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MEASUREMENT OF POTASSIUM AND CHLORIDE ION CONCENTRATIONS IN THE CUPULAE OF THE LATERAL LINES OF *XENOPUS LAEVIS*

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SUMMARY

1. Potential measurements were made with double barrel ion selective electrodes from the cupulae of lateral line organs in the aquatic toad *Xenopus laevis*.

2. A positive endocupular potential (ECP) of 15–50 mV was recorded within the cupula, immediately above the hair cells.

3. Increases in the Cl^- and K^+ potentials were recorded when the ion selective electrodes touched the cupula. Cupular Cl^- and K^+ varied between 35 and 70 mM and 24 and 100 mM respectively. This variation existed between, rather than within, different animals.

4. Subcutaneous injections of 0·4 ml. 2 mM ouabain greatly reduced the ECP and cupular K^+, whereas 0·4 ml. of *Xenopus* Ringer had no effect.

5. Changing the bath Cl^- a hundredfold had no effect on the ECP. It was concluded that the ECP was produced by an electrogenic K^+ pump which maintained high K^+ levels within the cupula.

INTRODUCTION

On the basis of the embryology and morphology of the sensory epithelium, the lateral line organs of aquatic lower vertebrates are considered to be related to the cochlea and vestibular sense organs. The sensory hair cells of these sense organs are mechanoreceptive and are mechanically coupled to an overlying gelatinous structure which transmits fine movements to them, e.g. the tectorial membrane in the cochlea, the cupulae of vestibular and lateral line organs.

In 1957 Davis proposed that a shearing displacement of the stereocilia on the apical surface of hair cells in the cochlea causes a change in the ohmic resistance across the top of the hair cells which modulates the flow of current through the hair cells. Von Bekesy (1951) established that movement of the basiliar membrane produced changes in the standing

current flow and concluded that the cochlear microphonic was produced by these movements.

This model is widely believed to describe sensory transduction in hair cells elsewhere in the acoustico-lateralis system (Flock, 1971). However the identity of ion species carrying the current through the apex of the hair cells and out through the body, and the driving force for the current must depend on the physiological environments of the hair cells in the different sense organs. The sensory hair bundles of hair cells in the cochlea and vestibular system in mammals project into endolymphatic fluid which has an ionic concentration presumably similar to the cellular contents of hair cells, being very rich in K^+ (150 mM) and Cl^- (150 mM) and with small concentrations of Na^+ (1 mM utricle, and 3 mM saccule) (Sellick & Johnstone, 1975). In the cochlea, the electrogenic endocochlear potential causes a large positive potential difference of +50 mV between the apex and the base of the hair cell. Thus it has been proposed in the cochlea that a K^+ current flows inward through the apex of the hair cell and outwards through the body, and that the driving force for this K^+ current is the resting potential of the hair cells and the endocupular potential (Johnstone & Sellick, 1972).

In contrast, the ionic composition of canal lymph in the lateral line canal organs of fishes may contain only little K^+ (2–25 mM) and larger amounts of Na^+ (114–509 mM) and the cupulae of free standing neuromast organs in amphibia project directly into the aquatic environment of the animal in which the dominant cation is Ca^{2+} (Liddicoat & Roberts, 1971). It is of considerable interest to know if the ionic composition of the millieu bathing the apical surfaces of the hair cells is similar to that surrounding the cupulae, or whether the apical surfaces of the hair cells have a microenvironment different in ionic composition from the fluid which surrounds the cupulae.

The object of the experiments described in this paper is to measure the K^+ and Cl^- concentrations in cupulae of free standing neuromasts in the lateral line systems in *Xenopus* with ion selective electrodes, and furthermore to see whether a positive potential similar to the endocochlea potential exists across the surfaces of the hair cells.

METHODS

Results were obtained from twenty-four adult and juvenile male and female *Xenopus laevis* weighing between 30 and 80 g. They were anaesthetized in 0·05% ethyl-*m*-aminobenzoate and transferred to a shallow experimental bath where they were pinned to a Silgard Base. Care was taken in handling the animals to avoid damaging the fragile lateral line cupulae. The animals were anaesthetized for 10–15 min before measurements were taken and usually survived the experiment,

except when they were injected s.c. with ouabain. The bathing solution consisted of the anaesthetic and 0·1 mM-KCl as a reference for the K^+ and Cl^- ion selective electrodes. Care was taken to keep the lateral line organs in the solution at all times and to prevent heating by the microscope lamp. Ouabain was made up in *Xenopus* Ringer (Russell, 1968) and injected subcutaneously beneath the organs being studied.

