J. exp. Biol. (1979), 83, 123–130
With 4 figures
Printed in Great Britain

MEASUREMENT OF CALCIUM ION CONCENTRATIONS IN THE LATERAL LINE CUPULAE OF *XENOPUS LAEVIS*

BY F. P. MCGLONE,* I. J. RUSSELL* AND O. SAND†

* *Ethology and Neurophysiology Group, School of Biological Sciences, The University of Sussex, Falmer, Brighton, Sussex* BN1 9QG,
† *Institute of Zoophysiology, University of Oslo, Norway.*

(*Received* 17 *November* 1978)

SUMMARY

1. Ion selective microelectrodes were used to measure the distribution of Ca^{2+} in the cupulae of *Xenopus laevis*, and to observe the effects of the Ca^{2+} blocker, La^{3+}, and a Ca^{2+} chelating agent (EGTA) on the endocupular potential and K^+ concentration.

2. Measurements of the endocupular Ca^{2+} and potential were found to be in the range of 2–30 μM and 55–10 mV respectively, and their relationship indicates that Ca^{2+} is passively distributed in the cupula.

3. The concentration of endocupular K^+ and endocupular potential was found not to be influenced by addition of either 1 mM EGTA or 100 μM–La^{3+} to the bathing solution.

INTRODUCTION

Intracellular recordings from hair cells in a wide range of acoustico-lateralis organs in different animals, from lateral line organs to the mammalian cochlea, show receptor potentials in response to mechanical stimulation (Harris, Frischkopf & Flock, 1970; Flock, Jorgensen & Russell, 1973; Mulroy *et al.* 1974; Hudspeth & Corey, 1977; Russell & Sellick, 1977). The synapses between hair cells and the afferent neurones are chemical, and the rate of transmitter release from the hair cells is controlled by the receptor potentials (Furukawa & Ishii, 1967; Flock *et al.* 1973; Sand, Ozawa & Hagiwara, 1975). However, little is known about the ionic basis for the hair cell receptor potentials. Davis (1965) suggested that shearing displacement of the stereocilia towards the kinocilium causes a conductance increase in the apical hair cell membrane, while displacement in the opposite direction causes a conductance decrease, thereby modulating the current flow through the apical membrane.

The apical hair cell membranes in the mammalian cochlea and vestibular system face an endolymph where K^+ (150 mM) is the predominant cation. The endocochlear potential exceeds + 120 mV, and the driving force for K^+ will thus be inward through the apex of the hair cell and outward through the cell body. It is therefore reasonable to suggest that K^+ carries the receptor current in cochlear hair cells (Johnstone & Sellick, 1972).

The superficial lateral line neuromasts of amphibia are exposed to an aquatic environment in which the dominant cation is Ca^{2+}. By observing the effects of varying the ionic composition of the external medium on the mechanical sensitivity of lateral line organs in the mudpuppy (*Necturus maculosus*), Sand (1975) found that this sensitivity was dependent upon the Ca^{2+} concentration of the medium, that it was inhibited by Ca^{2+} competitors and abolished in the absence of Ca^{2+}. He therefore tentatively proposed that Ca^{2+} carries the receptor current in the hair cells of the mudpuppy lateral line organs.

Russell & Sellick (1976) measured the endocupular potential and the potassium and chloride composition of the lateral line cupulae in *Xenopus laevis*, and found that the cupulae preserved a micro environment close to the hair cells which was remarkably similar to that in the cochlea. Cupular Cl^- and K^+ varied between 35 and 70 mM, and 24 and 100 mM, respectively, and the K^+ was proposed to be maintained by an electrogenic K^+ pump which produced the endocupular potential of 15–50 mV. In the light of this evidence it was suggested that K^+ and not Ca^{2+} carries the receptor current in hair cells of amphibian lateral line organs. The effect of Ca^{2+} and Ca^{2+} blockers observed by Sand (1975) could then be attributed to an indirect effect of Ca^{2+} on the K^+ channels (Meech & Standen, 1975; Clusin, Spray & Bennett, 1975 or Ca^{2+} might be essential for the operation of the K^+ pumping mechanism.

