CONFERENCE

Comparative studies on the transduction mechanisms and its controls in mechanoreceptors

Okazaki, Japan

7th and 8th December, 1979

The conference, held at the National Institute for Physiological Sciences in Okazaki, on December 7 ~ 8, 1979, is one of the meeting programs of the Institute. The institute is part of the National Center of Biological Sciences, which was established in 1977, and is to some extent modelled after the National Institute of Health in U.S.A.

The program of the conference was designed to discuss the transduction mechanism and its nervous control in mechano-receptors, in which hair cells in inner ear and lateral-line organ, cutaneous touch receptor and stretch receptor in muscle were included. Nine papers given in this meeting focused on the relationship between ionic permeability in the transduce membrane and its electron-microscopic structure in the different kinds of mechano-receptors. The program was organized by Fumio Ito.

The concept of mechano-transduction was derived thirty years ago. Katz,[1] recording the electrical responses from the sensory nerve fiber in the vicinity of the muscle spindle of frog, showed that the impulse discharge was generated by a graded and sustained depolarization of the sensory terminals. The first intracellular study of electrical responses of mechano-receptors was performed by Eyzaguirre and Kuffler[2] on the stretch receptor of lobster and crayfish. In agreement with the results obtained by Katz,[1] it was found that sensory stimuli produce depolarization of the cell membrane and that nerve impulses arise if the depolarization attains sufficient amplitude (a threshold for encoder). This depolarization became known as the "generator potential" (a term introduced by Bernhard and Granit[3]) or "receptor potential" (Davis[4]). Pioneering discussions on the production of the receptor potential have been made by Katz,[1] e.g., a capacitance change, chemical changes or permeability changes may be caused by the deformation of the membrane.

The following four hypothesis have been based on the view of Thurm[5] who have noticed the structural and functional characteristics on the energy supply in mechano-transduction. (1) The confrontation of a marked "anisotropy" of the receptor cell terminals to the higher "isotropy" of the basic organization of nerve axons is associated with differences in the functional basis of these cellular regions. Since the receptor current is generated in a terminal structure, the current circuit is locally fixed, in contrast to the local circuit which move along an axon. Whereas in an unmyelinated axon every membrane area becomes the site of current inflow as well as of outflow successively, in a receptor terminal one area is subject only to the inflow, the other area only to the outflow of the net current. Importance of structural and functional asymmetries in sensory transduction within receptor cell has also been pointed out in the frog muscle spindle by Ito *et al.*[6] (2) The sensory cells of most types are part of epithelia which separate two liquid-filled spaces. The

external medium can differ very much from the inter-cellular medium, just as for instance fresh water does or the cochlear "endolymph" which has a K^+/Na^+-ratio similar to the intracellular K^+/Na^+-ratio. Alexeev[7] found changes of K^+-concentration in the intra-capsular space of frog muscle spindle to be seven times slower than in the external solution, suggesting that the outer capsule of the frog muscle spindle acts as a diffusion barrier for potassium ions. High potassium contents in Pacinian corpuscle fluid have also been observed by Ilyinsky *et al.*[8] (3) Thurm[5] found the ratio of the density of mitochondria to be about 10 times higher in receptive regions in sinus hair cells of mini-pig than in axons of the same diameter. The mitochondria contain dense intramitochondrial granules which are known in other cells to contain concentrations of divalent cations (Lehninger[9]). This leads us to the fourth hypothesis. (4) Sodium channels of the axonal membrane along the sensory nerve may be replaced by calcium channels at the terminal membrane of the mechanoreceptor. The calcium channels may play a role of transduction.

On the base of above mentioned hypothesis, some important discussions were made in this meeting. Yoshioka developed a new method for measuring the membrane potential of small cells as auditory inner-hair cells of mammals, by means of [3H]-triphenyl methyl phosphonium ions. An important morphological basis on the function of mechano-receptors was given by Hama, who showed a special gap-junction between hair cells in the auditory and lateral-line organs. Yanagisawa showed a common functional property between auditory and lateral-line receptors in different ionic environments. The study on the integrative control function of brain stem to cochlear neuron by Murata was of interesting on the point that an amplifier mechanism in the mechano-reception is controled by the efferent system. Taniguchi studied HRP morphology of the efferent axon innervating the inner-hair cells, supporting the study of Murata. Evidence that considerably low resting potential of the inner-hair cells is due to the characteristic ionic environments was shown by Tanaka. Hisada presented an interesting morphological aspect of the mechanical transformer in crayfish tactile receptors. By means of newly developed technique, Uehara and Desaki showed scanning microscopic photographs of end-plate and muscle spindles in the frog skeletal muscles, by which the members of this conference were facilitated their scientic curiocity. Ito proposed a model of mechano-transduction in the frog muscle spindle. The model, like the IVth hypothesis mentioned above, represented that calcium channels may play a role as a transduction and also as an amplifier in transmission from the the transduction to encoding.

The success of this meeting was due to the hospitality and valuable suggestions of the Center staff, the president Yasuji Katsuki, the Dean Professor Kouji Uchizono and Professor Shun-ichi Yamagishi.

We wish to express our gratitude to the National Institute for Physiological Sciences, who sponsored the meeting.

FUMIO ITO

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Gap junctions between hair cells and supporting cells in the goldfish saccular macula. A freeze fracture study.

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Introduction

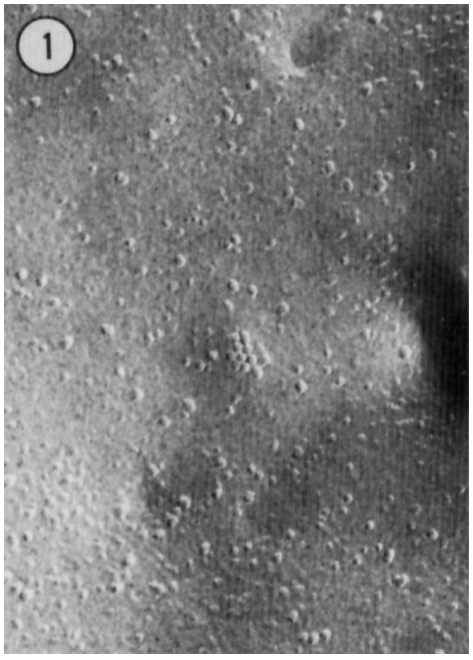
Gap junctions have been considered to be responsible for cell-cell communization and are found where electrical coupling or metabolic cooperation is present.[1,2] Many extensive gap junctions have been found between adjacent supporting cells in some acousticovestibular sensory receptors.[3] In the present study, gap junctions are found between hair cells and supporting cells in the goldfish saccular macula. The functional significance of these gap junctions is discussed.

