Shark Pit Organs: Enhancement of Mechanosensitivity by Potassium Ions

Abstract. *The mandibular pit organs of pelagic sharks, which respond sensitively to monovalent cations, often show neural discharges synchronized with respiratory gill movement. The mechanosensitivity of the organs is remarkably enhanced by application of potassium ions on the same end organ, respiratory movement remaining constant. In view of their mechanosensitivity to an increase of potassium ions in the cell environment, as well as their chemosensitivity, the pit organs of sharks, rather than the canal organs which have no chemosensitivity, may be designated as a better model of the inner ear of higher animals.*

In the cochlea of higher vertebrates two unusual phenomena have been observed. One is the d-c potential difference between the endolymph and perilymph, discovered by von Békésy in 1952 (*1*), and the other is the high potassium content in the endolymph, found by Smith, Lowry, and Wu in 1954 (*2*). These two phenomena have been considered to be intimately related and to support the extremely high sensitivities of cochlear hair cells to airborne sound. The amount of potassium in the endolymph and the development of the auditory endorgan in various animals, including elasmobranchs, have been extensively studied from the phylogenetical point of view (*3*). Whatever the hypothesis, however, the meaning of why the hair cells in the cochlea are immersed in potassium-rich medium, which is rarely found in other animal tissues, is still controversial.

When we worked on the lateral-line organ of fish (*4*), we found that almost half of the lateral-line nerve fibers, not only in sharks but also in many teleosts (*5*), are responsive to chemical stimulation of the skin, particularly to monovalent cations. Furthermore, application of the same chemical solutions to the canal pore, or even their introduction into the lateral-line canal, causes excitation, not by chemical stimulation but by fluid movement of the nerve fibers which conclusively innervate the canal organs. The end organs which respond sensitively to monovalent cations, therefore, can be said to be the pit organs in sharks, which are broadly distributed on the body surface. Neurohistological studies of the lateral-line system have already disclosed that both the canal and the pit organs in fish are innervated by the lateral-line nerve fibers (*6*).

To date it has been generally believed that the lateral-line canal and pit organs are the origin of the inner ear of higher animals because they seemed to be highly developed mechanoreceptors in structure and function (*7*). However, we have found a remarkable difference between the canal and pit organs.

The experimental materials were young sharks (60 to 100 cm long), particularly pelagic ones—for example, leopard shark (*Triakis semifasciata*) and gray smoothhound (*Mustelus californicus*). Ten leopard sharks and five gray smoothhounds, sent from California by air, were used. When they arrived in Honolulu they were kept in cold seawater, but they became adapted to warm water (20° to 22°C) within a few weeks. During the experiment the water was held at 21°C.

The pit organ is not easily found on the trunk surface of a relatively young shark. According to Tester (*8*), the pit organ is usually located in a pit beneath modified scales, but in histological structure it is not much different from the canal organ except for the cupula formation. The pit organs are free neuromasts with typical sensory hair cells and supporting cells, but there is some doubt that a cupula is included. The latest electron microscopic study by Hama and his associates on the pit organ of the shark (*Mustelus manazo*) confirmed that its microstructure is similar to that of the canal organ (*9*).

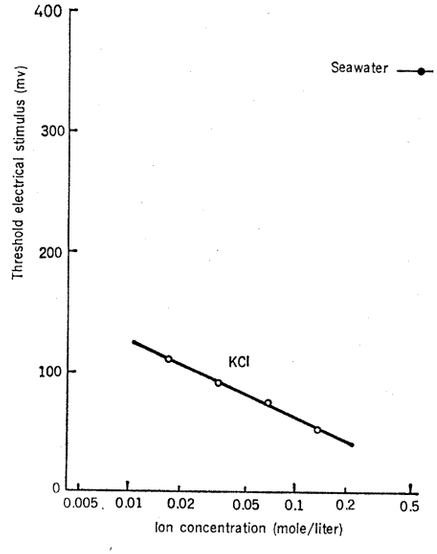

Fig. 1. Effect of KCl on electrical threshold of pit organs of leopard shark (*Triakis semifasciata*). Top right, electrical threshold in normal seawater.

Hama *et al.* observed many stereocilia and a single kinocilium on the top of sensory cells. Each two hair cells form a pair, and all pairs have the same directionality. Hence even a single pit organ may have a directional sensitivity to water flow in a particular direction. Pit organs found on the trunk surface, however, are rather insensitive to mechanical stimulation, although very sensitive to monovalent cations (*4*). This increase in chemosensitivity might be due to the possible absence of a cupula as indicated by Tester (*8*).

