EFFECTS OF Na^+ , K^+ , AND OUABAIN ON MICROPHONIC POTENTIALS OF THE GOLDFISH INNER EAR

S. MATSUURA, K. IKEDA and T. FURUKAWA

*Department of Physiology, Osaka City University Medical School, Asahi-machi,
Abeno-ku, Osaka 545, Japan*

Summary Microphonic potentials were recorded from the sacculus of goldfish with glass pipette electrodes to study the effects on the potentials of changes in the ionic composition of endo- and perilymph and of administered ouabain. Ions and drugs were applied with perfusion techniques and special attention was paid to the difference in effects between endo- and perilymphatic applications. The results obtained were as follows:

1. No marked changes were found in the amplitude of microphonic potentials when the endolymph was replaced either with 120 mM NaCl or with KCl.
2. Microphonic potentials were reduced in size when the perilymphatic K^+ concentration was raised, and this reduction was reversible when the solution returned to normal. The amplitude of microphonic potentials was linearly related to the logarithm of perilymphatic K^+ concentrations in a range between 25 and 120 mM.
3. A marked reduction in the amplitude of microphonic potentials was produced when over 5×10^{-6} g/ml of ouabain was applied to the perilymphatic space. An endolymphatic application of ouabain produced no effects even at much higher concentrations. This finding suggests the presence of an active extrusion mechanism of Na^+ across the membrane at the basal part of hair cells.

Functional differentiation between the membrane at the hair-bearing surface and at the basal part of hair cells and the functional significance of a high K^+ concentration of the endolymph were discussed.

It is well known that the endolymph in the inner ear of various animal species contains a much higher concentration of K^+ than ordinary tissue fluids (ENGER, 1964; JOHNSTONE *et al.*, 1963; MURRAY and POTTS, 1961; NAITO *et al.*, 1965;

Received for publication August 27, 1971

松裏修四, 池田勝浩, 古河太郎

SMITH *et al.*, 1954). The effects of changes in the ionic concentrations of the endo- and perilymph and of locally applied drugs on cochlear potentials have been extensively studied through iontophoretic injection or microperfusion techniques (KATSUKI *et al.*, 1966; KONISHI *et al.*, 1966; KONISHI and MENDELSON, 1970; KUIJPERS *et al.*, 1967; KUROKAWA, 1965; MATSUURA *et al.* 1968; TANAKA, 1963; TANAKA and BROWN, 1970; TASAKI and FERNANDEZ, 1952). Some functional relations between the cochlear responses and the characteristic ionic composition of the two fluids have been clarified, namely, the fact that the high K^+ concentration in the endolymph is indispensable for maintaining cochlear function (KONISHI *et al.*, 1966; TANAKA, 1963). The significance of the high K^+ concentration in the endolymph has been dealt with by TASAKI (1960), but the ionic mechanism for the generation of microphonic potentials and the role of high endocochlear K^+ concentration need to be clarified still further.

In this study, we perfused the goldfish sacculus (inner ear) to analyze the effects of ions and drugs on the microphonic potentials. In the goldfish the sacculus is a tubelike structure with hair cells forming part of its medial wall; the hair-bearing surface faces the lumen (FURUKAWA and ISHII, 1967a, b; HAMA, 1969). Results show differences in the effects of ions and drugs when applied either to the lumen of the sacculus or extraluminally. These results are different from those obtained in the mammalian cochlea. Some efforts have been made to explain these differences in terms of ionic mechanisms responsible for the generation of the microphonic potentials.

METHODS

Experiments were carried out on goldfish about 12 cm long. Fish were immobilized by gallamine triethiodide (*ca.* 1 μ g/ml of body weight) and fixed in a chamber similar to that described by FURSHPAN and FURUKAWA (1962). The fish were kept in good condition by feeding aerated water into its mouth. To expose the sacculus, the skull was opened and the brain tissue (medulla and vagus lobe) was pushed aside frontolaterally with a small glass rod after the medulla was sectioned and separated from the spinal cord.

