RECENT PROGRESS IN RESEARCH ON AUDITORY MECHANISMS

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REVIEW

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INTRODUCTION

After publication of my monograph (64) there were still many unanswered questions about reception of sound in the ear and processes in the audio tract. Most of the remaining questions have not been answered yet, but progress continues. I present here, a review of some investigations, their results, and a few possible conclusions that might be drawn.

Transmission of mechanical stimulation of the hairs is accomplished by relative shear motion between the tectorial membrane and the basilar membrane (23, 135), and it has been established that the edge of the tectorial membrane is attached to Corti's organ by a mesh rather than a solid membrane (121), so the subtectorial space is not isolated from the scala media. Hairs protrude from the apical surface of the cochlear hair cells in Corti's organ into the fluid in the subtectorial space. The tips of at least some, if not all, of the outer hair cells are firmly attached to the tectorial membrane, as shown by electron micrographs by Angelborg and Engström (3) and by Takasaka (119). It has been shown that the inner hair cells are not attached to the tectorial membrane (19, 99). The process of transduction of sound energy into electrical energy takes place in

the hair cells and probably starts in the hairs themselves (54).

Davis (23) proposed a variable resistance model of mechanoelectric transduction in the hair cell membrane. Russell and Sellick (99) reported measurements of d-c resistance and d-c potential changes in response to tones delivered to the tympanic membrane in guinea pig inner hair cells. They reported that d-c potential changes were proportional to d-c resistance changes for a broad range of tone levels and frequencies, and concluded that this result was inconsistent with the variable-resistance model proposed by Davis (23). Weiss *et al.* (125) showed that this proportionality is consistent with the Davis model, as a special case of the general property of Davis' proposal. Russell (97) later reported results from which he drew conclusions that were in agreement with the Davis model.

MECHANICS

The electron microscope has revealed cross-links between the stereocilia in the guinea pig organ of Corti. These include links between the side walls, and from the tip of shorter to the wall of the adjacent longer stereocilia. The authors suggest that distortion of the links might produce sen-

sory transduction (92). A somewhat similar kind of linkage was shown in the lateral-line organ of the sea eel (39).

Rhode (96) reviewed frequency selective mechanisms in the cochlea up to 1980. It is not yet clear whether the frequency selectivity of basilar-membrane motion is as sharp as that of the peripheral nerve-fibre activity.

Holton and Weiss (48) observed what they considered to be a frequency-selective mechanism between the motion of the basilar membrane of alligator lizards and generation of the receptor potential. They hypothesized that this additional mechanism is a product of mechanical resonance of the stereocilia of a hair cell with resonant frequency depending on tuft height. Correlation between frequency and tuft height, in the restricted range (2–3 kHz) for which they obtained measurements of both, led them to suggest that the frequency selectivity of the hair cell receptor potential and the nerve-fibre discharges are manifestations of a common, peripheral frequency-selective mechanism in three stages. Stage one includes the mechanical properties of the middle and inner ear; stage two is a zero-memory, non-linear transduction; and stage three represents the electrical properties of the hair cell. Their results were consistent with the hypothesis that tuning involves electrical resonance in the hair cell membrane (17). They presumed the non-linearity to be confined to the micromechanics of the stereocilia and/or to the mechanoelectric transduction of these displacements into membrane conductance changes or perhaps to some mechanism that couples these processes, as suggested earlier by Weiss (123).

Transduction and tuning were studied using *in vitro* preparations of the relatively simple and hardy inner ears of the saccular macula of the bullfrog (13, 14, 55), and the alligator lizard (47). The individual stereocilia of a hair bundle provide essentially full response with $\pm 2^\circ$ flex. The size of the response and the sensitivity of the transducer decline roughly as the cosine of the angular deviation (114).

The relationship between hair-bundle deflection and receptor potential had a time dependence that appeared to act as a high-pass filter causing attenuation and a phase lead in the response at low frequencies. This dependence is a property of the transduction process (27) which is extremely rapid. Responses followed step stimulation by no more than 45 μsec at 22°C, and by less than 15 μsec at 37°C (12). Response amplitude and rate of relaxation to a new steady state increased with increase of stimulus ampli-

tude (14).

