MINIREVIEW

Sound Reception and Synaptic Transmission in Goldfish Hair Cells

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There has been much progress in peripheral auditory physiology since the 1970s, especially in elucidating the mechanism of action of sensory hair cells and the role played by inner and outer hair cells regarding tuning in the cochlea. Since 1967, our group has been engaged in the study of peripheral mechanisms of sound reception in the goldfish sacculus, i.e., its inner ear [21, 23, 24]. Although the goldfish sacculus is an otolith organ, it is specialized for the reception of low frequency sound, in the range from a few hundred to a little over 1 kHz. In many aspects, it resembles a cochlea rather than a vestibular organ, as indicated by the absolute sensitivity which is about the same as in the human ear [65], and by the fact that the effect of efferent nerve stimulation is purely inhibitory, as in the cochlea [18]. A similar stimulation more often produces facilitatory rather than inhibitory effects in vestibular organs in fish as well as in mammals [36]. The goldfish sacculus, like the cochlea, is an organ with intense metabolic activity, as indicated by the high susceptibility to hypoxia (Suzue, Wu, and Furukawa, in preparation). The present review article deals with aspects of the goldfish sacculus which seem relevant to a better understanding of peripheral auditory mechanisms.

A. DIRECTIONAL SENSITIVITY AND NON-LINEARITY OF HAIR CELL ACTIONS

The proposal of a directional sensitivity of hair cells was formulated by Löwenstein and Wersäll [56] toward the end of the 1950's. They reasoned that a bending of hairs toward kinocilium must be excitatory, while the bending to the opposite direction is suppressive or non-excitatory. This epoch-making discovery was soon followed by report from Flock [15] who suggested that the transfer characteristic of hair cells must be non-linear. Intrigued by these reports which reached us through Prof. Y. Katsuki, we started investigating the goldfish sacculus.

1) Saccular macula of goldfish

Saccular macula is a small strip of tissue extending rostro-caudally for about
5 mm with a dorso-ventral width of about 1 mm, in goldfish which are about 12 cm length. By exploring with microelectrodes the saccular macula of anaesthetized fish, we found that the microphonic potentials recorded from the dorsal and ventral halves of the macula were evoked 180° out of phase [21, 23]. It thus became evident that hair cells in the dorsal and ventral halves of the macula are orientated in opposite directions and that two groups of hair cells are stimulated alternately by dorso-ventral movement of the saccular otolith. Based on the scheme proposed by FRISCH [17] on the manner in which vibration of the endolymph is transmitted to the saccular otolith (see also [64]), we assumed that hair cells located in the dorsal and ventral halves of the macula must be oriented with their kinocilium pointing dorsally and ventrally, respectively, as shown in ultrastructural studies of HAMA [30].

Scanning electron microscopy proved to be a useful tool for study of hair-bearing surface of the sensory macula. Details not only of hair cell orientation, but also of distribution over the macula of hair cells with different lengths and numbers of sensory hairs have been worked out [64].

2) Stimulus transduction and transfer characteristics of hair cells

The hair cell is a specialized epithelial cell which lines the endolymphatic space of hair cell organs. Thus the hair-bearing surface faces the K-rich endolymph, while the basolateral membrane faces ordinary tissue fluid. The general framework of variable resistance theory, as proposed by DAVIS [10] to explain the mode of action of hair cells, remains acceptable [4, 68]. Together with the contributions by BÉKÉSY [1] and also by KATSUKI [44], Davis' variable resistance theory was a landmark during the period when the physiology of peripheral auditory mechanisms was being elucidated. It must be mentioned, however, that proposals which clearly contradict the variable resistance theory were also presented from time to time.

In the 1970s, hair cell physiology entered a new era when RUSSELL and SELLICK [69] succeeded in obtaining intracellular recordings from cochlear inner hair cells. Their studies led to great progress in cochlear physiology, in that they demonstrated that the tuning curve of single inner hair cells was practically as sharp as the tuning curve of a single auditory fiber. They showed that the receptor potential of a single inner hair cell consists of DC and AC components. The amplitude of the former increased with an increase in sound frequency. The generation of the DC component was attributed to a non-linearity of the transduction channel. If it is assumed that the resistance of the channel varies linearly with the degree of hair deflection, then the transduction current would increase or decrease to the same extent as the hairs are bent alternately toward excitatory and suppressive directions. In such a case, no DC-component would result. Actually, there is a marked non-linearity in the transduction, as indicated by the finding that, while the inward flow of transduction current increases with deflection of hairs into the excitatory direction, only a slight reduction is observed in the transduction current by the bending of hairs in the opposite direction. It can be said that the transduction...
channel behaves like a half-wave rectifier. The DC component of the receptor potential results from the rectifying property of the transduction channel and from the electrical capacity of hair cell membrane which serves as the smoothing condenser.