As a first step towards determining the role of Ca^{2+} in the hair cell transduction process we have used ion selective microelectrodes to measure the distribution of Ca^{2+} in the cupulae of *Xenopus laevis*, and to observe the effects of the Ca^{2+} blocker, La^{3+}, and the Ca^{2+} chelating agent (EGTA) on the endocupular potential and K^+ concentration.

METHODS

The results reported here were obtained from 15 juvenile *Xenopus laevis* weighing between 30 and 40 g. This species has large and rigid cupulae, which facilitate stable endocupular recording. The animals were initially anaesthetized in 0·05% ethyl-*m*-aminobenzoate, immobilized with intramuscular injection of gallamine triethiodide, and transferred to a shallow experimental bath where they were pinned to a Sylgard base. The bathing solutions consisted of the anaesthetic and 0·1 mM-KCl as a reference for the K^+ ion selective electrodes, and 0·1, 1 or 10 mM-$CaCl_2$ made up in constant ionic strength with NaCl as reference for the Ca^{2+} ion-selective electrodes.

Double-barrelled liquid ion selective microelectrodes, similar to those described by Walker (1977), were used to measure the K^+ and Ca^{2+} concentrations in the lateral line cupulae. Theta section borosilicate glass tubing (2 mm o.d.) was chemically cleaned and pulled in a vertical pipette puller to produce electrodes with 1 cm shanks. The electrodes were bevelled on a grinding surface (Werblin, 1975) to produce 2–3 μm tips, which facilitate both the filling of the electrodes and their penetration of the cupulae. The tips of the electrodes were immersed for 5 s in a 5% solution of tri-*n* butyl chlorosilane in 1-chloronaphthalene after which there was a column of 250–300 μm of silane solution in each barrel of the electrodes. Double-distilled water was then back injected into one barrel to displace the solution in that barrel. The solution in the other barrel was permitted to air-dry over a period of 48 h in a clean

atmosphere. A column of liquid ion exchanger [K^+ exchanger from Corning, and Ca^{2+} exchanger from Simon (Oerne, Kessler, Simon, 1976)] approximately 250 μm long was introduced into the tip of the siliconized barrel by back injection. A solution of 100 mM-KCl or 1 mM-$CaCl_2$ was then back injected behind the resin. The water in the reference barrel was sucked out and replaced by 100 mM sodium acetate or KCl as reference for the K^+ and Ca^{2+} ion-selective electrodes, respectively. The shanks of both barrels were then topped up with light paraffin oil to prevent evaporation. Completed electrodes were stored for 4–24 h in solutions corresponding to those in their ion selective barrels prior to calibration.

Electrodes were calibrated in graded solutions of KCl between 0·1 and 100 mM for K^+ selective electrodes, and 0·1 and 10 mM-Ca^{2+} for Ca^{2+}-selective electrodes. Neither K^+ nor Ca^{2+} electrodes gave linear calibrations over the whole range, but approached the theoretical slopes of 58 and 28 mV/10-fold change in concentration at 20 °C, respectively. A WPI M701 amplifier was used to record potentials from the reference side of the double-barrelled electrode and a Burr-Brown Electrometer amplifier (34315) 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. The electrodes were advanced into the cupulae with a hydraulic micro-drive under visual control.

RESULTS

Ca^{2+} measurements

A typical recording of endocupular Ca^{2+} and potential is illustrated in Fig. 1. A stable positive potential of about 40 mV was recorded by the potential barrel when the electrode was advanced into the centre of the cupula just above the sensory epithelium. The response of the Ca^{2+} barrel indicated a marked decrease in endocupular Ca^{2+} with respect to the concentration of Ca^{2+} in the bathing solution, which was 1 mM in the example illustrated.