Materials and methods

Common goldfishes, about 10 cm in body length, were used. Saccular maculae were dissected out after decapitation without anesthesia. Specimens were fixed by immersion in 3 % glutaraldehyde and 0.1 M cacodylate buffer, pH 7.3, for one hour and cryoprotected in 25 % glycerol in the same buffer for two hours on ice. Specimens were frozen by immersion in Freon 12 at −155°C. Freeze fracture replicas were prepared using a Balzers freeze fracture device at −115°C.

Result

Indentations formed by nerve terminals are found on the basal half of the receptor cell. Many small gap junctions are observed outside the indentation on the receptor cell membrane. They are probably formed between the receptor cell and supporting cell since the baso-lateral surface of the receptor cell is covered by supporting cells except for the region attached to the nerve terminal. They are usually small and may consist of only 11 particles (Fig. 1). However, rather large ones consisting of over hundred particles are occasionally encountered (Fig. 2). The diameter of constituent particles is 8.77 nm ± 0.50 nm which is smaller than that of supporting cell-supporting cell gap junctions, 9.50 nm ± 0.63 nm



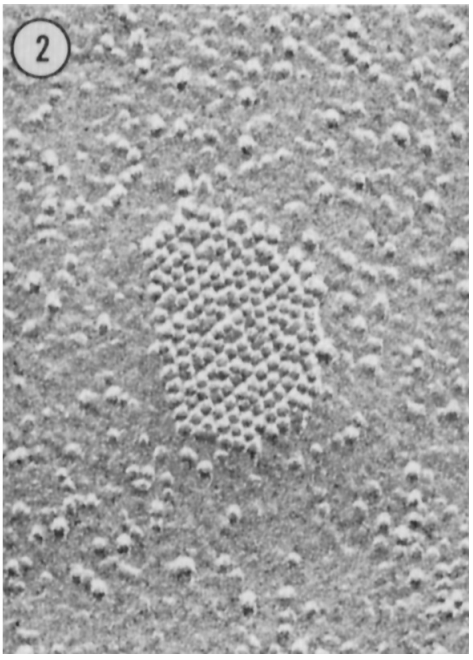


Fig. 1 Fig. 2

Fig. 1. A small gap junction between receptor cell and supporting cell consisting of only 11 particles. × 110,000

Fig. 2. Rather large gap junction between receptor cell and supporting cell. Particles are densely packed to form a regular hexagonal pattern. × 180,000

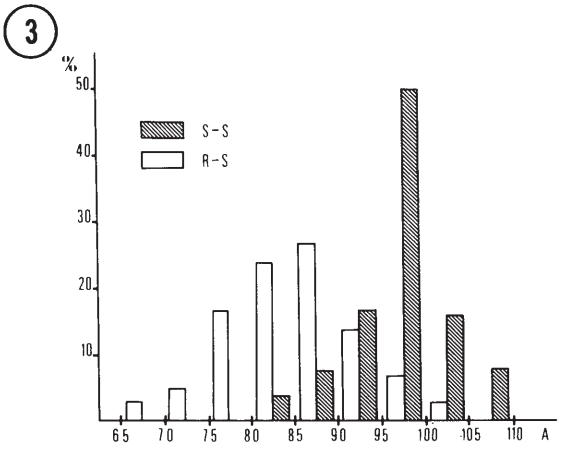


Fig. 3. Particle size distribution from supporting cells-supporting cells gap junctions (S-S) and receptor cells-supporting cells gap junctions (R-S).

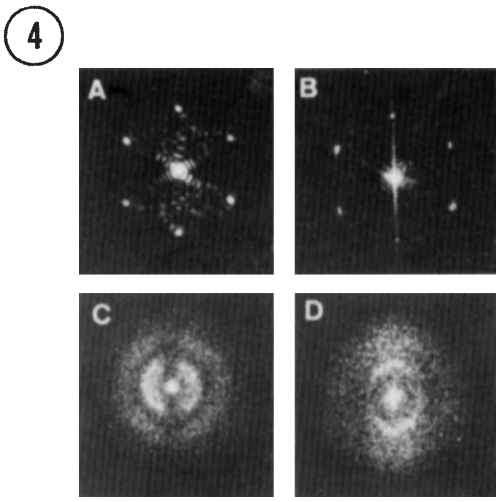


Fig. 4. Optical diffraction patterns.
A: from an aggregate of particles in a R-S gap junction.
B: from an aggregate of pits in a R-S gap junction.
C: from an aggregate of particles in a S-S gap junction.
D: from an aggregate of pits in a S-S gap junction.

(Fig. 3). They are packed into polygonal aggregate to form a regular hexagonal pattern. Optical diffraction patterns from these gap junctions indicate a regularity of particle and pit distribution. Whereas, those from supporting cell-supporting cell gap junctions do not (Fig. 4). They are frequently associated with desmosomes which is characteristic of the gap junction between receptor cells and supporting cells.

Discussion

Reports which demonstrate the electrotonic coupling between the hair cell and supporting cell in the vertebrate inner ears have appeared recently.[4,5,6] Although no electrical coupling has been directly observed between the hair cell and supporting cell in the saccular macula of goldfish, the small gap junctions described in the present study are probably related to electrotonic coupling between these cells. In the saccular macula of goldfish, since gap junctions were frequently found between hair cells and adjoining supporting cells and since adjacent supporting cells are extensively joined by large gap junctions[3,7] hair cells are conceivably connected by a low resistance pathway through surrounding supporting cells. Thus the electrical activity in one hair cell can influence the electrical activity in other hair cells through these connections. If so, the small gap junctions between hair cells and supporting cells might serve as a means for the processing of signals at the receptor cell level.

It has been postulated that, in freeze fracture replicas of gap junctions, size and distribution of intramembrane particles depends largely on tissue preparation methods.[8,9] Peracchia[10] has reported a decrease in particle diameter and an increase in regularity of particle distribution after uncoupling procedures. In the present material, since receptor cell-supporting cell gap junctions and supporting cell-supporting cell gap junctions are situated side by side in the same sensory epithelium, it is unlikely that the effect of tissue preparation procedures differs substantially among these two types of gap junctions. Thus

the present results may suggest either that receptor cell-supporting cell gap junctions are more easily uncoupled than supporting cell-supporting cell gap junctions by the same tissue preparation procedures, or these two types of gap junctions actually differ in structure reflecting the functional difference.