Double barrelled, liquid ion-exchange electrodes similar to those used by Khuri, Hajjar & Agulian (1972) were used to measure $[K^+]$, (Cl^-) and potential in the lateral line cupulae. 1 mm (i.d.) borosilicate glass tubing was cleaned in ethanol in a sonication bath and short lengths of this tubing were fixed together in pairs with heat shrinkable sleeving. The pairs were mounted in a Narishige vertical pipette puller, heated and twisted through 180° and then pulled in the conventional manner. Araldite (Ciba) was applied to the shanks of the electrode pair to give added strength. The electrode tips were broken under microscopic control to a diameter of about 2 μm so that 1% Siliclad (Clay Adams) could be drawn by capillary attraction 200 μm up the barrel intended to contain the liquid ion exchanger. Siliconizing solution was prevented from entering the barrel intended for the potential electrode by applying pressure via a rubber tube to this barrel. The electrodes were then baked at 200° C overnight. It was necessary to break the electrode tips again to a diameter of about 4 μm so that the shank could be filled by suction through the tip. The potential side was filled with NaAc since neither of these two ions were expected to interfere with K^+ or Cl^- measurements if leakage occurred from the tip. The ion exchange side of the electrode was filled with 100 mM-KCl and then either K^+ or Cl^- ion exchange was also sucked into the siliconized position of the tip. Electrodes were calibrated in graded solutions of KCl between 0·1 and 100 mM and gave responses of opposite polarities as expected. Neither K^+ or Cl^- electrodes gave linear calibration over the whole of the range but approached the theoretical slope of 58 mV/tenfold change for concentrations between 1 and 100 mM. A Bioelectric P1 amplifier was used to measure potentials from the potential electrode while a Burr Brown Electrometer amplifier (3431J input impedance $10^{14}\Omega$) was used for the ion exchange electrodes. The voltage from the potential electrode was subtracted from the ion exchange potential at the differential input of a Brush 220 chart recorder.

Electrodes were calibrated before and after measurements were obtained and the bathing solution was included in this calibration since ion leakage from the animal altered the reference level to a small extent. Electrodes were advanced into the centre of the cupulae with the aid of a hydraulic microdrive under visual control.

Single barrel micro-electrodes pulled from 1 mm diameter tubing, filled with KAc. and with resistances between 10 and 20 MΩ were used to measure the resistances of the cupulae. Signals from the electrodes were fed into the P1 amplifier which has facilities for simultaneous current injection and recording. Current pulses were passed through the electrodes when their tips were in the bath solution, and the resulting potential changes were balanced out with the amplifier bridge circuit. The electrodes were then advanced into the cupula, and the resulting change in resistance caused the amplifier bridge circuit to become unbalanced. The amplitudes of the out-of-balance potentials were measured and the resistance changes were calculated from the potential measurements and from the amplitude of the current pulses. The current injected through micro-electrodes was measured across a $10^9\Omega$ resistor between the preparation and the ground.

RESULTS

The potential changes encountered during penetration of the lateral line cupula with the three types of electrodes are illustrated in Fig. 1. The potential record (endocupular potential, ECP) increased steadily as the electrode was inserted until a maximum between +15 and +50 mV was reached in the centre of the cupula just above the sensory epithelium. Penetration in the epithelium to the sides of the cupula resulted in negative potentials. During some penetrations the electrode was advanced past the centre of the cupula resulting in a decreased positive potential, but if the electrode were withdrawn a small amount the full potential could be obtained. Once it had been obtained, the positive potential remained stable for at least 20 min.