Frequency-dependent compressive non-linearities similar to those found by Holton and Weiss (48, 49) in the receptor potential of alligator lizard hair cells have also been reported for basilar membrane motion (74, 95, 108), in receptor potentials of inner hair cells (38), and in discharges of cochlear nerve fibres (42, 101) in mammals. Non-linear basilar membrane damping due to compressive non-linearities might thus be found in more than one species, including mammals.

Tone induced receptor potentials in cochlear hair cells have been measured in several preparations: *in vitro* in the basilar papilla of the red-eared turtle (15–18, 30, 31); and *in vivo* in the basilar papilla of the alligator lizard (48); in the guinea pig, in inner hair cells (97–99, 107, 110, 111); in outer hair cells (20, 21, 99, 120); and in the Mongolian gerbil (38). Differences in results from these studies must consider maximum responses and sensitivities of the hair cells as well as mechanical and electrical characteristics of the ears.

Frequency selectivity is evident in the receptor potentials of all of these preparations. In the turtle, Crawford and Fettiplace (15, 17, 18) interpreted sharp frequency selectivity to indicate a broadly selective mechanical filter followed by sharply selective electrical resonance of the hair cell membrane. Holton and Weiss (49) maintain that the hair cell stereocilia in the alligator lizard have a sharp mechanical resonance, the transduction from stereocilia displacement to resistance change is non-linear, and the electrical properties of the hair cell membrane act as a low-pass filter that does not substantially sharpen the frequency selectivity of the mechanical input to the hair cell. They suggest more than one frequency-selective mechanism between the impressed sound and the neural results, with not all functioning to the same extent in every preparation.

Lewis and Hudspeth (75) found that isolated bullfrog sacculus hair cells oscillated in response to injected current pulses. The oscillation current frequency depended on the polarity and magnitude of the current. They also observed ionic currents, some of which appeared to be related to the oscillation. They concluded that the oscillations might depend on certain ion conductance changes and could be consistent with sharp frequency responses.

When measuring basilar membrane vibrations, some investigators did not monitor the condition of the cochlea. The mechanical fre-

quency response of the basilar membrane is susceptible to trauma which affects its frequency response, mainly in the peak region. This might lead to the relatively broad frequency response that has been observed by most early investigators. During experiments on cats, careful monitoring of the round window cochlear microphonic (CM) response and histological examination of the cochleae were used by Khanna and Leonard (69) to evaluate the condition of the cochlea. Light from a He-Ne laser was reflected from a gold crystal mirror on the basilar membrane. Motion of the membrane was assessed from interference between the incident and reflected light. Basilar membrane vibration was measured for sound pressure levels about 15 dB above the noise, corresponding roughly to displacement amplitude of about 10^{-8} cm or less. Basilar membrane tuning curves were compared to histologically determined cochlear damage. Cochlear damage decreased sensitivity and shifted the response peak slightly lower in frequency. From minimally damaged cochleae, the authors obtained response curves much closer to neural measurements than any previously reported. The exceptional precautions to minimize cochlear damage also resulted in peak basilar membrane responses larger than previously seen.

Sellick and his colleagues (89, 109) found that tuning curves were much sharper when the Mössbauer source was near the edge than when it was near the middle of the basilar membrane. They suggested that previous results probably included artefacts due to source position. While the organ of Corti moves in a direction approximately perpendicular to the planes of the basilar and tectorial membranes, these two membranes also move relative to each other in directions parallel to their planes. The apical ends of the hairs of the outer hair cells attached to the under-surface of the tectorial membrane are thus moved to-and-fro which bends the hairs. This process is discussed in more detail by Zwislöcki (135).

The following equation, with appropriate values for the constants a , b , c , can be used to approximately define the vibrations of the basilar membrane observed up to the present time. The only differences between earlier and later observations are the values of the constants.

$$Y = \left[\left(\frac{X}{X_0} \right)^a - \left(\frac{X}{X_0} \right)^b \right] \sin \left[\pi \Phi \left(\frac{X}{X_0} \right)^c \right]$$

Y is the transverse motion of a point on the basilar membrane at distance X from the stapes. X_0 is the point where the motion reduces to zero on

the apical side of the mechanical response peak. X_c is the point of maximum amplitude.

$$X_0 = X_c \left(\frac{\Phi}{\Phi - 0.5} \right)^{1/c} \quad (\text{approximately})$$

The envelope of the wave is determined by $(X/X_0)^a - (X/X_0)^b$. Φ accounts for the phase shift. The term $(X/X_0)^c$ accounts for the change in wavelength along the membrane.