The notion of non-linearity of the transduction can be traced back to Flock [15]. However, a curve which related hair bending with the current flow was not worked out. Because of favourable recording conditions in the goldfish saccular macula, we observed fairly clearly how the reduction of the channel current was clipped when hairs were bent into a non-excitatory direction (see Fig. 1B, D). We also demonstrated that non-linearity was absent for a weak sound, for it appeared only after the amplitude exceeded a certain limit. The transfer characteristic of hair cells could be drawn not only around the neutral operation point but also under different amounts of static deviation [23]. With intracellular recording, the non-linearity of the transduction channel was found to be marked also in hair cells of the frog sacculus [4] and turtle cochlea [6]. These investigators emphasized the importance of non-linearity for the reception of high frequency sound, a point which we stressed in earlier papers (e.g., [22]).
3) Directional sensitivity and non-linear transduction in the cochlea

According to a scheme commonly employed, displacement of the basilar membrane toward the scala vestibuli is said to bring about hair bending toward the excitatory direction, i.e., bending of hairs toward the periphery of the cochlea. Conforming to this scheme, Watanabe [83] found that nerve responses to a rarefaction click occurred with a latency shorter than to the condensation click. However, data indicating an opposite interpretation have appeared (see [50]). The issue is not settled, but a key to the riddle resides in the different levels of stimulus used. Kiang and coworkers found that the response phase of afferent firings was suddenly reversed by 180° when sound intensity exceeded a certain level [48].

In hair cells of lower vertebrates such as frog or goldfish, non-linearity of the transduction channel can be attributed to the intrinsic property of the channel [4, 6, 23].

The case in the cochlear hair cells is more subtle. The receptor potentials of the outer hair cells often appear in a "balanced" shape, indicating a probable absence of intrinsic non-linearity in the transduction channel of the outer hair cells [68, 79]. However, the difference in electrical response between inner and outer hair cells now tends to be interpreted as being imposed on them through the interaction with the tectorial membrane, since no essential difference was observed between their electrical responses when mechanical stimulus was delivered directly to their sensory hairs [68]. Dallos [8] found no difference in this respect between inner and outer hair cells in the guinea-pig.

There are further complicating aspects in the cochlea, for example, in the effects of modulation by ultra-low frequency sounds [73]. Since the purpose of applying a ultra-low frequency sound was to give a static deviation to the position of the hair, the experiment resembled our earlier one on goldfish [23]. However, while our objective was to study the effect on hair cells with static deviations, the aim of their study was to demonstrate suppression or enhancement in the sensitivity of inner hair cells effected through a change in the activity of outer hair cells to which static deviation was applied (Inner hair cells do not directly receive the effect of static deviation, since the tip of the hairs is not attached to tectorial membrane).

B. IONIC CHANNELS OF HAIR CELLS

Because the hair cell is a specialized epithelial cell, properties of its hair-bearing membrane and those of basolateral membrane differ. Although our attention generally tends to be more focused on the transduction channel which is located on the former, the properties of the basolateral membrane have to be given attention.

1) Transduction channel

The high concentration of K in the endolymph was once believed indispensable for a high sensitivity of hair cell's receptor function. It was also assumed that the hair-bearing surface of the cell was selectively permeable to K. By perfusing the...
goldfish sacculus, we demonstrated that the hair-bearing surface was not necessarily selectively permeable to K, but perhaps was also permeable to Na. We proposed that the function of a high concentration of endolymphatic K would reside in the fact that K ions, which entered hair cells by carrying the transduction current, could easily be disposed of without charging extra energy [57]. That would not be the case if the transduction current were carried by Na. Our hypothesis concerning the role of endolymphatic K is now receiving increasing support (see below).

