularly recorded action potential. Temperature, 23•Ž.

Japanese Journal of Physiology

RAPID MECHANICAL CHANGES IN SQUID AXON 73

exerted by the axon upon the stylus. The duration of the first phase is close to
that of the depolarizing phase of the action potential (see later). The second
phase represents the period during which the observed pressure is below the initial
level. In axons immersed in normal seawater, the amplitude of the second phase
is much larger than that of the first phase. We found that the second phase de-
cays slowly in a manner roughly analogous to the hyperpolarizing after-potential
of the intracellularly recorded action potential (see later). During the course of
a gradual recovery of the pressure, however, we regularly encountered a small
sign of abrupt shrinkage of the axon (see Fig. 4). In some instances, there were
signs of mechanical resonance of the recording system at a frequency around
2 kHz (see Fig. 2B, b).
In the majority of axons examined, the force developed by the axon at the
peak of the first phase of the mechanical response was in the range between 3
and 10 ƒÊg. Since the area of contact between the stylus and the axon was of the
order of 1.5 •~ 10-3 cm2, the pressure developed by the axon is then of the order
of 2 to 6 dyn/cm2.

2. Displacement of the axon surface during action potential
The experimental set-up used for detecting rapid movements of the axon
surface during action potentials is illustrated schematically in Fig. 1B. In re-
sponse to suprathreshold stimulating pulses applied near one end of the axon,
a diphasic movement of the axon surface could be observed with this set-up (see
Fig. 2B, a). Neither the amplitude nor the time course of the response was
affected by switching the site of electric stimulation from the proximal side of
the axon to the distal side, or by making two action potentials collide at the site
of mechanical recording (Fig. 3). This observation strongly suggests that the
flow of electric current associated with propagation of an action potential (i.e.,
local current) plays little role, if any, in the generation of movements of the axon

Fig. 3. Records of surface displacements induced by three different modes of stimulation
of an axon. The stimulating current pulses were delivered through electrodes E1,
through both E1 and E2, and through E2 alone. Note that collision of two action
potentials at the site of recording shortens the latency slightly. The bottom trace re-
presents an externally recorded electric response (stimulated at E2 and recorded at E1).
Temperature, 23•Ž.

Vol.32, No.1, 1982

74 I. TASAKI and K. IWASA

surface.
The mechanical response observed by this method was diphasic and had a

time course similar to that of the pressure changes . The first phase of the move-
ment of the axon surface was outwardly directed ; the magnitude of the first phase
was in the range between 0.5 and 1 nm. The amplitude of the second phase
,

representing shrinkage of the axon, was usually 1-4 times as large as that of the
first phase.
It is interesting to compare the record obtained with this optical device and

that taken from the same site using a piezo-ceramic bender. Figure 2B shows
an example of the results of such a comparison. By placing a piece of gold leaf
on an axon, we first obtained a record of axon surface displacement . Next, we
removed the Fotonic sensor and brought the stylus of a piezo-electric bender in
contact with the gold leaf. Finally, we lowered the bender by about 120
,am b

elow the point of contact and, without changing the stimulus strength , we took
a record of pressure changes.
The results thus obtained indicated that the latency of the pressure changes
was slightly shorter than that of the surface movement. The difference in latency
was variable, ranging between 0.1 and 0.35 msec. This difference probably
arises from the situation that the gold leaf used for optical recording was lying on

the surface of the connective tissue outside the axon, while the mechano-electric
transducer was firmly pressing the axon. The layer intervening between the
axolemma and the surface of the connective tissue appears to have produced a
small but variable delay in the movement of the gold leaf

.

3. Comparison between mechanical and electrical responses
To compare the time course of a mechanical response with that of the in-
tracellularly recorded action potential, the experimental set-up illustrated in
Fig. 4, top, was employed. The internal electrode used was an enameled silver
wire, about 50 ,am in diameter, with its 100-ƒÊm long tip kept uninsulated. The
separation between the stylus of the piezo-ceramic bender and the intracellular
electrode was about 0.5 mm. Action potentials and mechanical responses were
recorded alternately without changing the stimulus strength.
An example of the records obtained by this procedure is furnished in Fig. 4.
It is seen that the rising phase of the action potential coincides with the phase of
pressure rise fairly accurately. Similarly, the phase of pressure fall is seen to
proceed simultaneously as the falling phase of the action potential. Within the
limit of time resolution of the present measurements (approx. 50 ƒÊsec), the peak
of the positive pressure was found to coincide with the peak of the action potential.
Interestingly, there was a large difference between the mechanical and electrical
responses in the ratio of the amplitude of the first (swelling/depolarizing) phase
to that of the second (shrinkage/hyperpolarizing) phase.