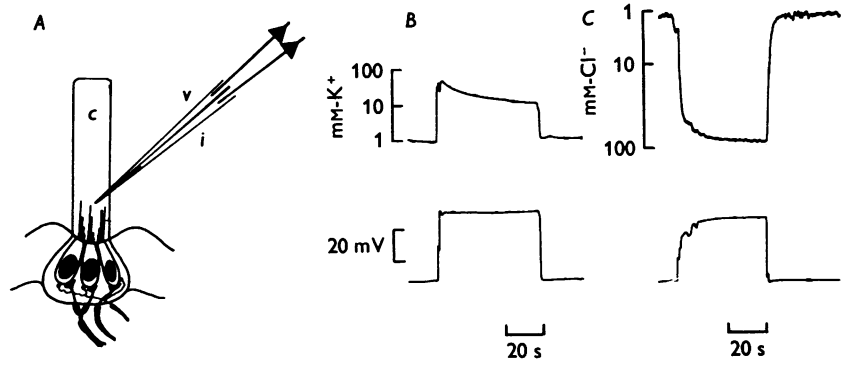

Fig. 1. Responses from the double barrel K^+ and Cl^- electrodes during insertion into the lateral line cupula of *Xenopus*. *A*, diagram of recording arrangement, cupula (*c*), hair cells (*h*), potential electrode (*v*), ion selective electrode (*i*). (*B*) upper trace: response from K^+ electrode (E_{K^+}), Lower trace: potential record (ECP). (*C*), upper trace: response from Cl^- electrode (E_{Cl^-}). Lower trace: potential record (ECP) Records in (*B*) and (*C*) were made from the same animal but in different cupulae.

Increases in the Cl^- potential (E_{Cl^-}) were observed as soon as the electrode tip touched the cupula, even before a positive potential was recorded, and the maximum [Cl^-] coincided with the maximum ECP. Cupular [Cl^-] varied between 35 and 70 mM.

The response from the K^+ electrode (E_{K^+}) upon penetrating the cupula was similar to the Cl^- response in that it increased as soon as the cupula was contacted but unlike E_{Cl^-} it was transient and fell to quite low levels after its initial peak. New peaks could be produced if the electrode was advanced further into the cupula until it reached the underlying epithelium. The transient response could not be caused by injury to the cupula

and subsequent leakage of ions since this did not occur during penetration with Cl^- electrodes having similar tip diameters. Also it would be expected that damage would cause a fall of the ECP and this was found not to be the case.

It is suggested that the transient nature of E_{K^+} is to do with the current

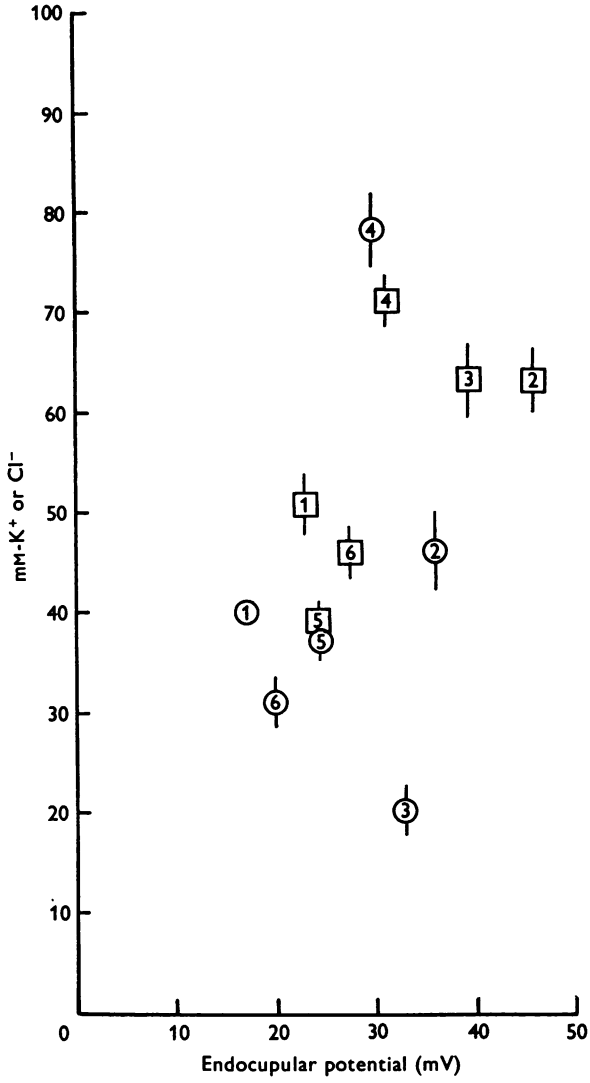

Fig. 2. The relationship between endocupular potential and cupular K^+○ and Cl^-□. The numbers refer to each of the six different experiments when these measurements were made. Each point is the average of ten measurements, S.D.s are indicated by vertical bars.