HUDSPETH and collaborators worked on the isolated frog saccular macula and observed intracellularly the effect of mechanical stimulus applied to sensory hairs [41]. Their main findings can be summarized as follows: 1) the resistance changes brought about by bending of sensory hairs occur with no detectable time delay, 2) the stereocilia are concerned with transduction and the kinocilium is not involved, and 3) the ion species which carry the transduction current are not limited to K but include other ions such as Na and Ca. Further, HUDSPETH [40] made it clear that the site of transduction was most probably located at the tip of the stereocilia.

OHMORI [62] used the patch clamp method and studied chicken hair cells. He clarified that the transduction channels were much more permeable to divalent cations such as Ca than to monovalent cations like Na or K. He also found that the current through the transduction channel changed in a step-wise manner. This was deduced from the finding that the conductances of transduction channel of a single chicken hair cell were multiples of unit conductance of about 50 pS, in Ca saline [63]. OHMORI [63] found that the transduction current was proportional to the angle of bending of hairs at their base, i.e., near their attachment to the cuticular plate. He pointed out that the site of transduction might be at the base of the stereocilia instead of at their tip, as indicated by the experiment of HUDSPETH [40]. Ohmori’s very interesting finding on the existence of quanta in the transduction current was not confirmed by HUDSPETH who observed no step-wise changes in the transduction current of single bullfrog hair cells [37, 41]. In Hudspeth’s model, each transduction channel would repeat an open/close event at a rate as high as 1,000/s and bending of hairs affects the average conductance of transduction channels by increasing or decreasing the probability of open time of each channel.

Aminoglycoside antibiotics, such as streptomycin, are potent ototoxic substances and with chronic systemic administration, produce permanent damage or loss of hair cells [9, 66]. When applied directly to hair cells, however, the aminoglycoside antibiotics produce blocking of the transduction channel [40]. Mechanisms of the blocking are yet to be worked out, but they may be similar to the action of tetrodotoxin on the Na channel of excitable membrane [61, 63]. In earlier studies on goldfish, we found that streptomycin could abolish the microphonic potentials only when applied to the luminal side, while an application to the basolateral side of hair cells was not effective, even at high concentrations [58]. This seems to be the first paper which explicitly states that application of streptomycin to the luminal side is much more effective than the application to the basolateral side. YANAGISAWA and coworkers demonstrated that stereocilia of cochlear hair cells
were selectively stained by the dye which was conjugated with the monoclonal antibody to triphosphoinositide [39]. Functional significance of the presence of triphosphoinositide at the outer surface of stereociliary membrane remains to be clarified.

**Function of supporting cells.** Hair cells in most hair cell organs are surrounded by supporting cells. Supporting cells are connected to each other via gap junctions [31]. In intracellular recordings from supporting cells of the goldfish sacculus, a slowly developing depolarization was observed, beside the microphonic potentials, during sound stimulation [19]. This observation indicates that supporting cells, like the glia cells during neuronal activity [51], may have a function in removing excessive K from the intercellular space during hair cell activity.

2) **Ion channels of basolateral membrane**

Corey and Hudspeth (1979) observed that the input resistance of frog saccular hair cells was highest at the resting potential while it decreased as the membrane was either depolarized or hyperpolarized from the resting level (see [4]). Later studies [41, 62] disclosed that the hair cell membrane lacks the Na channel, but has abundant Ca channels which provide inward currents upon depolarization of the membrane. Although the Ca channel exists at the presynaptic release site of the afferent synapse, it is not clear whether activation of these Ca channels is principally contributing to the Ca current observed in patch clamp experiments. The other channel which is opened by membrane depolarization is the Ca-activated K channel. This K channel is also well developed in the membrane of the goldfish hair cell. By recording with inside-out patch clamping, we discerned several Ca-activated K channels with slightly different unit conductances [78].