Japanese Journal of Physiology

RAPID MECHANICAL CHANGES IN SQUID AXON 75

Fig. 4. Top: schematic diagram of the set-up used for demonstrating the degree of timing coincidence between the mechanical and electrical responses of the axon. Ei represents
the electrode for recording the action potential internally. E5, stimulating electrodes.
Bottom: an example of the records obtained by using the set-up illustrated above.
Temperature, 23•Ž.

4. Effect of changing the tonicity of external medium
When the artificial seawater outside an axon is diluted with distilled water,
the hydrostatic pressure inside the axon is expected to rise and the membrane macromolecules to swell. A rapid, irreversible suppression of excitability takes place when a fraction of about 30 % or more of the external seawater was replaced with distilled water (cf. PICHON and TREHERNE, 1976). The effect of a rise in the intracellular pressure on the mechanical response of the axon was examined by using the experimental set-up shown in Fig. 1A.
Figure 5A shows an example of the results showing the effect of reducing the tonicity of the external medium. Initially, the axon under study was immersed in normal seawater. Next, a mixture of one part distilled water and two parts artificial seawater was introduced into the nerve chamber without removing the stylus pressing the axon. After this replacement, the amplitude of the first (i.e., swelling) phase of the mechanical response did not show a significant change. In contrast, there was a marked decrease in the size of the second (shrinkage) phase. This kind of asymmetric amplitude change in swelling and shrinkage was not noticed while lowering the stylus (see Fig. 2A). Under these hypotonic con-ditions, a close similarity in time course of the mechanical response to the in-ternally recorded action potential, as given in Fig. 4, was maintained. When normal seawater was re-introduced into the chamber, there was a sudden decrease in the amplitude of the recorded mechanical response.
Replacement of the normal seawater outside with a hypertonic solution pre-

Vol.32, No.1, 1982

76 I. TASAKI and K. IWASA

A B

Fig. 5. Effects of lowering (A) and raising (B) the tonicity of the surrounding medium on
the mechanical responses of the squid giant axons. The change in the tonicity was
roughly 30%. The filled circles represent the peak values of pressure rises

. The
empty circles show the peak values of pressure fall. Temperature

, 23•Ž.

pared by addition of sucrose is expected to lower the pressure inside the axon.
Such a replacement invariably reduced the amplitude of the mechanical response
(Fig. 5B). The observed reduction must be attributed to the fall in intracellular
pressure.

Addition of urea to the external seawater outside an axon is expected to
produce only a transient fall in the pressure inside, because the axon membrane
is highly permeable to urea (see JOHANSSON and JONSSON, 1968). To demonstrate
this expected effect, urea-seawater was prepared by adding urea at a concentration
of 0.33 mol/liter. When this solution was introduced into the nerve chamber
,

there was a rapid decrease in the amplitude of the mechanical response. During
the course of 10-20 min, however, there was a gradual recovery. When the
external urea-seawater was replaced with normal seawater after a period of 40-
60 min, there was a sudden increase in the amplitude of the mechanical response.
The amplitudes observed under these conditions were 3-10 times as large as that
seen at the onset of the experiment. In the following period, there was a rapid
decline in the response amplitude, accompanied by deterioration of the action
potential. A sudden rise in the intracellular pressure is considered the cause of
this injurious effect of the removal of urea.

5. Effect of Ca ions in the external medium
Partial replacement of the external seawater with an isotonic (330 mm) Ca
salt solution was found to exert a profound influence on the mechanical response
of the squid giant axon. When the external Ca ion concentration was increased

Japanese Journal of Physiology

RAPID MECHANICAL CHANGES IN SQUID AXON 77

Fig. 6. Effects of raising the external Ca ion concentration on the mechanical responses.
Record a was taken from an axon immersed in normal artificial seawater. Records
b, c, and d were taken 2, 8, and 10 min, respectively, after replacing normal seawater
with a Ca-rich solution (containing 220 mM NaCl, 200 mM CaCl2, and 20 mM Tris-HCl
at pH 8.1). Temperature, 23•Ž.