Perfusion of the endolymphatic space. A perfusion technique was employed for intraluminal administration of drugs and solutions of different ionic compositions to the sacculus. A micropipette (tip diameter: 20–30 μ) connected to a polyethylene tube was inserted into the canalis transversus with the aid of a micromanipulator and the perfusate was applied through the pipette (Fig. 1A). The perfusion rate was controlled by changing the height of the mercury column in the polyethylene tube and the flow rate was read by measuring the movement of a small air bubble in the tube (Fig. 1C). When perfusion was started and the flow rate was adjusted to the desired slow value, the canalis transversus became slightly expanded but there was no immediate change in the size of the microphonic poten-

Fig. 1. Structure of the hearing organ of goldfish. A: base of the cranial cavity after medulla and vagus lobes have been removed. Sites for insertion of the recording electrode and the perfusing pipette are shown. B: left labyrinth of goldfish, viewed from medial side. C: schematic diagram of the experimental methods for perfusion of the endolymphatic tube and recording of the microphonics.

tials. However, when the perfusion rate was too high, it led to irreversible damage of the structure. Therefore, the flow rate was usually increased very slowly, checking that it did not produce rapid deterioration of the microphonics. Generally, perfusion rates of 0.1–0.3 $\mu\text{l}/\text{min}$ could safely be used in the present preparation. To get some idea of the amount of fluid necessary for a complete replacement of the saccular endolymph, an effort was made to estimate the volume of the endolymphatic space. A glass cannula (tip: 30–40 μ) was inserted into the sacculus and the endolymph was collected by applying negative pressure. Thus, the endolymph on both sides in the sacculus, in the canalis transversus, and in the dorsal half of the lagena was replaced with air. In three trials, the amount of endolymph collected was 1.0, 1.2, and 2.0 μl respectively. The capacity of the space mentioned above was estimated also on the basis of serial histological sections, which gave a value of 1–2 μl . Therefore, the capacity of the sacculus is much less than 1.0 μl , indicating that saccular endolymph can be replaced by the perfusate within a few minutes with perfusion rates of 0.1–0.3 $\mu\text{l}/\text{min}$.

Extraluminal application. The perilymph communicates with the cranial fluids in the inner ear of goldfish. For an extraluminal application, 0.03–0.1 ml of test fluid was dropped gently from a small pipette onto the base of the skull so as to submerge the saccular macula in the fluid. To keep the effective concentration constant, the fluid was withdrawn from the base of the skull once every 5 min and was replaced with fresh solutions.

Recording of the microphonic potentials. Negative microphonic potentials were recorded with microelectrodes filled with 3 M KCl or 2 M NaCl (tip: 1–2 μ). For recording of negative microphonics, the recording microelectrode had to be inserted across the saccular macula into the sacculus, *i.e.*, into the small space between the saccular otolith and the macula (FURUKAWA and ISHII, 1967a, b).

The recording electrode was connected to a preamplifier with a cathode-follower input (Nihonkohden Type MZ-3B), the output of which was fed into an oscilloscope (Tektronix Type 502). An averaging computer (Nihonkohden ATAC-401) was used for measurement of the average amplitude of 80 responses. For sound stimulation, tone pips at about 1,000 Hz (duration: 30–40 msec) were delivered through a loudspeaker placed about 30 cm from the fish. The sound was monitored with a dynamic microphone placed under the fish chamber and displayed on the oscilloscope. The sound intensity used was about 90 dB SPL. An arrangement was made so that the sound wave appeared, on repeated sweeps, at the same place, *i.e.*, with the same phase relation in the oscilloscopic screen.

RESULTS

Replacement of endolymph with isotonic NaCl or KCl solutions

Figure 2 shows a typical time course of an experiment in which 120 mM NaCl solution was injected through a micropipette inserted into the sacculus. The

Fig. 2. Effects of intraluminal application of NaCl and KCl solutions on the microphonics. Sample records show the microphonics and the stimulus sound monitored. They were summed 80 times by averaging computer. Left shows the control before perfusion; middle and right, the records after perfusion with 120 mM NaCl and KCl solutions respectively.

microphonic potential showed a slight increase for the first few minutes after the initiation of perfusion. Thereafter, the potential tended to decline a little, but it became stationary at 15 min after the start of perfusion. The amplitude of the microphonic potential at the steady level was about 93% of the original value before perfusion. At 60 min, the perfusate was shifted to 120 mM KCl by application through a different micropipette. This produced no appreciable change in the microphonic potential, as can also be seen in Fig. 2.

Perfusion with 120 mM NaCl was carried out in 17 cases: 9 cases showed a slight decrease (about 10%) in the microphonic potential, 2 cases showed a slight increase but gradually returned to the original size after 30 min, and 6 cases showed no appreciable change in the size of microphonics. Perfusion with 120 mM KCl produced no appreciable change in the size of the microphonic potential in 9 out of 10 cases. A decrease of the amplitude was found in only 1 case, in which damage to the structure was suspected. These results with 120 mM NaCl differ from those obtained in the mammalian cochlea. This point will be discussed later.