On the other hand, the reduction of input resistance by membrane hyperpolarization is due to activation of the inwardly rectifying K channel. The inwardly rectifying K channel was initially found in skeletal muscle fiber, but is known to play an important role in producing the plateau potential in the cardiac muscle. This channel was also found to be well developed in the goldfish hair cell membrane. We found three different types of inwardly rectifying K-channels, i.e., inactivating, sustaining, and slow types (Sugihara and Furukawa, in preparation). Of these three types, the inactivating type was distributed most densely throughout the entire surface of the basolateral membrane. Distribution of the slow type was more limited and the sustaining type was only rarely recorded. As a common characteristic, these three types of inwardly rectifying K channels have a high rate of open/close events compared with similar K channels detected in other tissues. Except for the difference in the speed of gatings, the inactivating type of channel had gating properties which resemble those found in the inwardly rectifying K channel of cardiac muscle cells [72]. The sustaining type [77] has a large unit conductance of about 100 pS, and showed steady state activities which resembled those of the inactivating type. However, despite the apparent similarity, the voltage dependency of gatings was opposite between the inactivating and sustaining types. The slow type
resembled the inwardly rectifying K channel of skeletal muscles by absence of inactivation and by being blocked at a low internal pH.

This inwardly rectifying K channel seemed to prevent unnecessary hyperpolarization and to provide a high sensitivity to the sensory stimulus near the resting membrane potential. The inward rectifying K channel in the cardiac muscle contributes to generation and maintenance of the plateau potential by decreasing the conductance when the membrane is slightly depolarized from the resting level.

Electrical tuning in hair cells. Apart from a rather sophisticated frequency tuning mechanism present in the mammalian cochlea [11, 43, 48], frequency tuning in some hair cell organs was found to depend on tuning mechanisms particular for each hair cell. One well-known case is the freestanding region of besilar papilla of the lizard where hair cells are arranged in the order of increasing length of their sensory hairs. In this case, the sensory hairs of different lengths (10–30 μm) underly the selective frequency sensitivity of hair cells ranging from 1 to 4 kHz [38]. Systematic differences in the length of sensory hairs has also been observed in the guinea pig cochlea. Another tuning mechanism is the electrical resonance of hair cells.

Electrical resonance was first detected in turtle hair cells (see [7]), but was also found to exist in frog saccular hair cells. According to HUDSPETH [41], electrical oscillation in frog hair cells (below 300 Hz) occurs through interference of the Ca and Ca-activated K channels. Activation of the Ca channel, by enhancing the depolarization evoked by the stimulus and also by raising the intracellular concentration of Ca, would trigger the activation of Ca-activated K channel which in turn would repolarize the membrane. However, upon repolarization of the membrane, the activity of the Ca-activated K channel would halt and a new cycle of membrane depolarization would lead to oscillatory membrane potential changes. It must be noted that activity of the Ca channel would raise only the local concentration of Ca ions just beneath the surface membrane. Spontaneous slow oscillations of membrane potential in certain epithelial cells have been attributed to repetitive activation of Ca-activated K channels [81]. In this case, however, the origin of cyclic activation of Ca-dependent K channel was attributed to cytosolic Ca oscillation resulting from cyclic releases of Ca from the intracellular stores.

In the turtle hair cells, movement of sensory hairs is produced in response to a change in the membrane potential [7]. Electrical oscillation in turtle hair cells thus cannot solely be attributed to the activity of ionic channels of the membrane. Because of a high temperature-dependence of ionic channels, frequency stability of the tuning tends to be poor when it is solely dependent on channel activities. It is difficult to elucidate the mechanism of a regular array of hair cells having slightly different tuning frequencies on the basis of the difference in the channel activities of individual hair cells. On the other hand, a higher frequency stability as well as a tuning to different frequencies would be attained more easily in a system which includes mechanical elements.

Motor function of cochlear hair cells. A most intriguing problem in auditory
physiology is the cochlear amplifying mechanism and otoacoustic emission attributed to the function of outer hair cells [11, 47, 49]. The outer hair cells serve motor functions rather than sensory ones, and are involved in the amplifying mechanisms and in otoacoustic emission. As the outer hair cells are built into the rigid structural framework within the organ of Corti, minute changes in the length of outer hair cells would induce a vibration of the basilar membrane. Basolateral membrane of the outer hair cells is ultrastructurally unique, thereby suggesting a possible involvement in motor function [16]. Changes in the length of the isolated outer hair cells have been observed upon direct electrical stimulation [3]. While the outer hair cells are unique, the inner hair cells share properties with hair cells in organs other than the cochlea.