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The acousticolateralis system and ionic environments

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It has been known that the endolymph of the mammalian inner ear contains high concentration of K^+ and that this ion is indispensable for the transduction process of the cochlea. However, little is known about the ionic mechanism of sound reception. The lateral-line organs of aquatic animals are homologous to mammalian inner ear. The organ has been shown to be chemoreceptors for ions and that the mechanical sensitivity was altered significantly by various ions.[1] Russell *et al.*[2] reported the presence of large amount of K^+ in the lateral-line cupulae, the overlying gelatinous structure on the top of the hair cells. Sand[3] described the effect of different ionic compositions of the external water medium of this organ on the mechano-sensitivity. He reported that the mechano-sensitivity was a function of the Ca^{2+} concentration of the external medium. We confirmed the same Ca^{2+} effects. Recently a model was proposed for the mechanism of neomycin ototoxicity.[4] In this model the basic groups of the antibiotic bind directly to polyphosphoinositides which are thought to be the sites of calcium binding in the membrane. To investigate the effect of neomycin on the mechano-sensitivity of the lateral-line organ, different concentra-

tion of the drug in distilled water were applied to the organ of the mudpuppy. When a solution of neomycin with a concentration of 10^{-4}g/ml was applied to the neuromast for 5 min, the afferent synchronization to the mechanical vibration was completely suppressed. The suppressive effects of neomycin were reversed by application of excess calcium. These results are consistent with the proposed model of neomycin ototoxicity. We suggest that the action site of calcium for enhancement of mechano-sensitivity is related to membrane lipids. However, the chemical responses of the lateral-line organ to cations were unchanged by neomycin. From the results of experiments on the lateral-line organ of tadpoles, Yoshioka *et al.*[5] proposed the existence of chemical adsorption of ions on the receptor cell membrane. They explained the chemical responses with the aid of a site-binding chemical adsorption model and suggested that these sites were in the protein at the membrane surface.

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Auditory afferent and efferent neyrons in the mouse brain stem studied by axonal transport of horseradish peroxidase

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Anatomical evidence of the olivocochlear bundle which contains efferent fibers from the superior olivary complex to the cochlea was presented by Rasmussen.[1] Since then, it has been suggested that the olivocochlear bundle forms a feedback loop from the central nervous system to a peripheral receptor. Rasmussen identified two components of the olivocochlear bundle in the cat: one (approximately 400 fibers) was shown to originate from the contralateral superior olivary complex; the other (approximately 100 fibers) originated from the ipsilateral superior olivary complex.

Revision of a number of current conceptions regarding the olivocochlear bundle was suggested by Warr (1975). He determined the origin and number of olivocochlear efferent neurons of the cat brain stem by retrograde axonal transport of horseradish peroxidase (HRP) injected into the cochlea. Warr showed that 1700–1800 neurons were labelled by HRP in the superior olivary complex bilaterally, and that approximately 60% of the neurons in total were located on the side ipsilateral to the injection of HRP.

In the present study, distribution of HRP labelled neurons in the superior olivary complex and the cochlear nucleus of mice was investigated by injection of a 30 % solution of HRP into either the inferior colliculus or the cochlea. I intended to observe differences in distributions of the afferent and the efferent neurons in the superior olivary complex and those of the primary and the secondary auditory neurons in the cochlear nucleus.

Distribution of labelled cells within the superior olivary complex

The general distribution of labelled cells in the superior olivary complex following a large injection of HRP into either the inferior colliculus or the scala tympani of the cochlea is schematically illustrated in Fig. 1. A large injection of HRP into the inferior colliculus resulted in labelled cells within the ipsilateral medial and lateral superior olivary nuclei. Occasionally, a few labelled cells were observed in the contralateral medial superior olivary nucleus.

After a large injection into the cochlea, labelled cells were found bilaterally in the subdivisions of trapezoid body: medial, lateral, and ventral nuclei. These cells in the restricted regions may be the sources of the olivocochlear bundle. The bilateral distribution of the labelled cells in the superior olivary complex coincides with the previous result.[2)]

Distribution of labelled cells within the cochlear nucleus

A large injection of HRP into the inferior colliculus resulted in the scattered labelled cells within the contralateral cochlear nucleus as shown in Figs. 1 and 2. These labelled cells are the secondary afferent neurons projecting from the cochlea to the opposite inferior colliculus.

After a large injection into the cochlea, labelled cells were observed in the restricted region of the ventral cochlear nucleus ipsilaterally as shown in Figs. 1 and 2. These labelled cells were fusiform cells and identified as the primary auditory neurons. They may form the synaptic connections with the secondary neurons in the cochlear nucleus described above.

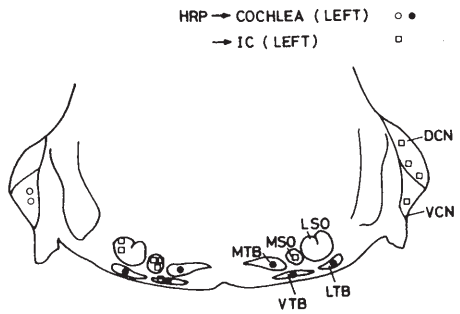

Fig. 1. Distribution of labelled neurons in the superior olivary complex and the cochlear nucleus. HRP was injected into either the left cochlea (○, ●) or the left inferior colliculus (□). DCN: dorsal cochlear nucleus; VCN: ventral cochlear nucleus; MTB: medial nucleus of the trapezoid body; VTB: ventral nucleus of the trapezoid body; VTB: ventral nucleus of the trapezoid body; LTB: lateral nucleus of the trapezoid body; MSO: medial superior olivary nucleus; LSO: lateral superior olivary nucleus; IC: the inferior colliculus.

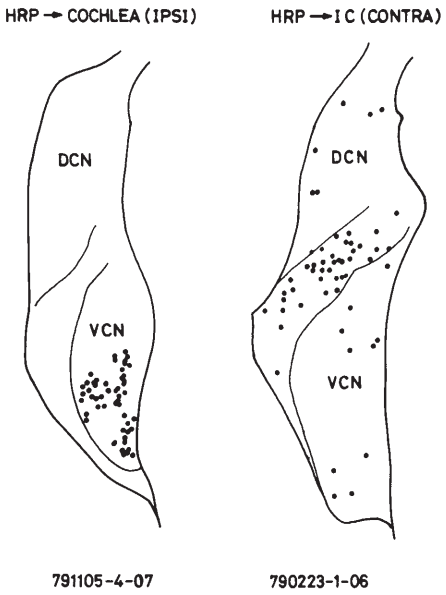

Fig. 2. Distribution of labelled neurons in the cochlear nucleus. HRP was injected into either the ipsilateral cochlea or the contralateral inferior colliculus. DCN: dorsal cochlear nucleus; VCN: ventral cochlear nucleus; IC: the inferior colliculus.