This is an empirical equation that approximates the response curves published by von Békésy (6), Rhode (95) and Khanna and Leonard (69). Replacement of X by f accounts for the frequency response at a fixed point, X , on the basilar membrane. The equation was developed for a computer analysis, and no pretense is offered for its physical meaning (Simpson, unpublished results).

COCHLEAR EMISSION

It has been known for some time that some human ears emit narrow-band acoustic signals spontaneously and continuously. Spontaneous cochlear oscillations suggest metabolic energy conversion to mechanical oscillation. Gold (37) suggested that an active process might enhance the selectivity and sensitivity of cochlear analysis, a suggestion that was supported by the work of Kemp (66), Kemp and Chum (68), Wilson (127), Wit and Ritsma (129), Rutten (100), Anderson (2), Kim (71) and Zurek (133). Findings have led to models of normal cochlear mechanics involving active and nonlinear processes (25, 67, 72, 128).

Zurek and Clark (134) demonstrated acoustic emissions in the ears of chinchillas. They reported continuous narrow-band otoacoustic emissions, apparently induced by exposure to a variety of high-intensity sounds and the absence of spontaneous emissions in ears of healthy chinchillas. Exposure to an octave band of noise centered at 0.5 kHz induced 4–7 kHz emission and this could be suppressed by external tones. They concluded from the frequency selectivity and time course of the suppression that the emissions originated in the cochlea.

Models of active involvement of hair cells have been proposed for generating sharply tuned basilar membrane responses (20, 29, 37, 67). Since the stereocilia of the outer hair cells are attached to the tectorial membrane (76), some models depend on active hair cells. It has been suggested that hair cell stereocilia produce a force proportional to displacement, or to velocity (as cited in 53). Either could be unstable and this could

account for objective tinnitus, sound emitted from the ear. If either model is valid, hair cells must undergo very rapid mechanical changes.

Emission induced by traumatic noise exposure suggests an active mechanical process that could assist normal cochlear functioning, but it might also be associated with damage to the organ of Corti and not with normal function. The high incidence of acoustic emission in humans and its absence in healthy chinchillas could indicate different histories of noise exposure, so its existence does not prove active mechanical assistance in normal cochlear functioning.

Hubbard and Mountain (53) reported electromechanical phenomena in the cochlea that were consistent with a hair cell force-generating mechanism. Sinusoidal electrical current in the scala media induced rapid mechanical changes that seemed to be related to the hair cell receptor potential. Alternating current in the scala media of the gerbil cochlea modulated the amplitude of a test tone measured near the eardrum. Variation with acoustic stimulus parameters and observed physiological vulnerability indicated a biophysical origin of the process, so cochlear hair cells might play an active as well as a passive role in cochlear micromechanics. Mountain and Hubbard (83) also measured phase and intensity of electrically induced cochlear emission. They suggested that the delay of the emission represented the travel time of the induced traveling wave, and that low frequencies predominantly stimulated the middle portion while high frequencies stimulated the basal end of the cochlea.

Two tone acoustic and electric stimuli produced distortion products in the ear canal at the frequencies of the sidebands. These acoustic distortion products disappeared or changed in damaged or dead cochleae. Acoustic components at the frequency of electrical stimulation and its second harmonic could be reduced in size after exposure to loud sounds indicating that they too were of physiological origin. Sidebands were related to the electrical frequency and not its harmonic, which was the larger of the two acoustic components at the eardrum. Acoustic emissions were not observed for electrical frequencies below 150 Hz, but these electrical stimulus frequencies produced large sidebands. It was concluded that sideband generation was a physiological, electromechanical effect and not simply a mechanically produced, two-tone effect. At the acoustic frequencies used, cochlear mechanics are thought to be dominated by the stiffness of the basilar membrane (53).