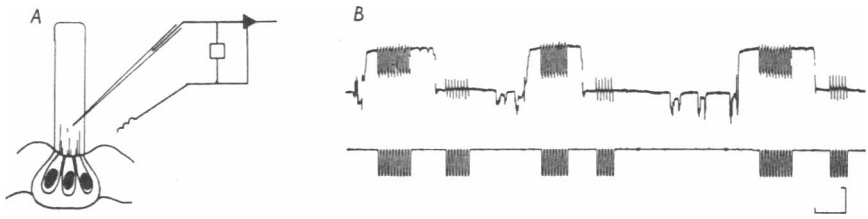

Fig. 3. The measurement of cupular resistance. *A*, diagram of recording arrangement. *B*, upper trace: record of potential and resistance measurements of successive penetration of three different cupulae. Note that current injection bridge returns to balance when electrode is withdrawn from the cupula. Negative deflexions in the trace are recorded when the electrode tip penetrates the epithelium adjacent to the cupula. Lower trace: record of current pulses injected through the electrode. Horizontal time scale: 10 sec. Vertical scale: upper trace 20 mV; lower trace 5 nA.

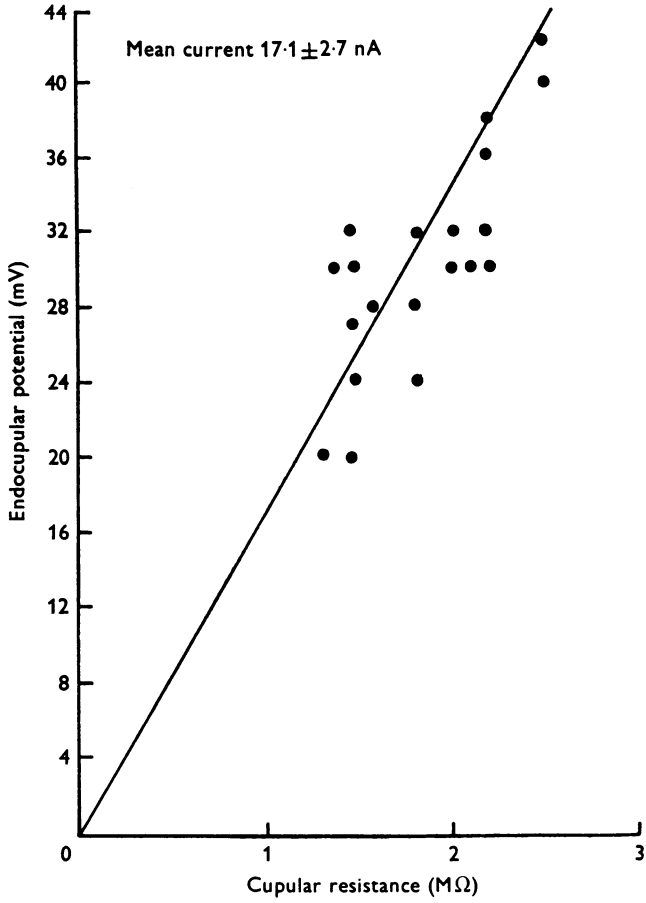

Fig. 4. Relationship between endocupular potential and endocupular resistance. The measurements are taken from three different experiments.

requirements of the K^+ electrode and that it might deplete the area of the cupula directly under the electrode tip. This effect was not apparent with the Cl^- electrodes perhaps because of the different properties of the Cl^- liquid ion exchanger. It was observed that the K^+ electrodes accurately recorded [K^+] in agar gels without the appearance of transients and it was

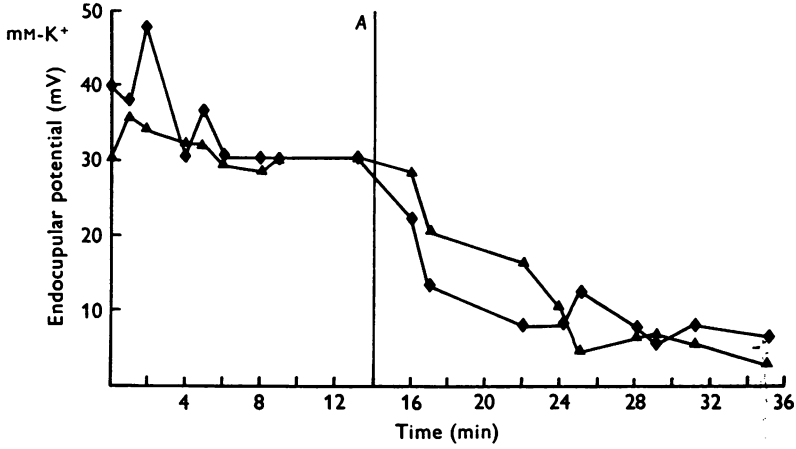

Fig. 5. Effect of s.c. injection of 0·4 ml. 2 mM ouabain on endocupular potential (▲), and cupular K^+ (◆). Injection of ouabain at *A*.