Recently, Ross *et al.*[3] reported the acute ototoxic effect of HRP injected into the cochlea of the guinea pig. They found no retrograde transport of HRP to spiral ganglion cells or to brain stem neurons when 1 % and 10 % solutions of HRP were used.

The uptake of HRP into the nervous system may depend on the concentration of HRP injected. Especially in the cochlea, HRP is considerably diluted by the perilymphatic fluid. Therefore, further studies are necessary to clarify this problem.

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Intracellular potentials of the organ of corti in guinea pigs

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Introduction

Cochlear microphonic potentials (CM) can be recroded from the intact organ of Corti, but it is still an open question whether they are essential phenomena in the sound reception of hair cells. In order to determine what motivates the release of chemical transmitters at the synaptic end of hair cells, potentials in response to sound stimulations have to be intracellularly recorded. The intracellular potentials are identified by means of a marking technique using Alcian Blue dye.

Methods

Guinea pigs weighing 250 to 500 g were employed as experimental animals. The animals were anesthetized with pentobarbital sodium injected intraperitoneally in a dose of 30 mg per kg of body weight and were kept in artificial respiration after tracheotomy and muscle relaxation with gallamine triethiodide were performed. The right tympanic bulla was opened through a ventrolateral approach. For the purpose of intracochlear illumination a piece of glass rod was attached to the surface of the bony wall of the basal turn.

Recording and dye-application electrodes were inserted into the organ of Corti through the round window. Sound stimulation in bursts of 2500 to 3000 Hz was delivered through a coupler between a Mitsubishi Diatone TW-25 tweeter and the external ear canal. DC potentials were fed to a Nihonkohden MZ-4 preamplifier and recorded with a pen recorder. AC potentials were led from a Tektronix 565 cathod-ray oscilloscope to an X-Y recorder through an averaging data processor.

Immediately after the potential recording the dye marking of the recording sites of potentials was performed. The glass micropipett electrodes, less than 1 μm in tip diameter were filled with 3 % Alcian Blue - 1.5 M KCl solution. The ionized Alcian Blue was expelled from the electrode by applying 1×10^{-7} A current pulses of 500 msec duration, once a second for 1 to 2 min. The cochlea was rapidly removed from the skull after decapitation. The widely-fenestrated cochlea at the round window and apex was fixed for about 12 hr at 4°C in 2.5 % glutaraldehyde solution. The organ of Corti on the hook portion of the basilar membrane was carefully picked out and was observed as a surface preparation. Following that the material was dehydrated in ethanol and embedded in Epon. The Alcian Blue spot was precisely located in cross sectional preparations.

Results and discussions

During the electrode penetration into the organ of Corti from the scala tympani to the scala media, a few negative deflections can be obtained prior to the recording of the positive endocochlear dc potential (EP). These negative potentials in the organ of Corti were thought to originate intracellularly when first recorded,[1)] and it was later ascertained

by using marking techniques.[2~4] In electrode tip positions where the negative deflections were usually obtained, very unstable negative or sometimes positive potentials were recorded. The dye marking to determine the recording sites of these potentials was executed. The detection of marked spots on surface preparations was insufficient for cell identification because it is very difficult to localize the marked spot in the vertical plane of the organ of Corti even by the microscope's foucussing. Therefore, marked spots were located in cross sections. Fourteen marked spots were found in outer hair cells and twenty three were in supporting cells. The other spots were in inner and outer pillor cells, Boetcher's cells and Claudius' cells.

The intracellular resting potentials of Deiters' cells were between $-45 \sim -90.0$ mV and their mean value was -67.8 ± 4.2 mV. The magnitude of CM inside the Deiters' cells, expressed as the ratio to that measured in the scala tympani, was 1.32 and its amplitude was 3.1 ± 0.4 mV. The negative dc potentials of outer hair cells were -5.2 to -29.3 mV and they were obviously smaller than the resting potential in the supporting cells. The intracellular dc potentials in several outer hair cells were found to have a positive polarity. The mean CM was 1.86 as the relative values of the measurement in the scala tympani and the mean amplitude was 3.8 ± 0.7 mV. The CMs of both outer hair cells and supporting cells were the same in phase as the CM in the scala tympani.

The potentials of the outer hair cells identified are compared with those of supporting cells in Figure. The intracellular negative dc potentials in the outer hair cells are recorded smaller than in supporting cells and the decrease of the dc potentials tends to relate to diminution of the CM amplitude. In supporting cells there is no relationship between the dc potentials and the CM.

Until recently there has been a controversy as to whether the negative potentials in the organ of Corti are intercellular or intracellular. The present marking study using Alcian Blue confirmed that the origin of the negative potential is intracellular through the observation of the marked spots on cross sections.

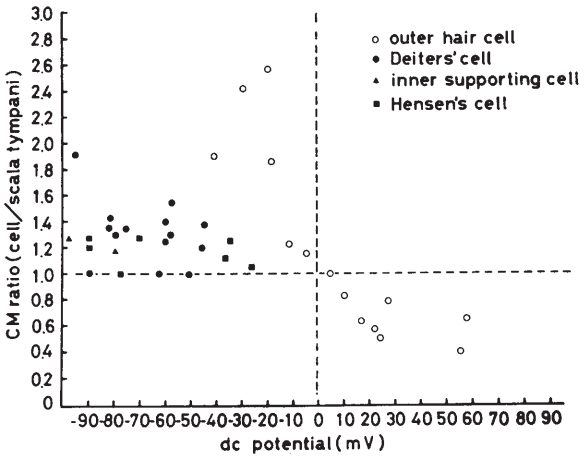

Fig. 1. Potentials in outer hair cells and supporting cells. CMs in those cells are expressed as the relative value of the measurement in the scala media.

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Scanning electron microscopic image of neuromuscular junctions

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Introduction

Although recent electron microscopical studies have added much information to the structure and function of neuromuscular junctions(NMJs), there is very little information in literature concerning three dimensional organization of NMJs at the fine structural level.

So far scanning electron microscopic studies of NMJs have not been achieved; the main obstacle resides in the presence of intramuscular connective tissue components which totally conceal the surface of NMJs when examined under the scanning microscope.

To overcome this problem, we have applied a modification of the HCl-Collagenase method which has been introduced by Evan *et al.*[1] in the survey of the basal aspect of tissues and cells.