The authors proposed that the stiffness of the basilar membrane was due in part to the stiffness of the hair cell stereocilia. They argued that the presence of actin (33, 102) and myosin (77), and their arrangement in cochlear hair cells (26, 34, 61, 122) might give hairs their stiffness and contribute to their electromechanical capability. Current through the organ of Corti could dynamically modulate that stiffness by modulating intracellular Ca^{2+} concentration, since the stiffness of frog hair cell stereocilia depends on Ca^{2+} concentration (88).

Kemp (65) used signal averaging to study response of the closed external acoustic meatus to acoustic impulses near the threshold of audibility. About 5 msec after the stimulus, the oscillatory response decay time constant was found to change from approximately 1 msec to more than 12 msec. This slowly decaying component was present in all normal ears tested, but not in ears with cochlear deafness. Its origin was suggested to be some nonlinear mechanism in the cochlea that responded to auditory stimulation, and depended on normal cochlear functioning, possibly through the actin in the sensory cell hairs (26, 33, 102, 122).

Brownell (8) reported dissociation and cell culture procedures for isolated cochlear hair cells. He found a nearly fourfold difference between the lengths of the shortest and longest outer hair cells, perhaps related to the morphology of the basal and apical cells. The mechanical properties of outer hair cells were quite different from those of other cells in the organ of Corti. They were stiff and resisted electrode penetration in the lateral walls. Some stress induced changes were reversible if the stress was removed. For example, the appearance gradually changed during several hours in 10 mM Ca^{2+} , and was restored by 0.3 mM Ca^{2+} .

Outer hair cells often contracted in response to a buzz while other cells were unaffected. Depolarizing intracellular current injections decreased cell length and increased width. Hyperpolarizing currents evoked the opposite response. This motile response ceased upon cell death. Transcellular stimulation with sinusoidal current changed length and width comparable to changes evoked by intracellular stimulation. The most effective transcellular stimuli seemed to be at optimal frequencies that varied from cell to cell and for the same cell over time. Optimal electrode positions were those that produced the largest potential gradients along the length of the cell, while gradients across the cell were ineffective. Ionophoretic application of acetylcho-

line to the synaptic end of the cell also evoked the motile response.

The results indicated the possibility of electro-mechanical coupling in outer hair cells and bidirectional transduction in the organ of Corti. The motile response to acetylcholine could indicate olivo-cochlear innervation of outer hair cells that act as motoneurons to modulate sensitivity and tuning in local areas of the organ of Corti.

INTRACELLULAR RECORDING

The values of resting potentials measured in hair cells vary widely, probably due to varying injury by the penetrating electrode. The resting potentials measured in guinea pig cochlear hair cells are rather low, ranging from -20 to -45 mV in inner hair cells (89, 99) and about -5 to -29 mV in outer hair cells (120).

Reported intracellular resting potentials and receptor potentials of various preparations are summarized in Table 1, along with the sources.

Intracellular recordings were made from outer hair cells in the third turn of the guinea pig cochlea, and the electrical characteristics of the cells were compared to those of inner hair cells, supporting cells, and extracellular spaces from the same recording region (21). Outer hair cells have higher membrane potentials than do inner hair cells, but they produce smaller a-c receptor potentials. In the frequency region where tuning is optimal, both cell types produce depolarizing d-c receptor potentials, but outer hair cells also generate hyperpolarizing responses at low frequencies.

Comparison of a-c and d-c receptor potentials

recorded from an outer hair cell reveal several differences. Outer hair cells produced both depolarizing and hyperpolarizing d-c responses, whereas the d-c output of inner hair cells was always positive (99, 111). The d-c response of outer hair cells appeared to be much more sharply tuned than the a-c component. Around the best frequency of the cell, the a-c and d-c response magnitudes were commensurate. The behavior of the d-c receptor potential was similar to patterns described earlier for the gross summing potential (22, 24, 50), which also changed polarity along the frequency axis. It has been shown that the summing potential is much more sharply tuned than the gross cochlear microphonic (19), further suggesting similarity between intracellular responses of outer hair cells and gross cochlear potentials. Intracellular a-c and d-c responses peaked at the same frequency when measured at low sound levels. At higher stimulus magnitude, both responses saturated, and the a-c response peak shifted to lower frequency.