C. AFFERENT AND EFFERENT INNERVATIONS

Most hair cells receive afferent and efferent innervations via chemical synapses. Mechanisms of action of these synapses, especially the manner in which information is coded into afferent impulses, deserves special attention.

1) Afferent hair cell synapses

By recording intracellular events in large afferent fibers of the saccular nerve of goldfish (our S1 fibers), the sound stimulus is seen to give rise to excitatory postsynaptic potentials (EPSPs) in the fiber terminals and afferent spikes are set up when the threshold is reached. A typical record of microphonic and nerve responses to a fairly strong sound is illustrated in Fig. 2. Here, spike potentials were blocked by locally applied tetrodotoxin. Note the presence of a synaptic delay of about 0.5 ms between each corresponding deflection of microphonic and nerve responses. The presence of such a delay attests to the chemical nature of transmission at the afferent synapse. Note also that the EPSPs are initially large in amplitude but do show a successive rundown thereafter, due to depletion of transmitter quanta at the presynaptic release site [21, 26, 27].

Graded activity of afferent synapse. In sensory synapses, the receptor potential, which serves as a presynaptic signal, varies with the stimulus intensity and is usually much smaller in amplitude than in the case of the ordinary synapse where the presynaptic signal consists of all-or-none spike activity. The output from the sensory synapse thus varies, depending on the stimulus intensity. It can readily be shown in records like Fig. 2 that EPSPs, though they are small for a weak sound, can reach an amplitude as large as 40 mV. Since the amplitude of the EPSPs is the result of a non-linear postsynaptic summation, the maximum amplitude of the EPSPs would exceed 100 mV, if the summation is linear. The maximum amplitude of the hair cell receptor potentials was 15–25 mV [42, 69].

Elucidation of the mechanism of the graded release of transmitter was facilitated by analysis of adaptive phenomena observed in the EPSP amplitude in the present material. The synaptic depression, i.e., decline or rundown in the EPSP...
size, attributable to a depletion of the transmitter quanta at the presynaptic release site has been noted with other synapses [53], but for the present case, the rundown takes place more markedly than at other synapses. Also, recovery from depression occurs much more rapidly (the time constant of recovery: 15–20 ms, compared to 4–5 s at the frog neuromuscular junction).

The meaning of "depletion" can be further elucidated by changing the stimulus

Fig. 2. Sample records of EPSPs and microphonics from the goldfish sacculus. Top: EPSPs recorded intracellularly from a S1 fiber; spike potentials were blocked with a local application of tetrodotoxin. Middle: intramacular microphonic potentials. Bottom: sound monitor (500 Hz, 95 dB SPL). From Furukawa and Matsuura [27].

Fig. 3. Incremental and decremental responses (A) and recovery from depression (B), observed as changes in the amplitude of the EPSPs. A: sound intensity was increased by 3 dB (from 98 dB SPL) in the top trace, and decreased by 1, 3, and 5 dB in the second, third, and fourth traces, respectively. EPSPs were completely suppressed until the time shown at arrows. B: test tone (same intensity as the adapting tone) was delivered at various intervals (dots) after cessation of the adapting tone. From Furukawa and Matsuura [27].
intensity. For example, even in a depressed state (in the presence of adapting sound), an increment in the sound intensity readily gives rise to additional releases of transmitter (incremental response). The EPSPs thus increased in amplitude run down again in the same manner as at the start of the sound. On the other hand, a decrease in intensity leads to a temporary reduction in the amplitude of the EPSPs (decremental response) (Fig. 3).

Apart from sensory synapses, the graded release of transmitter has been discussed also with conditions such as presynaptic inhibition or facilitation. The problem is whether or not the presynaptic impulse invades the terminal. While this is not the case at the endplate in the frog, block of the conduction seems to occur at some distance before the terminal in the crayfish neuromuscular junction [12]. Conduction block near the afferent fiber terminals is a matter of great concern also in the case of the mammalian spinal cord, for there is evidence which supports the view that an impulse of individual Ia sensory fibers may intermittently fail to invade some of the central terminals [34] (see also [52]).

Ultrastructural evidence. There is ultrastructural specialization which characterizes sensory synapses. Namely, the presynaptic site is marked with a presynaptic ribbon in the cochlear hair cells and retinal neurones or with a spherical dense body in hair cells of goldfish and other lower vertebrates [30, 32]. Synaptic vesicles are arranged in close association with these presynaptic organelles, hence the latter are assumed to attract synaptic vesicles from the cytosol and aid in moving them toward the presynaptic release site for a prompt replenishment [25, 35].