Materials and method

The sternothyroid muscle of the chinese hamster were used for this study. The muscles were fixed *in situ* buffered glutaraldehyde followed by postosmification. In order to remove connective tissue components, the muscle strips were treated with 8N HCl for 20 to 40 min at 60°C after rinsing them in distilled water according to the method reported by Evan *et al.*[1] It was found that the treatment with HCl alone removes collagen and basal lamina almost completely, and that the successive digestion with collagenase recommended in the original method was found to be omissible. Another method for the same purpose which consists of enzymatic digestion prior to fixation[2] was also tested but resulted in serious distortion of the tissues caused by muscle contraction during the specimen preparation. They were dehydrated through a graded series of ethanol and were immersed in isoamyl acetate for 30 min. After drying by the critical point method and spatter coating with gold, the specimens were examined in Hitachi S-500A scanning electron microscope.

Results and discussion

In the specimens where intramuscular connective tissues are adequately removed, such structures as muscle fibres, intramuscular nerves and blood capillaries are immediately recognized under the scanning microscopy.

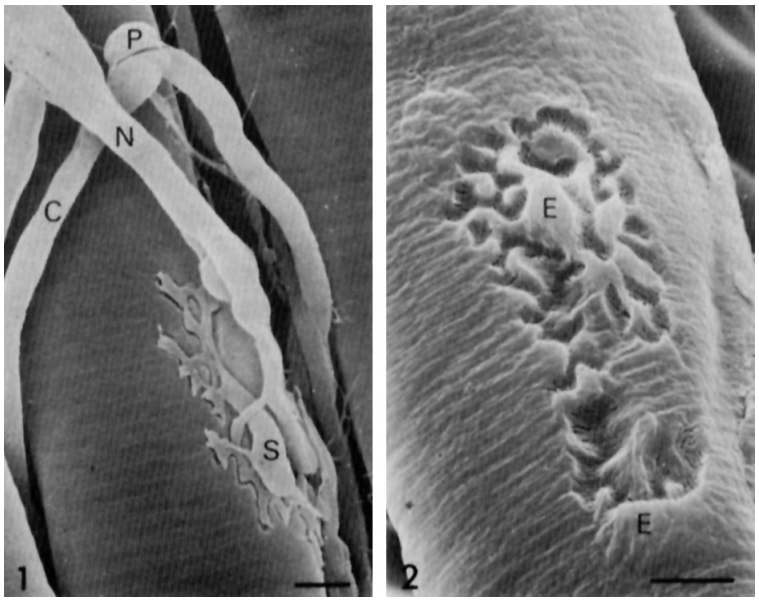

Fig. 1. Scanning electron microscopic image of NMJ of the chinese hamster. A branch of motor nerves (N) tapers to form a ramifying nerve ending which is fitted into subsynaptic depression of the muscle surface. (S); Schwann cell body. (C); Capillary with a pericyte (P).
Bar indicates 5 μm

Fig. 2. En face view of subsynaptic depression, showing the shape and arrangement of the junctional folds. Sarcoplasmic eminences (E) are evident. In this preparation, the nerve ending has been detached from the muscle surface during the HCl hydrolysis.
Bar indicates 5 μm

The nerves are found to ramify repeatedly in the vicinity of NMJs, and each branch tapers abruptly to give rise to a nerve ending; the branching point apparently represents the last node of Ranvier. The nerve endings are closely applied to the muscle surface lying in a set of depressions of muscle fibre surface to form a NMJs. The NMJs cover an oval area of about 15 by 30 μm in which bulged Schwann cell bodies are evident. A number of thin ramifying nerve endings are spread out of the cell bodies, which often appear to overlap and join each other to form a rather complicated texture (Fig. 1).

The surface of the nerve endings was exposed with this method but terminal axons themselves were not visualized owing to the presence of the Schwann cells covering. However, it is known from the previous transmission electron microscopic study that the Schwann cell covering is generally extremly attenuated, and therefore, the overall profile of the nerve endings is thought to conform closely to the shapes of the terminal axon, except in the region of the nuclei.

In addition to the surface features, the subsynaptic organization of the sarcolemma is visualized in those specimens in which the nerve endings have been detached from the muscle surface during the specimen preparation, possibly caused by hydrolysing materials of basal lamina in the synaptic clefts. In these specimens, the junctional sarcolemma exhibits deep and irregular synaptic depressions which are incompletely partitioned by ridges or folds of the junctional sarcoplasm. Unlike the schematic drawing of Couteaux[3] the junc-

tional folds are randomly disposed with respect to the long axis of the muscle fibre. The complexity in the shape and arrangement of these folds is fully displayed in this type of preparation (Fig. 2). Elevations of the muscle fibre which show a smooth surface and lack any cross striation are seen in and around the synaptic depression. The elevations undoubtedly correspond to the "terminal cone or eminence" of Doyére,[4] which are known to contain muscle (or fundamental) nuclei, an accumulation of mitochondria and sarcoplasmic reticulum.

The present study extends the previous light and transmission electron microscopical findings, adding new information to the morphology of NMJs which may allow quantitative study of the postsynaptic organization of the NMJs. Scanning microscopic study of muscle spindles is also in progress in our laboratory.

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High potassium effect on the mobility of sea urchin sperm

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Immobilization of sea urchin sperm by high concentration potassium cation was first described by Gray[1] and has been examined by various authors.[2,3] It would have been selected as a typical example for electrophysiological study of flagellum motility if the sperm was not so small. Recently, new techniques for measurement of membrane potential and intracellular pH were developed for such small cells using lipid soluble radioactive cation[4] and ^{31}P Nuclear Magnetic Resonance spectrometer.[5] In this report the membrane potential and intracellular pH of sea urchin sperm are described in connection with the sperm motility under high potassium concentration.

Sea urchin, *pseudocentrotus depressus,* was obtained from Misaki Marine Biological Station and its sperm was shed by conventional method. The preparations were made by dilution of the sperm into the artificial sea water of the various concentration of potassium with 10 % of volume concentration.

Tritiated TPP (Tri-phenyl-methyl-phosphonium) cation was purchased from New England Nuclear and the ethanol containing original solution was diluted 1000 times when it was used. The membrane potential was calculated from the following equations,

$$= 2.3 \frac{RT}{F} \log \frac{C_{sol}}{C_{cell}} \qquad (1)$$

$$= 2.3 \frac{RT}{F} \log \frac{v}{V} + 2.3 \frac{RT}{F} \log \frac{C_{sol}}{C^*_{sol} - C_{sol}} \quad (2),$$

where R is gas constant, F is Faraday constant, C_{sol} and C_{cell} means radioactivity of incubation solution and cells, respectively. V and v are volume of the incubation solution and cells. C* represents the radioactivity of the batching solution before incubation procedure starts.