The pit organ in the mandibular region is quite different. Here the pit organs are regularly arranged in a row, and the skin color is pale in comparison with the dorsal skin color. Therefore it is much easier to find the pit organ in the mandibular region. The innervation of these pit organs is known to be the posterior division of the external mandibular nerve, a branch of the seventh nerve. The same nerve also innervates the mandibular ampullae of Lorenzini and the mandibular canal neuromasts together (*10*).

When the external mandibular nerve was exposed at the lateral side of the head and was separated into tiny bundles, each composed of a few nerve fibers, touch, chemical, and electrical stimulation of the end organs were performed under a binocular microscope in order to determine from which end organs the nerve response would come.

The canal organ could be located with light touch followed by the typical burst discharges of that organ. The ampullary and pit organs responded to chemical stimulation; mostly potassium ions were used. Final discrimination between the ampullary and pit organs could be made by electrical stimulation.

The location of the pit organ was determined by electrical stimulation. Once an increase or decrease of nerve fiber discharges was found with a large bipolar chlorinated silver wire electrode (polar distance, 1.0 to 1.5 cm), a very small stimulating electrode (polar distance, 1.0 to 1.5 mm) was substituted, and the end organ responsive to electrical stimulation was determined in a quite restricted area. The results obtained from the nerve fibers innervating those three organs by three stimulations are shown in Table 1.

The numerical values of electrical thresholds do not always indicate the excitability of the receptor cells because the structure of the end organs and of

Table 1. Responses of the canal, pit, and ampullary organs to three stimulations, mechanical, chemical, and electrical. Electrical threshold values are approximate.

Organ	Results of stimulation		
	Discharges		Electrical (threshold mv)
	Mechanical (touch)	Chemical (KCl)	
Canal	Burst	None	1000
Pit	A few	Burst*	100
Ampullary	A few	Burst*	10

* Proportional to concentration.

their accessory organs are different and the current density (dI/cm^2) at the end organ is unknown. However, the different values give us a good clue to the nature of three kinds of end organ.

Furthermore, thresholds of the organs for electrical stimulation were measured after application of potassium ions on the end organ. In the case of the pit organ, the thresholds changed remarkably, while in ampullary and canal organs no change was noticeable. The relation between potassium concentration and threshold for electric stimulation is shown in Fig. 1. The higher the potassium concentration, the lower the threshold. The mechanism of lowering of pit organ threshold may be the effect of potassium ions on the receptor cell itself and not that on the nerve ending because there is almost no threshold change in the other two cases.

During the experiment we sometimes observed that spontaneous unitary neural discharges show burst discharges synchronized with the respiratory gill movement. In such cases application of potassium ions to the pit organ caused a marked rise in the enhancement of synchronized burst discharges (Fig. 2), which was obtained by the use of a rate meter (*11*). Through this apparatus the temporal and sequential change of the discharge rate of a single nerve fiber can be precisely computed. This device was composed of a storage counter and a discharging circuit. For steady neural discharges the average rate was obtained. For the temporal sudden change of discharges, such as burst discharges synchronized with respiratory gill movement, the number of impulses per burst was computed as shown in Fig. 2. At the left the spontaneous discharge of the nerve fiber shows the periodic change of impulse frequency synchronized with respiratory movement. Soon after application of potassium solution (1/16 dilution of 0.55*M*) the maximum frequency of impulses gradually increased, while the minimum frequency also increased; the difference between the maximum and the minimum frequency, however, became bigger. This change of impulse frequency, appearing as a train of burst discharges, is modulated by the triphasic change of impulse frequency, which may be due to the direct ionic effect of potassium—that is, the initial phasic peak and a following slow tonic phase.

When the end organ is rinsed with seawater, a phasic increase of discharges, more marked than the initial peak, appears again; then the impulse frequency returns to the original level of the mechanical response rather rapidly, although the aftereffect of the potassium ions remains. The second large peak of impulses due to rinsing with seawater may come partly from the enhanced mechanosensitivity and partly from the chemical off-effect from potassium dilution.