There is a great deal of similarity in ultrastructure between sensory and other ordinary synapses. Freeze fracture revealed that active zones of synapses generally occupy only a small proportion of the area of the presynaptic terminal facing the postsynaptic cell [31, 35]. The active zone in the lizard neuromuscular junction consists of short, single or two parallel rows which are rich in membrane particles [82]. Synaptic vesicles are arranged in parallel with the particle row or rows, i.e., on one side of it in the case of a single particle row (in slow fibers) or in between the two particle rows (in twitch fibers). The active zone particles would be calcium channel complexes through which this ion enters the terminal to initiate transmitter release [35, 67]. Thus, there would be differences between single and two-rows cases with regard to the accessibility of Ca to the synaptic vesicles. The active zones of hair cell-afferent fiber synapse conform to these general pictures. Each active zone of the ribbon-type synapse has a single particle row [71], while the active zone of the afferent synapse of the goldfish hair cell, which contains a spherical dense body, has multiple parallel particle rows [31, 32]. This leads to the idea that the afferent synapse of the goldfish hair cell is suited for a phasic release of transmitter while the mammalian hair cells are suited for a slower action.

Mechanism of graded transmitter release. In the squid giant synapse, postsynaptic response (i.e., EPSPs) is detectable when the presynaptic depolarization exceeds 30–40 mV, and further increase in the depolarization leads to a steep rise in the amplitude of the EPSP [46, 51]. In sensory synapses, a release of transmitter is
triggered by presynaptic depolarizations much smaller in amplitude than in the squid giant synapse. The difference most likely is related to intrinsic differences in the voltage sensitivity of the Ca channels at the active zone. Ca currents, recorded from isolated frog and chicken hair cells using patch clamping, showed properties roughly in accord with the above inference [41, 62].

It may simply be stated that a large presynaptic depolarization would bring about a large Ca inflow, and this, in turn, would result in a release of a large amount of transmitter. However, whether the effect of change in the presynaptic depolarization is similar to the effect of change in the Ca concentration of the medium requires further attention (see [84]). Here, we directed attention to the quantal release hypothesis of transmitter release [45, 51].

A fundamental assumption of the quantal release hypothesis can be stated as \( m = n \times p \), where \( m \) is the mean number of the quanta released upon stimulation, \( n \) is the number of available quanta at the presynaptic site (see below), and \( p \) is the release fraction. We have to know whether changes in the transmitter output \( m \) are mediated by changes in parameter \( p \) or \( n \). The value of these parameters can be determined experimentally, using binomial statistics [2, 20, 25].

**Multiple release sites model.** Our multiple release sites model was designed to explain graded release and adaptive phenomena in the hair cell-afferent fiber synapse [26, 27]. It is assumed in the model that (1) there are numerous release sites on the presynaptic membrane, and (2) each release site has its own threshold, so that release can occur from the site only when the threshold is reached. It is further assumed that (3) at the most, a single synaptic vesicle is allocated to each release site, and (4) when release does occur from an occupied release site, the site becomes empty and remains so until a replenishment from the store.

It follows from these assumptions that depletion would take place only at release sites, the threshold of which is reached. The incremental response can thus readily be explained, because an increment in sound intensity would trigger a new release from the sites with a threshold above the level of the adapting sound, sites that would have remained fully occupied.

**Results of quantal analysis of transmitter release.** Supporting evidence for multiple release sites was obtained by calculating release parameter \( n \) and \( p \) from statistical analysis of the amplitude of the EPSPs [20, 25]. Results of these studies indicated that it was the value of parameter \( n \) that changed under different conditions, including adaptive rundown, incremental as well as decremental responses, while the value of parameter \( p \) remained largely unchanged. It must be noted that \( n \) does not represent the number of physically existing synaptic vesicles. It is appropriate to our multiple release sites model to define \( n \) as the number of occupied release sites activated by sound intensity.