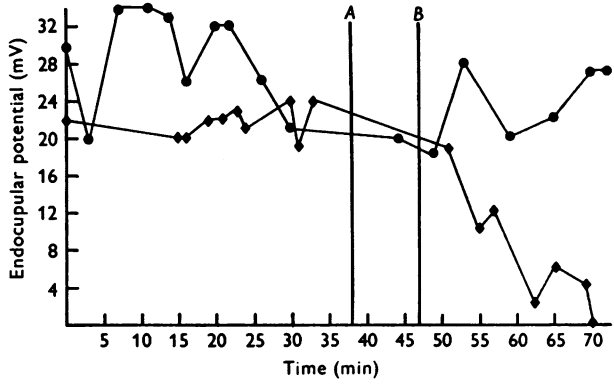

Fig. 6. The effect of injection of 0·4 ml. 2 mM ouabain in *Xenopus* Ringer (◆) and 0·4 ml. *Xenopus* Ringer (●) on endocupular potential. *A*, inject *Xenopus* Ringer; *B*, inject ouabain.

concluded that the cupula has different properties to a water structured agar gel. If the above explanation of the E_{K^+} transients is correct then the peak of the transients should correspond to the true cupular [K^+], and hence [K^+] was measured from this point, and was found to vary between 24 and 100 mM.

The variation in ECP, K^+ and Cl^- existed between animals rather than between individual neuromasts in the same animal (Fig. 2) and it is evident that a relationship exists between ECP and cupular K^+ and Cl^-; however, due to variability in the data it is not possible to define this specifically.

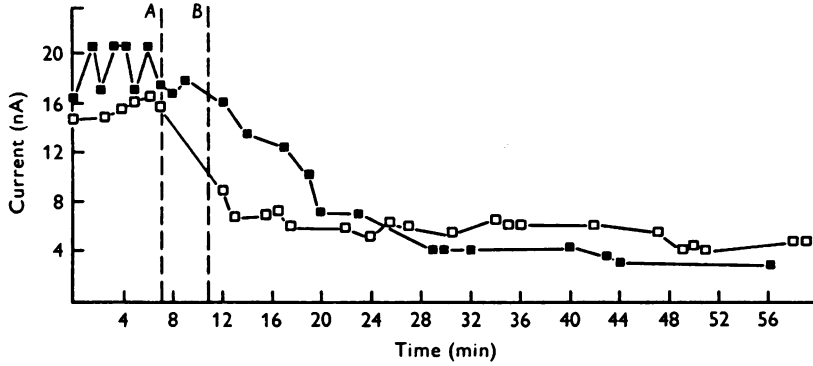

Fig. 7. Effect of subcutaneous injection of 0·4 ml. 2 mM ouabain on endocupular current calculated from resistance measurements in two different animals. □ (50/75), ■ (51/75). *A*, inject 50/75; *B*, inject 51/75.

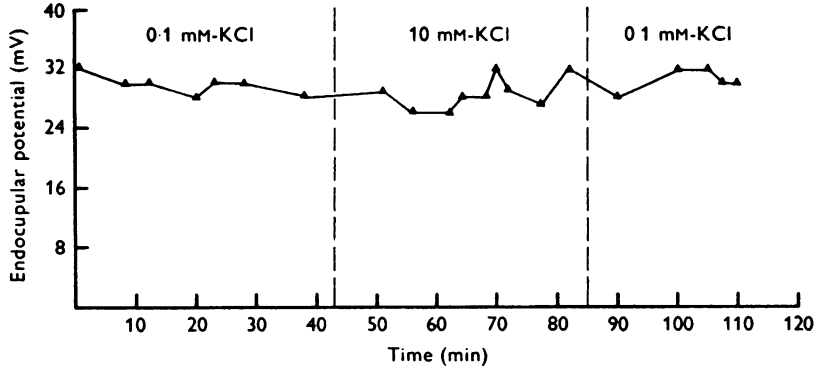

Fig. 8. Effect on endocupular potential of changing the bathing medium from 0·1 to 10 mM-KCl.