to 100-200 mM, there was an immediate decrease in the amplitude of the first
(swelling) phase of the response (see Fig. 6). The amplitude of the second
(shrinkage) phase was slightly enhanced in some preparations. Following this
initial change, there was a gradual decrease in the amplitude of the response.
This effect of raising the external Ca ion concentration was partially reversible.
The effect of lowering of the external Ca ion concentration was examined by
adding an isotonic (530 mM) NaCl solution to the external seawater. This pro-
cedure brought about, in most axons examined, a slight enhancement of the first
(swelling) phase of the mechanical response. Lowering the external divalent
cation concentration to a level below about 10 mM evoked, as expected, repetitive
firing of action potentials. The properties of the mechanical responses associated
with repetitively fired action potentials will be discussed elsewhere.

6. Effect of removal of endoplasm
Using the technique of intracellular perfusion preceded by mild protease
treatment, it is possible to remove almost all of the endoplasm of an axon without
suppressing the excitability (TASAKI, 1968). As long as a thin (less than 1 ,ƒÊm
in thickness) layer of ectoplasm is left intact, the action potentials developed by
the axon remain practically unaltered (METUZALS and TASAKI, 1978). Mechanical
responses were examined using such axons devoid of endoplasm.
Initially, a 10 mm long portion of an axon was internally perfused for about
2 min with a potassium salt solution (see METHODS) to which pronase was added
at a level of 0.05-0.1 mg/ml. Next, the pronase inside was washed out with the
standard perfusion fluid for a period of 20-40 min. An example of the mechanical
responses recorded from such axons using a piezo-ceramic bender is furnished in
Fig. 7A. It is seen that axons devoid of endoplasm are capable of producing
mechanical responses and also that the amplitudes of these responses are not

Vol.32, No.1, 1982

78 I. TASAKI and K. IWASA

A B

Fig. 7. Mechanical responses recorded from axons devoid of endoplasm. The axons used
were internally perfused first with a KF solution containing 0.05 mg/ml pronase for
2 min, then with a pronase-free KF solution for 20 min. Temperature, 22-23•Ž.

very different from those observed in intact axons. However, the rate of rise and
fall of the pressure developed by such axons was found to be considerably lower
than those in intact axons. Probably this change in the time course of the me-
chanical responses is produced by the loss of rigidity of the axon resulting from
the removal of the axoplasm. Similar results were obtained by using a Fotonic
sensor in place of a piezo-ceramic bender (Fig. 7B).

7. Effects of several biochemical and pharmacological agents
To examine the effect of breaking protenaceous cross-bridges between the
surrounding Schwann cells and the axolemma, trypsin (1 mg/ml) was added to
the external seawater. (Note that one of us, I.T., found that the layer of con-
nective tissue and Schwann cells could be detached mechanically in such trypsin-
treated axons.) After immersion of axons in such trypsin-containing seawater
for a period of about 60 min, mechanical responses of the axons were examined
by using either a Gulton piezo-ceramic bender or a Fotonic sensor. No clear
effect of protease treatment was detected by this procedure.
Immersion of a giant nerve fiber in a medium containing ouabain is expected
to bring about an abnormal rise in the intracellular Na and Ca ion concentration
in the Schwann cells. Immersed in a medium containing 0.1 mm ouabain, small
nerve fibers are known to lose their excitability within a relatively short period of
time (see e.g., WHITE et al., 1974); the excitability of giant axons of the squid re-
mains unaffected by ouabain for a much longer period. Using the experimental
set-up shown in Fig. 1A, the effect of ouabain added to the external seawater at a
level of 1 mm on the mechanical responses of giant axons was examined. Within
60-90 min, no significant effect was observed.
There is a collagen layer outside the Schwann cells surrounding the giant

Japanese Journal of Physiology

RAPID MECHANICAL CHANGES IN SQUID AXON 79

axon. Axons are treated with 1 mg/ml collagenase dissolved in artificial seawater
at room temperature for 60 min. Although the axons become soft after the treatment, no clear effect on the mechanical response was recognized. Injection of 1 mm phalloidine, which favors a polymerized form of actin, did not yield any marked change in mechanical responses. On the other hand, injection of 10 mm cholchicine and 0.2 mm cytochalasin D, which depolymerizes microtubules, increased the magnitude of the mechanical responses without changing its diphasic pattern. The peak value of the swelling pressure showed an almost 10-fold increase 2 hr after colchicine injection. The concentration of these drugs in the axon interior is estimated to be about 10 % of that in the in-jection solution.