Effects of extraluminally applied K^+

Figure 3 shows the typical time course of an experiment in which the K^+ concentration of the perilymph was successively changed, as shown at the top of the figure. Solutions of various K^+ concentrations were prepared by mixing 120 mM KCl and NaCl solutions. First, 120 mM NaCl solution was administered. After that, solutions of successively higher K^+ concentrations were applied in steps

Fig. 3. Time courses of changes in the size of the microphonic potentials following changes in extraluminal K^+ concentration.

until finally a concentration of 120 mM was reached. Finally, the solution was returned again to 120 mM NaCl. Figure 3 shows that isotonic NaCl produced a small increase in the microphonic potential, while high K^+ solutions reduced the microphonic potential. In other experiments, the microphonic potential either did not change or showed a slight decrease in isotonic NaCl.

With the administration of K^+ -rich solutions, however, an immediate effect was always observed and the microphonic potential began to decrease in size very soon after the application. Reduction in size advanced rapidly for the first 15–20 min, but the slope gradually became flat. The microphonic potential, thereafter, would reach a steady level after 30–40 min. Upon applying a solution of higher K^+ concentration, the microphonic potential size again started to decline with a similar time course. This rather slow progress of the action of K^+ may be attributable in part to dilution of the solution with the tissue fluid, but a more important factor is the time required for K^+ to reach the base of hair cells by diffusion, for the hair cells are surrounded at their basal part by supporting cells. There are also other structures such as eighth nerve fibers, capillaries, basement membrane, and so forth.

The effect of changing K^+ concentrations on the microphonic potential was reversible. When the K^+ -rich solution was later replaced with isotonic NaCl solution, the microphonic potential recovered to the original size almost completely. The microphonic potential that had once been decreased by applying K^+ -rich solution showed a gradual recovery if the solution was not replaced. This spontaneous recovery of microphonic potentials could be related to the presence of a rich capillary network in the macula.

The amplitude of the microphonic potentials in the experiment shown in Fig. 3 was replotted in Fig. 4 against the logarithmic concentration of K^+ . Microphonic potentials for each K^+ concentration were measured after they had

Fig. 4. Relation between the size of the microphonics and the K^+ concentration in the extraluminal perfusate.

reached steady levels following the application of the solution. The ordinate, *i.e.*, the size of the microphonic potentials, is shown as a percent of the control (filled circle). An approximately linear relationship was obtained at concentrations above 30 mM. These results could be explained by assuming that the reduction in the size of the microphonic potential was produced as a result of a decrease in the resting membrane potential of hair cells. This assumption appears reasonable, for it is quite probable that hair cells, like nerve and muscle, could be depolarized by K^+ , and depolarization would lead to a reduction of the microphonic potentials. The resting potential of hair cells exclusively supplies the electromotive force for the generation of the microphonic potentials in the goldfish's ear, since potentials comparable to the endo-cochlear DC potentials do not exist (see DAVIS, 1965, for the mode of generation of the microphonic potentials).

Effects of ouabain

Ouabain inhibits specifically active transport of Na^+ and K^+ through the cell membrane at proper concentrations. Therefore, it was interesting to see whether the activity of hair cells could be modified by ouabain. Ouabain was applied from either surface of the hair cells. Ouabain (G-strophanthin; M.W. 728.77) was dissolved in isotonic KCl for intraluminal administration, and in isotonic NaCl or Ringer's solution for extraluminal administration.

Figure 5 shows an experiment in which ouabain at a concentration of 5×10^{-4} g/ml was applied successively intra- and extraluminally in the same preparation. Intraluminal administration of ouabain had no effect on the microphonic potential.

Fig. 5. Effects of intra- and extraluminal application of ouabain on the microphonic potential.

Fig. 6. Changes in the size of the microphonics following extraluminal application of various concentrations of ouabain.