A record of the ECP during resistance measurement is illustrated in Fig. 3 and the relationship between ECP and cupular resistance is illustrated in Fig. 4. It is obvious that there is a linear relationship between ECP and simultaneously measured cupular resistance, and it can be seen that most of the variation in ECP is due to variation in cupular resistance since the current flow through the cupula is relatively constant, i.e. about

17 nA in different cupulae in different animals. The variation in cupular resistance is presumably due to slight variation in electrode position within the cupulae or to different states and dimensions of the cupulae.

Injection of 0·4 ml. 2 mM ouabain (S.C.) greatly reduced the ECP and cupular K^+ (Figs. 5 and 7), whereas 0·4 ml. *Xenopus* Ringer had no effect (Fig. 6). In view of the fact that the K^+ and ECP fall simultaneously one must consider the possibility that the ECP could be a Cl^- diffusion potential. In order to test this hypothesis, the bath [Cl^-] was changed a hundredfold from 0·1 to 10 mM. This had no effect on the magnitude of ECP and we have therefore excluded the possibility that a Cl^- diffusion potential is responsible for or contributes to ECP (Fig. 8).

DISCUSSION

It is clear from the results of experiments reported here that a second role can be attributed to the cupulae of superficial lateral line organs. In addition to providing the means of transmitting mechanical stimulation to the hair cells, the cupulae also preserve an ion rich microenvironment above the sensory epithelium. Since we have shown that the ECP is not a diffusion potential and that it is abolished by ouabain we propose that it is electrogenic in nature. Furthermore since the major anions and cations found in the cupulae are K^+ and Cl^- it is likely that the electrogenic pump is a K^+ pump. However, in view of the uncertainty about the K^+ measurements, it is possible that there may also be transport mechanisms for other anions, e.g. Na^+ or Ca^{2+}.

The complex microstructure of the cupula is freely permeable to ions. This is clearly seen in Fig. 5 where, as a result of S.C. injection of ouabain, K^+ and ECP decline at the same rate. This is an indication that K^+ diffuses rapidly away from the cupula, and that its levels within the cupula must be quite actively maintained. This result is in agreement with other observations that lateral line cupulae are freely permeable to salts and dyes (Jierlof, Spoor & de Vries, 1952; Frishkopf & Oman, 1973).

The site of ion secretion is unknown, but from their ultrastructure (Flock, 1967; Roberts & Ryan, 1971; Jorgensen & Flock, 1973), it is likely that this occurs at the mucosal surface of the supporting cells. Another reason for suggesting that ion transport occurs at the mucosal surface rather than the serosal is that the latter site would necessitate a large intracellular positive potential. Only negative potentials have been recorded from supporting cells in lateral line organs of *Ambystoma* (Å. Flock (unpublished data), *Necturus* (Sand, 1975) and *Lota* (Flock, Jorgensen & Russell, 1973).

Free standing neuromasts, therefore, may be compared with other sense

organs of the acoustico-lateralis systems in that the apical surfaces of the hair cells are bathed in an electrogenically maintained high K^+ environment. It has yet to be shown that high K^+ micro-environments occur beneath the cupulae of open lateral line canals that do not contain high K^+ endolymph, e.g. dogfish (Liddicoat & Roberts, 1971). High values of K^+ are found in closed lateral line canals in which flow between canal lymph and the environment is restricted. Even so, these values are lower than the values we have measured in *Xenopus* cupulae, and it is possible that the values observed in these systems may not reflect the values existing at the surface of the hair cells.

The presence of large amounts of K^+ in the cupulae makes it tempting to suggest that this ion might carry the receptor potential current. Sand (1975), however, has shown that the presence of EGTA in the medium bathing the apical surfaces of hair cells in the neuromasts of *Necturus*, or the presence of competitors for Ca^2, reduces the mechano-sensitivity of hair cells, while increased concentrations of Ca^2 increases their mechano-sensitivity. From the observations he tentatively proposes that the Ca^{2+} carries the receptor potential current.

In the light of the observations reported in this paper an alternative interpretation of Sand's (1975) results is presented. Namely, that Ca^2 does not carry the receptor potential, but has a different role in the transducer process; for example, Ca^{2+} may be involved in regulating the ionic permeability of the transducer membrane. Ca^{2+}-activated K^+ channels are known in neurones (Meech & Standen, 1975) and in sense organs (Clusin, Spray & Bennett, 1975), and recently. Thornhill (personal communication) has used an electron probe to show the existence of a store of free Ca^{2+} in the hair cell kinocilium and at its base. It is clearly of interest to discover the identity of the current which carries the receptor potential in hair cells, and the factors which regulate it.

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