The intracellular pH of the sperm was determined by measurement of the chemical shift of the resonance peak of inorganic phosphate using ^{31}P-NMR spectrometer (JEOL FX-60). About 1.5 ml of sample was packed in a NMR sample tube (8ϕ × 200) with 2 cm thickness. In order to determine the chemical shift value within an accuracy of 10 %, it took 10 min for measurement of each sample.

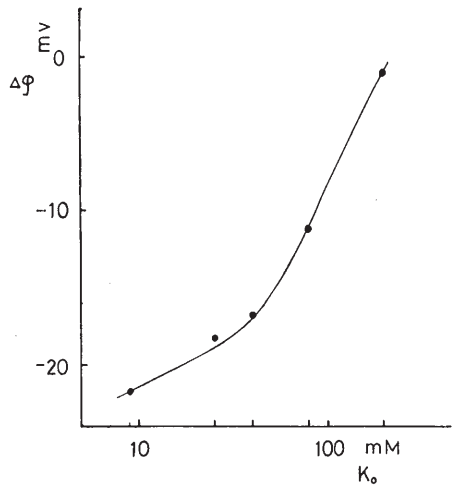

Fig. 1. Relation between membrane potential and potassium concentration of external solution. Membrane potential was calculated using eq. (2).

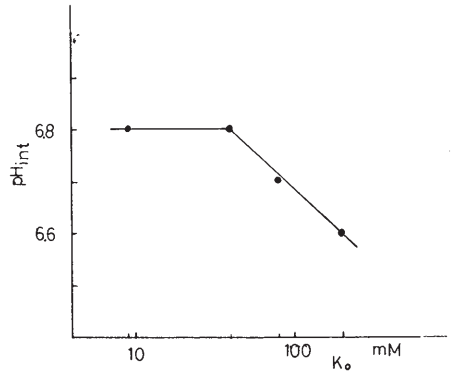

Fig. 2. Intracellular pH (pH_{int}) changes with the concentration of potassium in the incubation solution. The pH_{int} was determined by the chemical shift of resonance peak of inorganic phosphate.

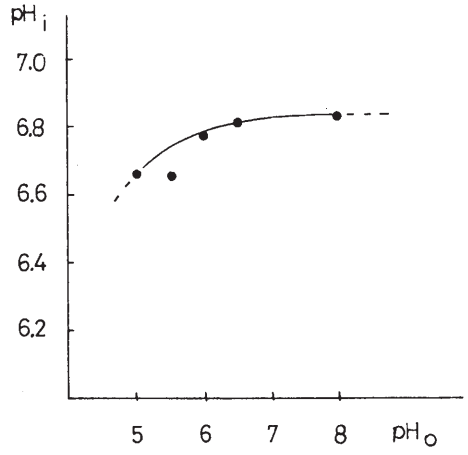

Fig. 3. Changes of intracellular pH against the external pH. External pH was adjusted by the titration of 0.1 N HCl and 0.1 N NaOH.

Cell motility was measured by the degree of the fluctuation of the light intensity which pass through optical microscope (× 150) observing systems. The photodiode was mounted on the eye-piece lense and its output was fed into the operational amplifier and finally connected to memory oscilloscope. The degree of cell motility was determined by the average amplitude of the intensity fluctuation caused by sperm moving.

In Fig. 1, the membrane potential ($\Delta\varphi$) of the sperm was plotted against the various concentration of potassium. Under the normal condition of incubation solution, the membrane potential of the sperm was about -20 mV, and it was decreased linearly with potassium concentration. Finally, it became nearly 0 when potassium concentration reached 200 mM.

Under the same range of potassium concentrations, the intracellular pH was measured by using ^{31}P-NMR. The results are shown in Fig. 2. From 10 mM to 40 mM of potassium concentration, the pH_{int} was constant (=6.8) and then decreased linearly with K^+ concentration. The same kind of decrease in pH_{int} was also obtained when the pH of the incubation solution was changed to acidic (Fig. 3). In both cases, high K^+ concentration (=200 mM) or low pH (=5.0) of external solution, pH_{int} was decreased about 0.2 unit and surprisingly, cell motility was inhibited absolutely.

From these results, we can conclude that external high potassium concentration led to the acidification of pH_{int} and reached to the immobilization of sperm. The mechanism why high potassium resulted in low pH_{int} and why pH_{int} stops flagellum motion is not clear at present but it might be very convenient to assume the existence of H^+-K^+ exchange mechanism.

Considering the simillarity in the structure of sperm flagellum and hair cell cillia,[6] these results suggest that high potassium content in endolymph of cochlea might be necessary to inhibit self moving of the hair cell cilia and increase the sensitivity to the mechanical stimulation on it..

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Inhibition on cochlear nerve fibers by the sound-activated olivocochlear bundle

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The olivocochlear bundle (OCB) described by Rasmussen descends from the ascending auditory pathway, the superior olivary complex, to the receptor site in the cochlea. In addition to 500 myelinated OCB fibers estimated by Rasmussen, numerous unmyelinated

efferent fibers were histochemically found and the total number of OCB fibers were estimated at 1700-1800. An abundant distribution of the olivocochlear efferent synapses in the cochlea suggests the central control of transduction. The functional significance of the OCB, however, is not yet well understood in spite of the extensive studies and several proposals concerning its possible functions.

Considering that the afferent activity in the cochlea is inhibited by the electrically activated crossed OCB (COCB) and the OCB neurons are activated by a sound, it is an attractive hypothesis that the transduction is modified by the descending OCB which is activated by the input sound, *i.e.* the OCB contributes to a feedback loop. Pfalz denied the assumption from his observation that an intense sound stimulation to an ear, by which the OCB might be bilaterally activated, did not inhibit the cochlear action potentials in response to clicks presented to the other ear.

In the present study interactions in the cochlea between two sounds presented to the both ears were analysed to examine whether or not the activity of a single cochlear nerve fiber can be modified by the sound-activated OCB.

The neuronal activity was isolated under Nembutal anaesthesia with Flaxedil paralization from the cochlear nerve of cats whose another cochlear nerve was completely transsected. A pure tone burst to the ear contralateral to the recording site (c-tone) activated a cochlear nerve fiber, when the c-tone was enough intense and its frequency was not far from the neuron's best frequency. The difference in threshold between the c-tone and an ipsilaterally applied another tone burst (i-tone) at the same frequency exceeded 40 dB.