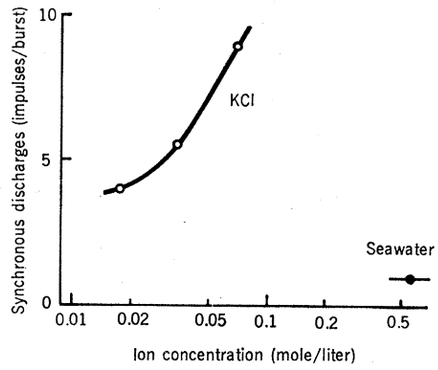

Fig. 3. Change of mechanosensitivity of pit organ of leopard shark with potassium concentration. The applied solution was diluted with isotonic 0.55*M* NaCl solution from the original KCl solution. Ordinate shows the number of burst discharges synchronous with respiratory gill movement of the animal.

The response of the same organ to 1/32 dilution of 0.55*M* KCl solution is also shown in Fig. 2 (right). The tendency is almost the same as shown in Fig. 2 (left), but the increase of impulse discharges is smaller than the other.

Figure 3 illustrates the relation between the concentration of potassium ions (abscissa) and the number of discharges in a single train (ordinate). The higher the concentration of potassium ions, the greater the number of discharges in a single train. One possible explanation of this phenomenon is that the mechanosensitivity of the pit organ receptor cell is increased by potassium ions in the cell environment. Increase in sensitivity occurred if the application of potassium ions were in a restricted area and the lateral-line nerve bundle had already been cut so that any information from the end organ did not reach the brain to change the respiratory movement. This increase of the mechanosensitivity of the receptor cell was reversible. Receptor cells of the pit organ seem to have dual functions, namely, mechano- and chemosensitivity, The inaccessibility of potassium ions to the canal neuromast cell might be due to the formation of a solid cupula, as opposed to the probable lack of a

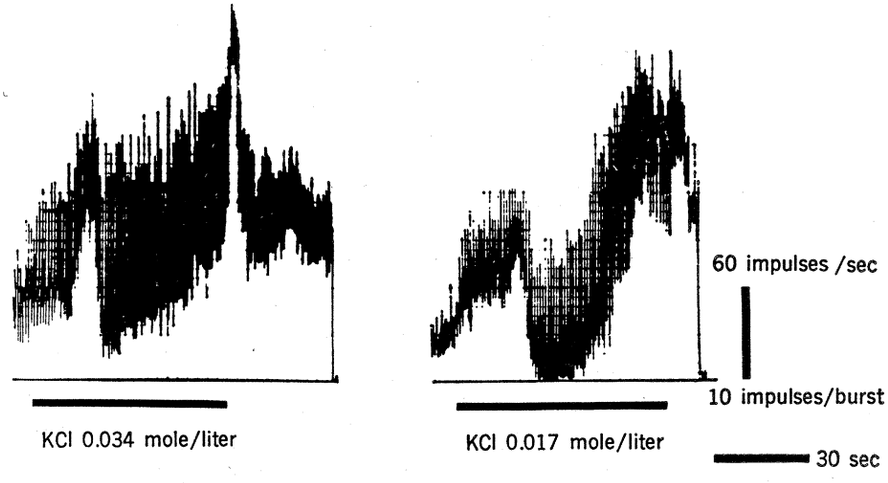

Fig. 2. Single fiber response of pit organ of leopard shark, recorded through a rate meter, to application of KCl solutions. Abscissa shows time; a horizontal bar shows the period of application of KCl solution. At the end the receptor was rinsed with seawater to exclude K^+ effect. Ordinate shows the number of impulses; a perpendicular bar shows 60 impulses per second or, in case of burst discharges, 10 impulses per burst.

cupula in the chemically sensitive pit organ.

From the pit organ in the dorsal body surface, such sensitive mechanoresponses were hardly observed. This may be because the dorsal skin of the shark is very thick and tough and may prevent the deformation of skin from reaching the end organ, whereas the mandibular skin is rather thin and soft and much closer to the gill than the dorsal skin. We have observed the same phenomenon in the free neuromasts near the operculum of the mullet, one of the teleosts, which are sensitive to chemical stimulation.

Tasaki (*12*) once said that the giant axon membrane of squid develops higher mechanosensitivity in more concentrated potassium ionic medium (*11*). The pit organ of the shark, however, may provide more direct evidence of the enhanced activity of the receptor cell when it is immersed in high potassium medium.

We think the pit organ of the shark may be a better model of the inner ear of higher animals than the canal organ because the pit organ has both chemo- and mechanosensitivity.