Basilar membrane motion has been compared with intracellular recordings from inner hair cells (89, 109) at similar locations in different guinea pig preparations. They concluded that the mechanical input to the inner hair cells is nonlinear and frequency dependent while the transduction process is also nonlinear, but independent of frequency.

CENTRAL ORGANIZATION

Tonotopic organization was reviewed by Aitkin (1) and updated by Katsuki (64). Imig and

Table 1 *Intracellular Potentials Observed in Certain Hair Cells*

Species	Organ	Resting potential (mV)	Receptor potential (mV)	Source
Fish	lateral-line	10-65	≤ 2	(35)
Mudpuppy	lateral-line	—	0.03-0.8	(41)
		20-69	—	(130)
		20-60	0.1-0.5	(104)
		60-80	15.5	(7)
Bullfrog	sacculle	56-60	5-10	(56)
Lizard	lagena	75-103	≤ 3	(124)
Turtle	lagena	~ 50	30-45	(15)
Guinea pig	cochlea (IHC)	25-45	5-17 (DC) ≤ 4 (AC)	(99)
	cochlea (OHC)	≤ 29	~ 4 (AC)	(120)

IHC, inner hair cell; OHC, outer hair cell. Taken from reference 64

Brugge (59), combining electrophysiology with retrograde and anterograde horseradish peroxidase and tritiated proline tracing, found that summation columns were rich in callosal innervation. Imig and Reale (60) studied anterior and posterior auditory field projections to the primary auditory area, AI, using tritiated amino acids. These projections connected similar best frequency regions in each field with terminations predominantly in the suppression columns. Cortical neurons along isofrequency lines were subdivided into functional groups. Middlebrooks and co-workers (81, 82) concluded from directional sensitivity found in cat AI that there are two systems: one contributing to detection and/or analysis of non-spatial features of sound, and the other suited to sound localization and scrutiny. These two systems might correspond to the excitatory-excitatory and excitatory-inhibitory stripes found by Imig and Adrián (58).

In subcortical nuclei, functional organization has been explored to a limited extent. Different parts of the cat's inferior colliculus contain different types of 'binaural' neurons (112). In the Mexican free-tailed bat, *Tadarida brasiliensis mexicana*, one part of the inferior colliculus can process biosonar information, while another part may be used for communication (11).

Calford *et al.* (10) examined large samples of single unit tuning curves at various levels of the auditory pathway. Their conclusion, based on bandwidth at 20 dB above threshold, was that the sharpness of the tuning curves was independent of frequency and did not change with level in the auditory pathway. This seemed to contradict the much earlier work of Katsuki (62, 63) on the cat and monkey and the more recent results of Suga and Manabe (117) showing progressive sharpening of the frequency response from the periphery to the medial geniculate body of the bat.

In a recent analysis of the results of many investigators, including their own, Suga and Tsuzuki (118) determined that audio pathway tuning curves are not independent of sound pressure level. They suggested that 50 dB above threshold might be a more appropriate level to make such measurements, although this depends on the ultimate purpose of the investigation. They point out that Calford might obtain results more nearly in agreement with their own (117) and those of Katsuki (62, 63) if the measurements were made at 30 or 50 dB above threshold.

Suga and Tsuzuki state that Katsuki (62, 63) gave the impression that all responses become sharper at higher levels of the audio tract, but not all do so. This author agrees with both points.

They also discuss lateral inhibition and level-tolerance at peripheral and central locations, especially in relation to functional stimuli.

Niimi and Matsuoka studied thalamic afferents to the auditory cortex in the cat by retrograde axonal transport of horseradish peroxidase (85). They describe projections from the medial geniculate body to the primary auditory area (AI), the secondary auditory area (AII), the suprasylvian fringe, the ectosylvian area, the insular area, the temporal area of the second somatic sensory area (SII); and from the pulvinar nuclear group to the auditory cortex. Many of the connections that they describe strongly suggest tonotopic organization.