By contrast, extraluminal administration resulted in a marked reduction in the size of the microphonics. The reduction started at about 3 min after the application of the drug, and the microphonic potential reached 20% of its original amplitude within 25 min. The reduction in microphonic potential amplitude took place rapidly and was very marked in this case. But, the time course of the reduction seemed to vary greatly, depending on the concentration of the drug used. Although ouabain was able to produce a marked reduction of the microphonic potential even at a much lower concentration than that used in the experiment shown in Fig. 5, a considerable length of time was required before the onset of the effect in such cases. Figure 6 illustrates the time course of changes in amplitude of microphonic potentials after different concentrations of ouabain were applied to the saccular macula. The time lapse before a decrease in microphonic amplitude began was much longer at lower concentrations. An interesting finding in this respect is that the reduction of the microphonics, when compared at the final level, was no less marked even at a low concentration such as 2.5×10^{-5} g/ml. The effect of ouabain was irreversible; the depressed microphonics did not recover even after repeated washing. Therefore, it was not possible to compare the effects of different concentrations of ouabain on the same preparation.

The threshold concentration of ouabain necessary to reduce the microphonics was usually about 2.5×10^{-5} g/ml, but it differed from one preparation to another. Intraluminal application did not produce any changes in the size of the microphonics even at concentrations as high as 5×10^{-3} g/ml. The results obtained in 30 cases are summarized in Table 1.

Table 1. Effects of locally applied ouabain on microphonic potentials (summary of 30 cases).

Route of application	Concentration of ouabain (g/ml)	Total number of cases	Microphonics	
			Decrease	No change
Extraluminal	5×10^{-6}	4	1	3
	1×10^{-5}	3	1	2
	2.5×10^{-5}	3	3	0
	5×10^{-5}	4	4	0
	2.5×10^{-4}	2	2	0
	5×10^{-4}	3	3	0
	5×10^{-3}	2	2	0
Intraluminal	2.5×10^{-4}	2	0	2
	5×10^{-4}	5	0	5
	5×10^{-3}	2	0	2

Perfusion of solutions of high osmotic pressure

The microphonic potential did not seem very sensitive to a slight change in the osmotic pressure of the perfusate. But perfusion with solutions of much

Fig. 7. Changes in the size of the microphonics following intraluminal application of hypertonic NaCl and KCl solutions.

Fig. 8. Effects of intra- and extraluminal application of sucrose solutions on the microphonics.

higher concentrations produced a reversible reduction of the microphonic potential. Figure 7 shows such an example in which the sacculus was perfused successively with 200 mM NaCl and KCl. Reduction in the size of the microphonic potential was about the same with these two solutions, indicating that the reduction is probably due to the action of high osmotic pressure rather than a specific action of different ion species. On the contrary, no marked change in the microphonic potential was produced when the concentration of the electrolyte in the perfusate was decreased to one-half normal. Similarly, hypertonic solutions did not produce a comparable reduction when applied extraluminally.

Perfusion with isotonic sucrose usually decreased the size of the microphonic potential and the suppression in this case was reversible (Fig. 8). Out of 5 cases studied, the decrease reached 40% of the control only in 1 case and the reduction

was about 20% in the others. We expected that perfusion with isotonic sucrose would produce a much greater decrease in the size of the microphonic potential, for it would result in complete removal of electrolytes such as K^+ and Na^+ inside the sacculus. The relatively mild effects of intraluminally applied sucrose may be explained in part by the high viscosity of the sucrose solution, making complete substitution of the endolymph rather difficult. In fact, micropipettes with a relatively large tip diameter (25–35 μ) had to be used for the perfusion of sucrose solution.

Intraluminal perfusion with hypertonic sucrose resulted in a much greater reduction in the size of the microphonics, demonstrating again the suppressive action of hypertonic solutions. Extraluminal application of isotonic sucrose did not produce any marked change in the size of the microphonics.

Action of CaCl_2 and ethylenediamine tetraacetic acid (EDTA)

Intraluminal perfusion with isotonic KCl containing 4 mM CaCl_2 produced no marked change in the microphonic potentials. However, the microphonic potential was reduced when the concentration of CaCl_2 was increased to 20 mM (KCl was reduced to 100 mM). This reduction was to some extent reversible (Fig. 9). Marked suppression of the microphonic potential was observed when the lumen was perfused with 120 mM CaCl_2 . In this case the reduction of the microphonics was almost irreversible.

Effects of EDTA were also examined. The solution used consisted of 3.6 mM $\text{EDTA} \cdot 4\text{Na}$, 1.4 mM $\text{EDTA} \cdot 2\text{Na}$, and 110 mM KCl, the pH of the solution being

Fig. 9. Effects of intraluminal application of CaCl_2 and EDTA on the microphonic potential.

adjusted to 7.4. When this EDTA solution was injected into the sacculus, the size of the microphonic potential decreased gradually and it reached 25–30% of the original amplitude in about 50 min. The reduction was irreversible. The effect of EDTA on the microphonics may be attributable to either the disappearance of free Ca^{++} from the endolymph or its direct action on the saccular otolith or on hair cells.