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Chromosome Number of a Small Protist: Accurate Determination

Abstract. *Electron micrographs of serial sections through a meiotic prophase nucleus of the mycetozoan* Labyrinthula *sp. show that there are nine separate and distinct synaptinemal complexes. Since each complex represents a set of paired homologous chromosomes, it follows that the haploid chromosome number of this protist is nine.*

Unicellular organisms that have a nucleus of less than 4 μ, often have chromosomes that are difficult to resolve with the light microscope, because of their shortness, their small width, and their limited ability to bind stain. Electron microscopy is usually not helpful because the chromosomes are rather diffuse and because they have poorly defined boundaries. If a sexual cycle exists, however, each set of paired homologous chromosomes forms a synaptinemal complex during meiotic prophase (*1*), and the individual complexes are clearly distinguishable in electron micrographs. Where the nucleus is less than 4 μ in diameter, it is practical to make serial sections of a complete nucleus. The individual synaptinemal complexes can then be traced, and the chromosome number can be determined unambiguously.

Methods used to isolate and maintain the marine mycetozoan, *Labyrinthula* sp., as well as conditions for obtaining zoosporulation have been described (*2*). While still attached to agar blocks, sporulating cells are fixed for 1 hour in 2.5 percent glutaraldehyde buffered at *p*H 7.4 with 0.2*M* Millonig's phosphate buffer (*3*). The blocks are then rinsed in four changes of 0.2*M* phosphate buffer in 0.15*M* NaCl; they are then fixed in 1 percent

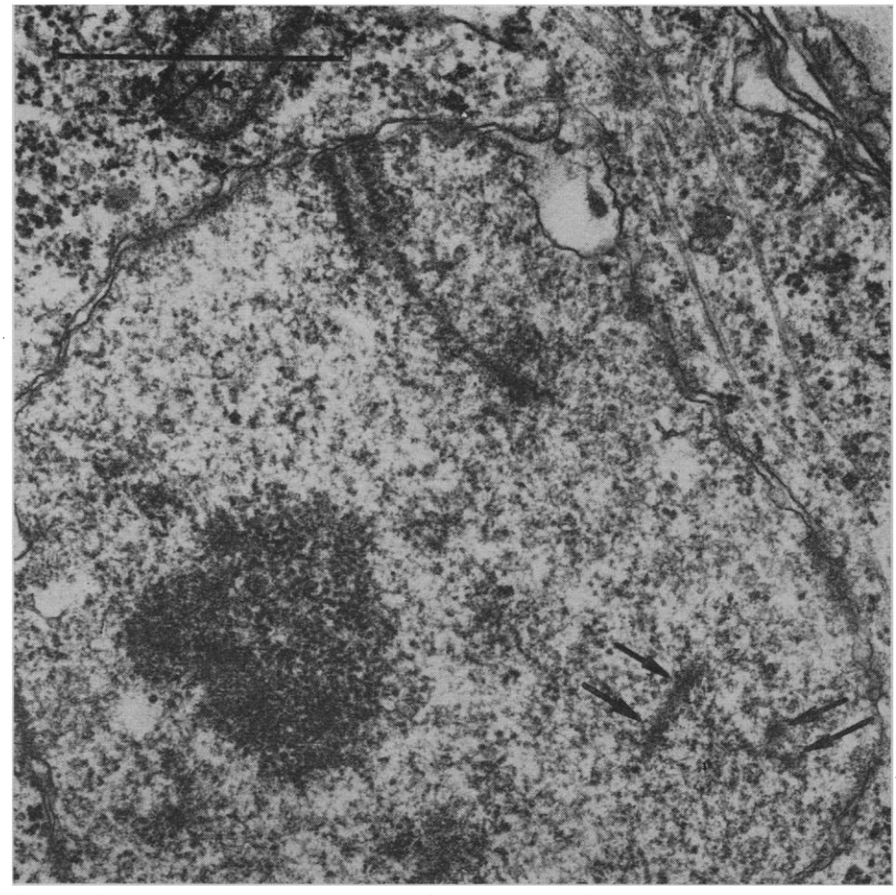

Fig. 1. Meiotic prophase nucleus of *Labyrinthula* sp. One synaptinemal complex in longitudinal section ends on the nuclear membrane. The two dense parallel bands are the lateral elements. In cross section the complex consists of two dark dots, marked by arrows.