Deoxyglucose autoradiography revealed tonotopic organization in the auditory tract. Isofrequency laminae were identified except in the medial geniculate body and the auditory cortex (80). The deoxyglucose method, developed to measure simultaneous glucose utilization in components of the central nervous system in conscious animals, promises to facilitate the study of cortical organization, among other brain characteristics (116). The same carrier that transports glucose across the blood-brain barrier also carries 2-deoxyglucose (2-DG) bidirectionally between blood and brain. In cerebral tissues it is phosphorylated like glucose by hexokinase to produce 2-deoxyglucose-6-phosphate, but unlike glucose-6-phosphate it cannot be metabolized further. There is relatively little glucose-6-phosphatase and even less deoxyglucose-6-phosphatase activity in the brain. Thus, 2-deoxyglucose-6-phosphate, remains essentially trapped in cerebral tissue for at least 45 min (115). The use of ^{14}C to synthesize the 2-DG, or ^{18}F to replace one of the H atoms and make ^{18}F -fluorodeoxyglucose permits detection of the trapped metabolites.

Autoradiography has been used to measure blood flow (94, 103) in the study of various physiological and pathophysiological states in animals, but the radioactivity can also be detected by other means. Computed tomography (CT) is the scanning of a region of interest with a moving detector and coordinating the data with a computer (52, 90). Ido *et al.* (57) and Barrio *et al.* (5) have described the preparation of ^{18}F -fluorodeoxyglucose for CT.

Positron computed tomography (PCT) can reveal functional anatomy, so the effects of sensory stimulation and deprivation can be examined in human subjects. PCT experiments with sensory stimuli, including visual, auditory, and tactile modalities have revealed stimulus-

response relations in the brain of humans that could previously be obtained only from animal studies using invasive techniques (93). Finklestein *et al.* (32) described relative left-right differences in oxygen and glucose metabolism in normal subjects under resting conditions, and left-right symmetry of sensory inputs has been demonstrated (4, 36, 79). CT including PCT and other forms such as X-ray scanning can now be used to reveal complex results of higher cortical functions (91).

TRANSDUCTION AND ACTION SITES

Ohmori (86, 87) studied ionic currents in enzymatically isolated vestibular hair cells of the chick by a whole-cell-clamp variation of the patch voltage clamp, with single channel recording. The hair bundle was directly stimulated by a glass rod, and transduction currents were recorded from the cell body. Triangular wave shape of the stimulating probe motion generated stepwise transduction current between discrete levels of amplitude. Ionic currents through Ca channels, Ca-induced K channels and anomalous rectifier channels were found.

To determine the site in or around a hair bundle at which transduction begins, potential differences due to current flow toward sinks at transduction channels were measured in the medium immediately surrounding a continuously stimulated hair bundle (54). The strong signals near the distal ends of the hair bundles, suggested active transduction channels near the tips of the stereocilia or within the region where adjacent stereocilia contact each other.

Corey and Hudspeth (12) reported that the transduction channel appeared to them to be a pore, about 0.6 nm in internal diameter, with little capacity to distinguish among small cations. These authors found that iontophoretic application of aminoglycosides to the hair bundle reversibly blocked transduction within milliseconds. They suggested that the positively charged amino or guanidinium groups of aminoglycoside antibiotics and other cationic ototoxins enter the transduction channel and block it because of their bulky sugar moieties. Simultaneous recording of endocochlear potential and Na^+ activity during noise stimulation or kanamycin treatment by Konishi and Mori (73) indicated that Na^+ permeability of the endolymph-perilymph barrier does not contribute to the results of noise damage or kanamycin toxicity.

Ototoxic effects of aminoglycosides have been clinically observed (43, 106). The site of this

toxicity on polyphosphoinositides (PPIs) was reported by Schacht (105). The site of action has been confirmed and studied. The effects are probably due to competition for the binding site between Ca^{2+} and the aminoglycoside (46, 78, 113).

Shiozawa and Yanagisawa (113) showed that neomycin caused complete suppression of afferent synchronization to mechanical stimulation, but had no effect on the hair cell chemical response to NaCl. When CaCl_2 was added to NaCl, the response to NaCl was suppressed markedly, but this effect was also unchanged by neomycin. The reversibility of the suppression by neomycin agreed with the conclusion of Wersäll and Flock (126) that streptomycin primarily affected the surface membrane of the receptor cells.