DISCUSSION

Perfusion of the endolymphatic space. Replacement of endo- and perilymph in the inner ear with artificial fluids has been performed by many authors to examine the action of the fluids on hair cells (KONISHI *et al.*, 1966; KONISHI and MENDELSON, 1970; KUIJPERS *et al.*, 1967; KUROKAWA, 1965; MATSUURA *et al.*, 1968; TANAKA, 1963; TANAKA and BROWN, 1970; TASAKI and FERNANDEZ, 1952). In the present experiment, fluids that were applied into the sacculus leaked out through the ruptured portions of the semicircular canals. This was ascertained by perfusing with isotonic KCl solution containing a small amount of methylene blue (below 0.01%). Furthermore, when ototoxins such as dihydrostreptomycin (5×10^{-4} g/ml) were perfused into the space the microphonic potential decreased in size soon after the application and eventually disappeared almost completely (MATSUURA *et al.*, 1968, 1971). These facts indicate that the test fluids acted on saccular hair cells when injected into the endolymphatic tube. However, it is not easy to assess how completely the endolymph was replaced by test fluids. In this respect, not only anatomical structures but also the perfusion speeds have to be taken into consideration. Since the capacity of the endolymphatic space is less than 1–2 μl (see METHODS) and since we perfused it at a flow rate of 0.1–0.3 $\mu\text{l}/\text{min}$, the endolymph was totally replaced within several minutes. However, the perfusate was applied to that portion of the sacculus where the canalis transversus connects with the sacculus, which makes a blind sack at its caudal part. Therefore, the endolymph in that part can be replaced with the perfusate only by diffusion or by swirling. However, since the microphonic potential was recorded from the portion of the macula which lies rostral to the canalis transversus (FURUKAWA and ISHII, 1967a), the difficulties caused by this anatomical relationship may not have been serious. An additional factor is that the space between the otolith and the hair-bearing ends of hair cells must be very narrow, making the exchange of fluid inside this space a slow process.

As to extraluminal application, perfusion problems seem to be less serious, for K^+ and ouabain acted on hair cells and reduced the microphonics. It is therefore certain that the basal end of hair cells communicates more or less directly with the perilymph in the saccular macula of goldfish, although the presence of an abundant capillary network in the macula may modify the effects of ions and drugs applied extraluminally. Also, the microphonics are easily influenced by hypoxia

(DAVIS *et al.*, 1955; IINUMA, 1967; MISRAHY *et al.*, 1958; WEVER *et al.*, 1949). However, this influence may be negligible in the present experiments because of the presence of sufficient blood circulation to the macula. Moreover, the reduction of the microphonics by hypoxia in goldfish sacculus is at most moderate in degree (FURUKAWA and ISHII, unpublished observations).

Effects of ouabain. Ouabain, an inhibitor of Na^+ - and K^+ -activated adenosine-triphosphatase, suppresses active transport of Na^+ across the cell membrane only when it is present on the side toward which Na^+ is pumped out (GLYNN, 1964). Certain directional effects have been demonstrated in glandular and epithelial cells; ouabain suppresses active transport of Na^+ only when applied to the luminal surface in the goose salt gland (HOKIN, 1967) and when applied to its inner surface in the frog skin (KOEFOED-JOHNSEN, 1957). This is related to the fact that glandular and epithelial cells have a directionality in their function (FAWCETT, 1962). Namely, epithelial cells participate in the active transport of Na^+ only on some parts of the membrane. The finding that ouabain reduces the saccular microphonics of goldfish only upon its extraluminal application is satisfactorily explained by assuming an analogous situation. One then comes to the conclusion that active transport of Na^+ is continuously operating at the basal membrane of hair cells to expel Na^+ from the cell toward the perilymphatic space. Consequently, the reduction of the microphonic potential by ouabain can be attributed to accumulation of Na^+ inside the cell, leading to a depolarization of hair cells.

As to the concentration of ouabain necessary to suppress the microphonics, the threshold was about 2.5×10^{-5} g/ml (5×10^{-5} M). This concentration is a little higher than that needed for 50% inhibition of the membrane ATP-ase in isolated erythrocytes (GLYNN, 1964). However, HOKIN (1967), for example, used 10^{-4} M for the salt gland which is in the same range of concentration as that used in the present experiments.