Proof for the quantal release hypothesis was obtained in frog neuromuscular junction placed in medium with a reduced Ca or increased Mg concentrations [45]. Since parameter \( p \) becomes small under such a condition, the amplitude of the endoplate potentials in a large series of observations is expected to distribute in a
characteristic manner as described by Poisson's law. Low extracellular Ca concentration was found to lower parameter $p$ also in the hair cell-afferent fiber synapse of goldfish [26]. To study the situation under normal Ca concentrations, it is necessary to use the binomial distribution (see [2, 51]). In the frog neuromuscular junction, Ca concentration in the medium influences not only the value of parameter $p$, but much more greatly the value of $n$ [2]. Our results mentioned above indicate that the amplitude of presynaptic depolarization mainly influences transmitter release by affecting the value of parameter $n$; however, the influence on parameter $p$ cannot be disregarded, particularly when sound intensity is varied over a wide range.

Anatomical support for the multiple release sites model. The model, as depicted above, should conform to the actual anatomical situation in the goldfish saccule. How many release sites would be available for each S1 fiber? The problem is important in view of the very large size of the EPSPs recorded and the small value of quantum size (about 0.4 mV in S1 fibers).

To explain the growth of EPSP amplitude in response to various amounts of sound intensity increment (in the presence of fixed intensity of adapting sound), one must assume that the amplitude of the EPSPs should increase with the sound intensity to a value at least as great as 100 mV (in the assumed absence of the effect of non-linear summation). If $p$, the release fraction, is set at 0.5, then there must be at least 500 release sites per each S1 fiber [27].

Calculations based on morphological findings seem to satisfy the requirement, if the number of release sites existing at each synaptic site is assumed to be about 25 [32]. Studies with intraaxonal injections of Lucifer yellow indicated that each S1 fiber had, on the average, 7 dendritic terminals [74]. It was observed electron microscopically that each terminal process contacts 1–3 hair cells and that each contact can have several synaptic sites. These observations suggest that irrespective of the terminal size of the S1 fibers, each S1 fiber may have the same numerical range of total synapses, which would exceed 20.

In fish and in turtles, the number of hair cells in the sensory macula increases with growth of the animal. Since the number of afferent fibers remains unchanged, the number of hair cells innervated by each afferent fiber must increase with the age [5]. In the mammal, however, the number of hair cells always remain constant after birth (see [5]).

Impulse initiation in small S2 fibers. Afferent fibers from goldfish sacculus include, beside large S1 fibers, much more numerous small S2 fibers, which arise from the caudal part of the saccular macula. Because they exist in large numbers, S2 fibers are considered important for hearing. Most S2 fibers showed more or less spontaneous firings, and their sound-evoked firings showed an adaptation much smaller than that observed in S1 fibers. The mechanism of impulse initiation in S2 fibers was studied by Kyogoku et al. [54]. Afferent firings in S2 fibers, as in S1 fibers, seem to occur when EPSPs at their terminal exceed the threshold. This was observed for both sound-evoked and spontaneous firings. However, the amplitude of the unit

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EPSP (at least a few mV) was much larger than in S1 fibers (0.4 mV). The large size of the unit EPSP is mostly attributable to the large input resistance of the S2 fibers. A smaller degree of adaptation during the sound stimulation is mostly attributable to the fact that, because of the synchronized release, the transmitter substance released during sound stimulus can set up afferent impulses much more efficiently than those which are released spontaneously. Namely, the rate of transmitter release that little exceeds the rate during the spontaneous activity would be sufficient to maintain the high rate of afferent firings during sound stimulation. A similar explanation might be applied to the observation of non-adaptive firings in the frog auditory system [59].

The branching pattern of single S2 fibers in the saccular macula has been studied by SENTO and FURUKAWA [74]. Each S2 fiber, as in S1 fibers, was found to synapse with many hair cells, but the diameter of the terminal area over which each S2 fiber spreads in the saccular macula (79.4 ± 6.8 µm) was much wider than that of S1 fibers (37.6 ± 3.9 µm).

Apart from fine dendritic arborizations, there is an interesting aspect in afferent fibers of goldfish sacculus in relation to dorso-ventral orientation of hair cells within the macula [21, 24]. Some S1 fibers were found to synapse with both dorsally and ventrally located hair cells, receiving input from both. Therefore, afferent firings in such fibers may occur at a frequency twice that of sound. However, situations with S2 fibers differ, because each S2 fiber is connected to either dorsally or ventrally located hair cells only [74]. Those S2 fibers which are connected with hair cells of different orientations, form separate fiber bundles which can be traced for some distance after they enter the medulla.