Phosphoinositides turn over much more rapidly than other lipids. Schacht (106) demonstrated a high rate of PPI metabolism in the inner-ear, and interference with this by neomycin. Reversal of the neomycin suppression by application of excessive calcium verifies the competitive occupation of sites on the membrane by neomycin and Ca^{2+} (132). Evidence that hydrolytic attack of the phosphomonoesterase splits off the monoester phosphate to liberate Ca^{2+} and change membrane permeability to cations stems from experiments on erythrocytes demonstrating correlation between PPI metabolism and Ca binding (9). Intermittent sound pulses at 75 dB sound pressure level increased the metabolic activity of PPI monoester and the uptake of ^{32}P by monophosphoinositide in the auditory organ of noctuid moth (70). PPIs thus seem to be involved in the transduction of sound energy to electric energy.

There are three well known, possible means of ion transfer across lipid bilayers (28). The energy necessary to transfer many hydrophobic ions is not excessive (84), so ions of this type, which are frequently adsorbed on membrane surfaces, could readily move through the membrane without requiring special channels. Another method of membrane transport involves carrier molecules and the third is by way of polar heterogeneities or channels. The last affords selectivity, but the best electron microscope studies to date have shown no indication of protein molecules in the hair membranes that have come to be associated with membrane transport (40, 119), although such proteins might exist. Triphosphoinositide (TPI) did not affect membrane permeability in bilayer lipid membranes, but its lysotype compound was a highly effective channel type ionophore (45). It was concluded that the

Ca^{2+} -TPI interaction regulated membrane conductance by closing the ion channel gates.

Interactions between drugs, Ca^{2+} and PPIs were measured by means of ^{31}P nuclear magnetic resonance (44). Ca^{2+} at low concentration interacted with the 4- and 5-phosphates of TPI, simultaneously; but not with the phosphodiester of the lipid. At high Ca^{2+} concentration the phosphodiester reacted to Ca^{2+} , while the two phosphomonoesters, which were probably saturated, were not affected further. The reaction to neomycin was less than that to Ca^{2+} alone, but pretreatment with neomycin prevented further reaction to Ca^{2+} . This was attributed to neomycin occupation of all available reaction sites.

Maeno and Enomoto (78) analyzed the interaction of Ca^{2+} and streptomycin using 4-aminopyridine, a Ca influx facilitator. They proposed a Ca binding site, X, and formation of an X- Ca complex. The X site might be TPI. If so, their results verified that the Ca^{2+} -TPI interaction is also important in neuromuscular transmission.

Yoshioka and Inomata (131) examined the cross reactivity of a monoclonal antibody to TPI with other phospholipids together with the suppressive effect of neomycin. The monoclonal antibody against TPI reacted with phosphatidylinositol phosphate (DPI), phosphatidylinositol (PI) and phosphatidic acid (PA) in the order $\text{PA} > \text{DPI} > \text{PI}$, but not with phosphatidylcholine. It was concluded that the monoclonal antibody against TPI can recognize the monoester phosphate as well as the inositol ring, but not the diester phosphate of phospholipid, so it can recognize the number of phosphates in the inositol ring. Since neomycin suppressed the reactivity of the antibody to TPI, and neomycin bound to the 4- and 5-phosphates of TPI selectively interfered with Ca binding (44), and neomycin can selectively bind to TPI, the authors assumed that the neomycin binding site and the antibody binding site of TPI might be the same. The polar head of TPI, is known to change in nature from hydrophilic to hydrophobic upon combining with Ca^{2+} . This characteristic of TPI could constitute an ion gate that can be controlled by Ca^{2+} .

Horikoshi *et al.* (51) stained stereocilia of guinea pig cochlear hair cells by using anti-TPI serum with peroxidase-antiperoxidase (PAP) staining. Stereocilia of both inner and outer hair cells were stained, but not the cell bodies of hair cells or supporting cells. The stereocilia were stained uniformly. After the antiserum was absorbed by incubation with excess TPI, the reactivity of the antiserum to TPI was reduced. When the antiserum was applied in the presence

of neomycin (10^{-3} – 10^{-4} M), PAP staining intensity was reduced. Decreased reactivity of the antiserum after incubation with excess TPI, or in the presence of neomycin suggests that both bind to the same site, probably TPI, in the cochlea.

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