Changing the phase difference between a simultaneously presented c- and i-tone from the same oscillator, the discharge rate of neuron was changed according to the phase difference. Difference between the most favourable phase angle, and the least favourable, to activate the neuron was found to be π. If the frequency difference between the c- and i-tone was slight and their phases were locked at particular angles at their respective starts in every stimulus trial, the pst-histogram of responses to the binaural stimuli showed ripples which synchronized with the beat between the two sounds. Holding the i-tone at the neuron's best frequency and just above its threshold, the frequency and intensity of c-tone were widely changed. The i-tone response was suppressed by the c-tone whose frequency range was higher and/or lower than the best frequency. The latency and time course of the suppression were exactly same as those of the *two tone suppression* caused by the monaurally applied two sound.

These binaural phenomena observed in the deafferented animals can be explained from their physical characteristics as direct mechanical interactions in the ipsilateral cochlea between an i-tone and a crosstalk of c-tone from the contralateral ear.

In normal cats with the intact right and left cochlear nerve the same mechanical interactions between binaurally applied two sounds were observed on most cochlear nerve fibers as those in the deafferented animals. Among these neurons we observed quite a few neurons which showed a binaural interaction other than the mechanical. The minority of neurons had no inhibitory area which might be observed on secondary neurons for an i-tone stimulation. Unlike the majority of neurons, they were not activated by a c-tone at the neuron's characteristic frequency (c-CF-tone) even at its available maximum intensity, but their spontaneous activity was clearly suppressed by an extremely intense c-CF-tone. During presentation of an i-tone at the neuron's best frequency (i-CF-tone) and just above its threshold, the sound-evoked discharges were suppressed by the c-CF-tone similar to the

spontaneous activity. The pst-histogram of responses to the simultaneously presented two tones at the frequencies slightly different from each other had no ripples corresponding the beat between the two sounds regardless whether or not their phases were locked at their starts, but the histogram showed a slowly progressing monophasic suppression. The suppression by the c-tone on the spontaneous activity and on the sound-evoked activity grew up slowly to its maximum and maintained the plateau with slightly progressive decrease till cessation of the suppressing sound. The discharge rate then recovered gradually to its control level with or without rebound.

The latency, the peak latency and the recovery time of the suppression after cessation of a c-tone ranged 10-28 msec, 20-84 msec and 60-280 msec respectively. These values were not inconsistent with those for the inhibition caused by the electrical stimulation of the COCB. Increasing the intensity of one of the two sounds by more than 15 dB, the mechanical interaction observed on the majority of cochlear nerve fibers became no more recognizable, whereas the suppression on the minority of neurons became more dominant and the extent of suppression increased with elevation of the suppressing c-tone level.

The c-tone frequency was just the neuron's best frequency and the c-tone leakage from the contralateral ear was strong enough at the cochlea under observation to activate the neuron. The c-tone did not activate the neuron, but suppressed it. The suppression can not be considered to be due to a direct action of the leaked c-tone on the opposite cochlea but it may result from the interaural neural inhibition. As any neural connections are not morphologically found between afferent dendrites and the efferent synapses in the cochlea are solely originated from the OCB, the inhibition might come from via the OCB.

An effective c-CF-tone level for the inhibition was so high that it exceeded 60 dB SPL and was around 100 dB SPL in some neurons. The population of cochlear nerve fibers which were inhibited by a c-CF-tone was small; less than 10 % of the fibers examined precisely in the present study. It was reported that the electrical stimulation of the crossed OCB beneath the fourth ventlicle inhibited most of cochlear nerve fibers. These facts imply that the OCB might not be expected to be an automatic gain control system, but, besides an input sound, synaptic convergence of any other additional gating signals to the OCB neurons in the superior olivary complex, probably from the higher level of the CNS, might be necessary to activate the OCB extensively enough to inhibit the transduction in the cochlea.

Structural bases of crustacean mechano-sensory hair function

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Crustacea and insect are the main member of arthropod, whose external surface is protected with sclerotized chitinous cuticle. Those animals therefore must have cuticular structure on the exoskeleton as sensory interface with the environment. Hair structure is employed as the crustacean cuticular mechanoreceptor. Antennular basal segment of crayfish carries several groups of mechano-sensory hairs including those of the statocyst

organ. Functional polarization of the statocyst hairs and its role in encoding the positional information has been reported.[1] Another group of mechano-sensory hairs was previously identified as the sensory input of a large interneuron in the CNS.[2] Structural bases of the receptive mechanism of these sensory hair were studied here with both scanning and transmission electron microscopy. The structure was identified as of the scolopidial type. This structure has so far been reported only in chordotonal organ and Johnston's organ.

External hair structure is found to be connected to the sensory cells with an extracellular chitinous thread. Thus the external force deflecting the hair is transferred to the sensory cells via this subcuticular 'chorda' thread in form of tension change. The chorda is surrounded by several layer of sheath cells and 1 to 2 μm in diameter and, in an extreme case, close to 5 mm in length. The transducing element is spindle shaped scolopidium with dimension of 40 μm in length and 4 μm in diameter. Scolopidium consists of three sensory cells together with one scolopale cell and several sheath cells. The scolopale cell contains electron dense fibrous material, i.e. scolopale, in the cytoplasm. The scolopale cell forms a spindle shaped cavity in which three sensory processes are enclosed and the scolopale seems to act as the framework of the cavity.

Sensory neurons are bipolar cell of 10 μm in diameter and 15 μm in length of somata. Each neuron has one sensory process protruding into the scolopale cavity. The distal part of the sensory process is slightly dilated and is rich in microtubules. The distal end of the scolopale constricts a set of three dilations of the sensory process. The middle part of the process is a ciliary segment with 9 + 0 type ciliary organization. The proximal processes are rich in mitochondrion. Three sensory cells adhere tightly together by desmosomal contact at this part of the process. A ciliary rootlet or axial filament runs through the proximal process from the base of cilium to the perikaryon.

Three sensory processes run together along a particular side of the inner wall of the scolopale cavity. Thus the ciliary process follows the curvature of the scolopale which itself warps along the cavity. This ciliary bending seems to play a significant role in sensory transduction as in the hair cell of vertebrate acoustico-lateralis. The schematic reconstruction is given in Fig. 1. It should be noted that the scolopidial structure is exaggerated about 10 times in size compared with the cuticular hair.

The structure revealed is in good accordance with the functional polarization observed in these hairs. Deflection of the hair in a direction of pulling of chorda can exert a stretch in the ciliary part of the sensory cell. And an in due increase in firing frequency of the cell results.

The structure described here resembles closely to the sensory element of the chordotonal organ found in shore crab.[3] Similar scolopidial structures were reported in insect chordotonal organ[4] and in Johnston's organ.[5] However, these sensory organs are generally regarded as the subcuticular sense organs and thought to have no corresponding structure on the exoskeleton. On the contrary, sensory hairs and campaniform sensillum are thought to be of a different type of sensory structure, and have been classified as the cuticular sense organs.