DISCUSSION

Under RESULTS, we have described various properties of rapid mechanical
changes of the squid giant axon associated with the production of action poten-
tials. We have examined, in the first place, whether or not the mechanical changes
are expressions of some events occurring in the layers which surround the giant
axon. Since treatments of the axon with trypsin, collagenase, or ouabain did not
affect the mechanical changes, we inferred that the collagen layers and Schwann
cells are not directly involved in the production of mechanical changes. Next,
we have demonstrated that similar mechanical changes can be evoked after removal
of the endoplasm. Thus, there seems little doubt that the rapid mechanical
changes represent physicochemical processes located in the superficial structure
of the axon proper.
Propagation of an action potential is known to be accompanied by an ex-
change of extracellular Na ions for the intracellular K ions. The quantity of
alkali metal ions involved is of the order of 10- 11 Eq/cm2 per impulse (see HODGKIN
1964; TASAKI, 1968). The difference in the hydrated molar ionic volume be-
tween Na and K ion is approximately 20 ml (GREGOR, 1951). The change in the
volume of the axon associated with this cation exchange is then given by (20 cm3•E
Eq-1)•~(10-11 Eq•Ecm-2) which amounts only to 0.02A. Based on this simple
calculation, we believe that the Na-K ion exchange associated with propagation
of an action potential is totally inadequate to explain the origin of the movement
of the axon membrane.

To explain the observed outward movement of the axon surface of 1 nm by
assuming an influx of water, roughly 3•~107 H2O molecules/ƒÊm2 is required.
The number of Na ions which enter the axon during one cycle of action po-
tential production is of the order of 6•~104/ƒÊm2. We thus find that the num-
ber of water molecules involved is about 500 times as many as that of sodium
ions.

We now examine electroendosmosis associated with production of an action

Vol. 32, No. 1, 1982

80 I. TASAKI and K. IWASA

potential as a possible cause of the observed mechanical changes in the nerve. If we assume that the swelling of the axon is the result of water flux caused by the rise in the membrane potential, the pressure rise in the axon is expected to lag behind the rise in the membrane potential by approximately 0.5 msec. (Note that the pressure rise produced by this mechanism should be proportional to the integral of the water flux in such a case.) Since the pressure was found to rise simultaneously with the membrane potential, it appears safe to conclude that the swelling is not brought about by simple electroendosmosis.
The peak of the pressure rise was found to coincide within about 0.05 msec with the maximum of the membrane potential. Judging from the time required for relatively small molecules or ions to travel by diffusion, it is reasonable to assume that the structure involved in the production of the mechanical changes is located in the axolemma and/or in the ectoplasm. Replacement of Ca ions within the axolemma-ectoplasm complex with Na ions is expected to lead to a marked swelling of this layer. It is now known that this structure contains tubulin and other proteins and is very sensitive to a variety of electrophysiological manipulations of the axon (METUZALS and TASAKI, 1978; PANT et al., 1978; BAUMGOLD et al., 1981). Therefore, we suggest that the cation exchange processes occurring in the axolemma-ectoplasm complex are at the base of the phenomena described in this paper. The shrinkage of the axon observed during the hy-perpolarization phase of the action potential may be regarded as the consequence of the Ca influx which takes place while the structure is in a swollen state.
In artificial membranes, it is well known that the intramembrane water con-tent is one of the most important factors which determine the membrane resistance (see e.g., GREGOR and SOLLNER, 1946; LAGOS and KITCHNER, 1960). In acidic macromolecules, a marked increase in water content may be produced by an ex-change of hydrated Na ions for divalent cations (see e.g., KATCHALSKY and ZWICK, 1955). In squid giant axon, the polyatomic cations that are capable of sub-stituting the external Na ions without suppressing excitability are known to possess hydrophilic side groups (TASAKI, 1968). The present study gives further em-phasis on the importance of water molecules in the process of nerve excitation.