The effect of ouabain was also studied in the inner ear of guinea pig, but its exact site of action remained unclear; the cochlear microphonics were suppressed either by perilymphatic perfusion (injection into the scala tympani or vestibuli) or by endolymphatic administration (injection into the scala media) (KUIJPERS *et al.*, 1967; TANAKA and BROWN, 1970). A more detailed study reported recently by KONISHI and MENDELSON (1970) is in agreement with our results in that the effects of ouabain was more marked when applied to the perilymphatic than to the endolymphatic space. Still, the decline in the cochlear microphonics took place in parallel with a reduction in the endocochlear DC potential. In the inner ear of the guinea pig, ATP-ase was found, besides the organ of Corti, in various tissues such as the stria vascularis, Reissner's membrane, and spiral ligament (IINUMA, 1967; NAKAI and HILDING, 1966, 1967). The reduction in the guinea pig cochlear microphonic potential produced by the endolymphatic application of ouabain could therefore depend on the inhibition of ATP-ase activity in structures such as the stria vascularis.

Ionic composition of endolymph. The endolymph in the inner ear has high K^+ and low Na^+ concentrations, while the perilymph has a high Na^+ concentration like ordinary extracellular fluids (ENGER, 1964; JOHNSTONE *et al.*, 1963; NAITO *et al.*, 1965; SMITH *et al.*, 1954). JOHNSTONE and others (1963) have reported that the Na^+ concentration in the endolymph of guinea pig is as low as 1.4–2.5 mM/liter. The endolymph in goldfish contains 79 mM K^+ and 53 mM Na^+ (NAITO *et al.*, 1965), while the endolymph of elasmobranchs contains much lower K^+ and higher Na^+ concentrations (ENGER, 1964; MURRAY and POTTS, 1961). Further, in octopus the endolymph has approximately the same concentration as sea water (AMOORE *et al.*, 1959). Thus, the ionic composition of the endolymph shows a great variation among different animal species. But, at least in vertebrates, the K^+ concentration of the endolymph becomes high in more advanced animal species. At its extreme, almost all the cations of the endolymph consist of K^+ in the mammalian cochlea.

In guinea pigs, the cochlear microphonic potential is suppressed by perfusion of the scala media with NaCl, while perfusion with isotonic KCl has a much smaller influence on the potential (DAVIS, 1957; DAVIS *et al.*, 1955; KONISHI *et al.*, 1966; MISRAHY *et al.*, 1958; TANAKA, 1963). These findings indicate that the high K^+ concentration in the endolymph is indispensable for maintaining cochlear function, and many explanations of its physiological significance have been proposed. For example, TASAKI observed that the squid axon membrane treated with K^+ -rich media and subjected to anodal polarization became extremely sensitive to mechanical distortion of the membrane. From this finding, he proposed that the high K^+ concentration in the endolymph might contribute to elevating the sensitivity of the cochlear hair cells as a mechanical receptor (1960). This interpretation has been supported by KATSUKI and HASHIMOTO's recent report on mechanosensitivity of shark pit organ (1969).

In goldfish, however, perfusion of the endolymphatic space with isotonic NaCl did not have any influence on the microphonic potential. Therefore, our results clearly contradict those obtained on guinea pig cochlea. The cause of this discrepancy is not necessarily clear, but there is one aspect that has so far remained unnoticed on the role of high K^+ concentrations in the endolymph. As to the mode of activity of hair cell organs, DAVIS postulates that there is a steady inward flow of electric current across the hair-bearing surface of hair cells (1965). In guinea pigs, whose endolymph is constituted almost exclusively of KCl, the current may be carried solely by the inflow of K^+ , while in lower forms such as goldfish the ionic composition of the endolymph is such that a considerable portion of the current had to be carried also by Na^+ . It deserves notice here that Na^+ , once it has entered the cell, has to be pumped out, while K^+ can be dissipated passively. These considerations seem to suggest that the active extrusion mechanisms for Na^+ are much better developed in hair cells of lower forms than in those of the mammalian cochlea. If this were the case, the above-mentioned differences

regarding the effects of perfusing with isotonic NaCl could easily be explained.

The authors are indebted to Professor C. Eyzaguirre for his helpful criticism of the manuscript. This investigation was supported by grants from the Ministry of Education.

A preliminary report of this work was published in *Science*, **160** (1968): 1117-1119.

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