Present finding of the scolopidial structure as the sensory element of the crustacean external sensory hairs necessitates a new unified view of the homologous origin of these sensory structures of both cuticular and subcuticular sense organs in arthropods.

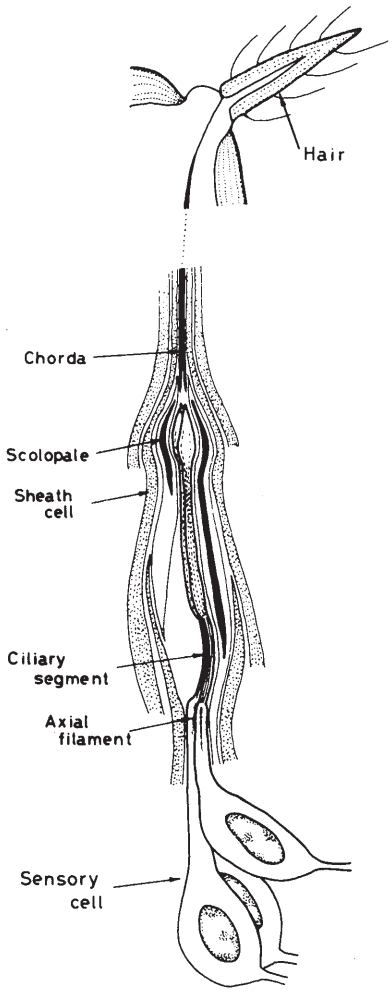

Fig. 1

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A model of transduction in the frog muscle spindle

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Introduction

It has been known that a receptor potential may occur at the non-myelinated terminals in the frog muscle spindle and the receptor currents may be integrated in a Ranvier node of the myelinated branches at which afferent impulses may initiate.[1] The hypothesis that the receptor potential may be caused by an inward flow of sodium ions[2] is supported by recent works on mammalian muscle spindles[3] and crustacean stretch receptors.[4]

We, however, found calcium spikes at the sensory nerve terminal of the frog muscle spindle,[5] in addition to abortive spikes which were assumed to be sodium spikes propagating along the non-myelinated filaments in the terminal.[6,7] As the calcium spikes and abortive spikes can be induced by stretch of the muscle spindle and also they are supposed to play a role of trigger for afferent impulses,[5,7] several steps from the transduction to the encoding have to be considered in the frog muscle spindle. The present note describes a model of the ionic mechanisms for explaining the complex processes in the mechanoreception.

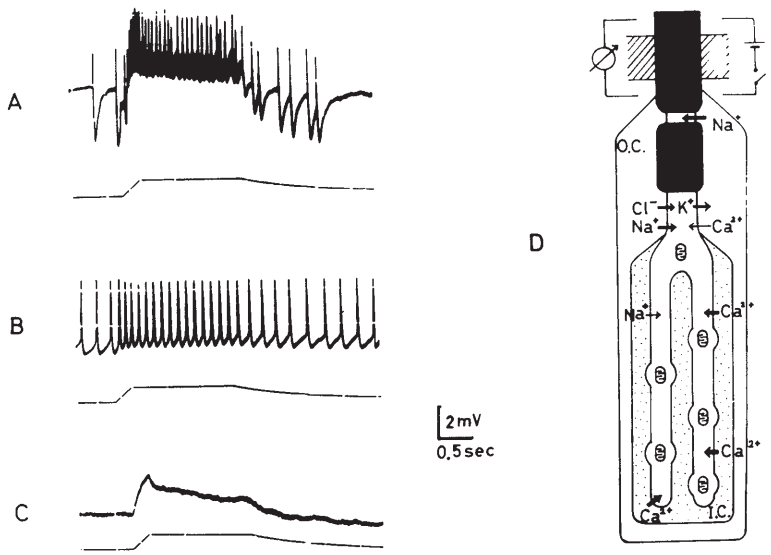

Fig. 1. Effects of 5 mM $CoCl_2$ (B) and Na^+ deprivation (C) on responses during stretch of a muscle spindle in comparison with the response in normal Ringer solution (A), and a schematic diagram of the distribution of ionic channels along the membrane of the nodes and terminals (D). O.C.; outer capsule. I.C.; inner capsule.

Methods

Muscle spindles were isolated from sartorius and semitendinosus muscles of the frog *Rana nigromaculata.* The sensory axon which innervates the spindle was isolated for 1 - 2 mm near the spindle capsule; the motor axon was removed. Just outside the capsule the myelinated segment of the axon was laid across an air-gap between two pools of Ringer solution made on two glass plates. Potential differences between the two pools were recorded through two Ringer-agar bridges immersed in the pools; each bridge was in turn connected to a calomel electrode which led to a differential high input impedance amplifier. The spindle was suspended between two stainless steel rods, connected to the tip of the levers of a differential electromagnetic puller, which stretched preparations symmetrically towards both poles at constant velocities (0.5 - 20 mm/sec).[8]

Results and Discussion

In a representative response of a muscle spindle during stretch in normal Ringer solution (Fig. 1A), dynamic and static discharges of sodium spikes occurred superimposed on the dynamic and static deflections of a spindle potential respectively. For 1 - 30 min after treatment of the spindle nerve terminal with 5 mM $CoCl_2$ or $MnCl_2$ which is known to block reversibly calcium spikes,[5] the discharges of sodium spikes occurred more rhythmic than that before the treatment, without any deflection of the spindle potential (Fig. 1B). The discharges and responses to stretch disappeared approximately 30 min after application of the calcium blockers. These effects were reversible if exposure to the drugs was not prolonged beyond 1 hr. Fig. 1C shows a spindle potential during stretch of the same preparation in Na^+-free Ringer solution after the recovery. The above results suggest that afferent discharges in normal Ringer solution may consist of two kinds of spikes triggered by calcium and abortive spikes and also that the spindle potential is probably a secondary response which may be due to a change in calcium permeability at the nodal membrane in the immediate vicinity of the site of origin of afferent discharges. The fact that all the responses to stretch are blocked by long-lasting application of calcium blockers leads us to another assumption that a genuine receptor potential may be due to an increase in calcium permeability at the non-myelinated terminals enclosed in inner capsule where is supposed to be more distal to the site of abortive and calcium spikes.

We would like to propose a model as shown in Fig. 1D for elucidating coherently the above suppositions. The density of Ca channels may increase instead of Na channels from the nodes of the myelinated branches to the terminal of the non-myelinated filaments. The Ca channels at the non-myelinated terminal may play a role as a mechano-transduction, but those near the Ranvier nodes as an encoding mechanism.

Acknowledgement

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