2) Situation in the cochlea

An important feature of afferent innervation in the cochlea is that the great majority of thick afferent fibers terminate only on the inner hair cells. But since the number of afferent fibers are in excess of the number of inner hair cells, each inner hair cell is redundantly innervated by afferent fibers, even though individual cochlear afferent fibers do not branch [48].

Patterns of activity in individual afferent fibers in the cochlea have been studied in detail, since such recording became feasible in the 1950s [13, 44]. Characteristics of cochlear afferent firings can be summarized as follows: a) spontaneous firings; b) increase in the rate of afferent firings during sound (level function); c) phase-locked firings; d) adaptation during sound stimulus (perstimulus adaptation); e) suppression of activity at the end of sound (poststimulus adaptation); and f) tuning curve with a characteristic frequency. How do these features relate to the finding shown in Fig. 2? After examining various evidence, we reached the conclusion that there are similarities and differences between the responses of cochlear afferents and goldfish auditory fibers.

Similarity is marked with the adaptive phenomena (d and e of the above), although it is also more or less observed regarding a) to c). Adaptive phenomena
which correspond to those demonstrated in goldfish sacculus [27] seem to occur with regard to the firings of single cochlear afferents, when the rate of firings was taken as its index. Of special importance is the fact that the time course of the phenomena is practically identical, in either preparation [75, 76]. It seems certain that the forward masking, psychophysically observed in human subjects, is attributable to the decremental response at the hair cell-afferent fiber synapse [33].

On the other hand, the mechanism for frequency detection differs. Since firing in neither S1 nor S2 fibers shows any sharp frequency tuning, exact synchronization of afferent firings to sound or the difference in sensitive frequency range existing between S1 and S2 fibers [74] might be used for frequency discrimination in the fish. Afferent synaptic mechanisms are also different. While the firings in goldfish saccular afferents show rather exact synchronization to the sound wave, the phase-locking in cochlear afferents shows some temporal scatter around the exact synchronization (see [13]). Difference between these two modes of firing may probably result from the finding that a fairly large number of transmitter quanta must be released to trigger an impulse in goldfish, while a release of only one or two transmitter quanta would be adequate to set up an impulse [28]. In the former case, the effect of temporal scatterings resulting from probabilistic release of individual quanta would be cancelled in the course of summation of small EPSPs [24].

**D. EFFERENT INNERVATION TO HAIR CELL ORGANS**

Effects of efferent inhibitory action in the cochlea have been attributed to "presynaptic" inhibition, i.e., postsynaptic inhibition to hair cells [14]. This held true in the case of efferent inhibition in the goldfish sacculus [18]. Interpretations of the mode of action of efferent inhibition in the cochlea have been re-assessed since it was found that efferent innervation by thick myelinated fibers covers only outer hair cells, while the inner hair cells receive efferent innervation by thin non-myelinated fibers (see [55]). The inhibitory effect, so far observed in the cochlea by stimulating the crossed olivocochlear bundle at the bottom of fourth ventricle, is now interpreted as being produced via suppression of the cochlear amplifying mechanism mediated by the outer hair cells. Response to sound of the inner hair cells is suppressed by such efferent stimulation, with no change in the membrane potential or input conductance.

In contrast to the irregular pattern of afferent firings, natural firings of efferent fibers occur regularly. The rate of efferent firing increases during sound stimulation [55].

*Transmitter substances.* Although the efferent nerve activity is mediated by acetylcholine, the nature of the transmitter substance at the hair cell-afferent fiber synapse has not been clarified. Glutamate, GABA, or related substances are possible candidates [29, 60, 80].
SUMMARY

Great advances in studies on the mechanisms of stimulus reception and transmission in hair cell organs have been made. With the use of intracellular recording, mechanisms related to transduction have been elucidated. The cochlea possesses mechanisms much more sophisticated than in other hair cell organs. In this review, the author attempted to explain these points, in relation to investigation on the goldfish sacculus (i.e. its inner ear).

Key words: hearing, hair cell, goldfish, synapse, ionic channel, cochlea.

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