REFERENCES

BAUMGOLD, J., TERAKAWA, S., IWASA, K., and GAINER, H. (1981) Membrane-associated cyto-skeletal proteins in squid giant axon. J. Neurochem., 36: 759-764.
BETHE, A. (1920) Nervenpolarizationsbilder and Erregungstheorie. Pflugers Arch., 183: 289-302.
BRYANT, S. H. and TOBIAS, J. M. (1955) Optical and mechanical concomitants of activity in Carcinus nerve. I. Effect of sodium azide on the optical response. II. Shortening of the nerve with activity. J. Cell. Comp. Physiol., 46: 71-95.
COHEN, L. B. (1973) Changes in neuron structure during action potential propagation and synaptic transmission. Physiol. Rev., 53: 373-418.
GREGOR, H. P. (1951) Gibbs-Donnan equilibria in ion exchange resin systems. J. Am. Chem.

Japanese Journal of Physiology

RAPID MECHANICAL CHANGES IN SQUID AXON 81

Soc., 73: 642-650.
GREGOR, H. P. and SOLLNER, K. (1946) Improved methods of preparation of electropositive permselective protamine collodion membranes. J. Phys. Chem., 50: 88-96.
HILL, B. C., SCHUBERT, E. D., NOKES, M. A., and MICHELSON, R. P. (1977) Laser interferometer measurement of changes in crayfish axon diameter concurrent with action potential. Science, 196: 426-428.
HILL, D. K. (1950) The volume change resulting from stimulation of a giant nerve fibre. J. Physiol. (Lond.), 111: 304-327.
HOBER, R. (1920) Zur Analyse der Calciumwirkung. Pflugers Arch., 182: 104-113. HODGKIN, A. L. (1964) The Conduction of the Nervous Impulse, Liverpool Univ. Press, Liver-pool, p. 44.
IWASA, K. and TASAKI, I. (1980) Mechanical changes in squid giant axons associated with production of action potentials. Biochem. Biophys. Res. Commun., 95: 1328-1331.
IWASA, K., TASAKI, I., and GIBBONS, R. C. (1980) Swelling of nerve fibers associated with action potentials. Science, 210: 338-339.
JOHANSSON, B. and JONSSON, O. (1968) Cell volume as a factor influencing electrical and me-chanical activity of vascular smooth muscle. Acta Physiol. Scand., 72: 456-468. KATCHALSKY, A. and ZWICK, M. (1955) Mechanochemistry and ion-exchange. J. Polym. Sci., 17: 221-234.
LAGOS, A. E. and KITCHNER, J. A. (1960) Diffusion in polystyrene-sulphonic acid ion-exchange resins. Trans. Faraday Soc., 56: 1245-1251.
LOEB, J. (1906) The Dynamics of Living Matter, Columbia Univ. Press, New York, pp. 78-95. METUZALS, J. and TASAKI, I. (1978) Subaxolemmal filamentous network in the giant nerve fiber of the squid (Loligo pealei L.) and its possible role in excitability. J. Cell Biol., 78: 597-621. PANT, H. C., TERAKAWA, S., BAUMGOLD, J., TASAKI, I. and GAINER, H. (1978) Protein release from the internal surface of the squid giant axon membrane during excitation and potassium depolarization. Biochim. Biophys. Acta, 513: 132-140.
PICHON, Y. and TREHERNE, J. E. (1976) The effect of osmotic stress on the electrical properties of the axons of a marine osmoconformer (Maia squinado brachyura crustacea). J. Exp. Biol., 65: 553-563.
SANDLIN, R., LERMAN, L., BARRY, W., and TASAKI, I. (1968) Application of laser interferometry to physiological studies of excitable tissues. Nature, 217: 575-576.
TASAKI, I. (1968) Nerve Excitation, Charles C. Thomas, Springfield, Illinois, p. 80, pp. 141-143. TASAKI, I. and IWASA, K. (1980) Shortening of nerve fibers associated with propagated nerve impulse. Biochem. Res. Commun., 94: 716-720.
TASAKI, I., IWASA, K., and GIBBONS, R. C. (1980) Mechanical changes in crab nerve fibers during action potentials. Jpn. J. Physiol., 30: 897-905.
TEORELL, T. (1962) Excitability phenomena in artificial membranes. Biophys. J. (Suppl.), 2: 27-52.
WHITE, G. L., SCHELLHASE, H. U., and HAWTHORNE, J. N. (1974) Phosphoinositide metabolism in rat superior cervical ganglion, vagus and phrenic nerve : Effects of electrical stimulation and various blocking agents. J. Neurochem., 22: 149-158.

Vol. 32, No. 1, 1982