The relationship between the endocupular Ca^{2+} and endocupular potential was examined in many different cupulae and for different concentrations of $CaCl_2$ in the bath solution. Fig. 2. presents recordings of the endocupular potential and Ca^{2+} of different cupulae from a single animal which was made progressively anoxic by a subcutaneous injection of 0·4 ml, 2 mM oubain in the vicinity of the stitch (which reduced the endocupular potential to 15 mV in about 30 min). Fig. 3 shows data from experiments where the endocupular potential and endocupular Ca^{2+} were measured in cupulae of normal animals, but with different external Ca^{2+} concentrations. From the data presented in these two figures it is clear that the relationship between the endocupular Ca^{2+} and the endocupular potential shows close agreement to the relationship to be expected if the Ca^{2+} were passively distributed in the cupulae.

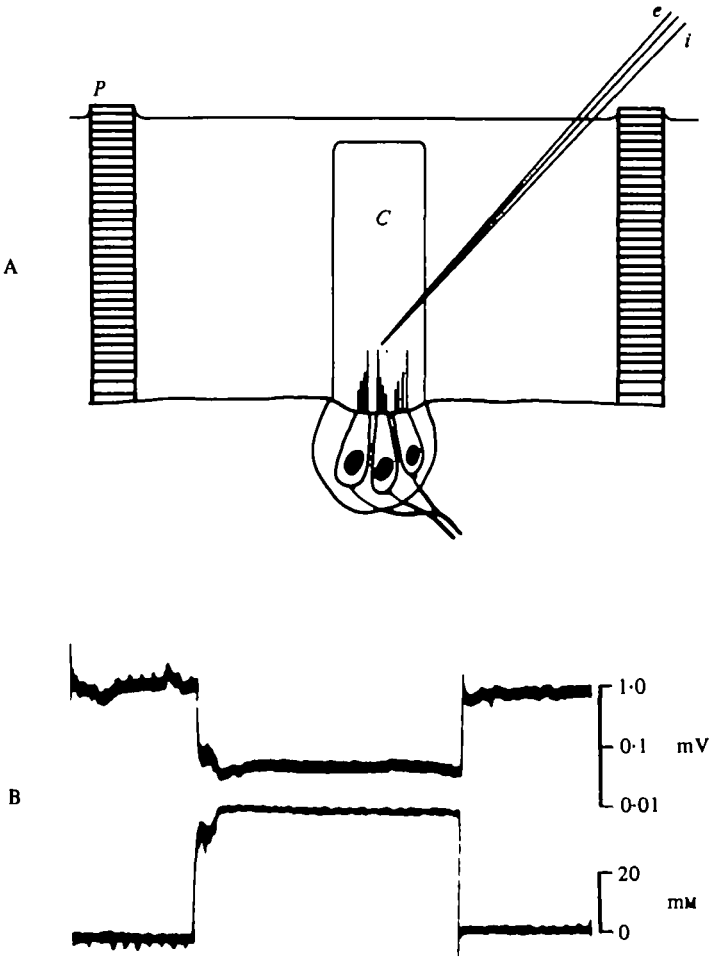

Fig. 1. Responses from the double barrel Ca^{2+} electrodes during insertion into the lateral line cupulae of *Xenopus*. (A) Diagram of recording arrangement, cupula (C), potential electrode (*e*), ion selective electrode (*i*), perspex ring (*P*). (B) Upper trace: response from Ca^{2+} electrode (*E*, Ca^{2+}), lower trace: potential record (endocupular potential).

K^+ measurements

In a different series of experiments the effects of La^{3+} and EGTA were observed on the distribution of K^+ in the cupula. The bath solution in this case contained 1 mM-KCl as a reference for the K^+ selective electrodes. Fig. 4 represents results from one of the two animals tested. The initial control measurements showed normal endocupular K^+ and potential of about 50 mM and 30 mV, respectively. The response from the K^+ electrode upon penetrating the cupula was transient, and fell to quite low levels after the initial peak, as described by Russell & Sellick (1976). They suggested that the cupular space directly under the electrode tip might be depleted of K^+ due to the current requirements of the electrode, and the peak of the transient should thus correspond to the true cupular K^+. Less than 100 μM EGTA blocks the mechano sensitivity of *Necturus* lateral line organs (Sand, 1975), but 1 mM EGTA had no evident effect on either the endocupular potential or the endocupular K^+ in *Xenopus*

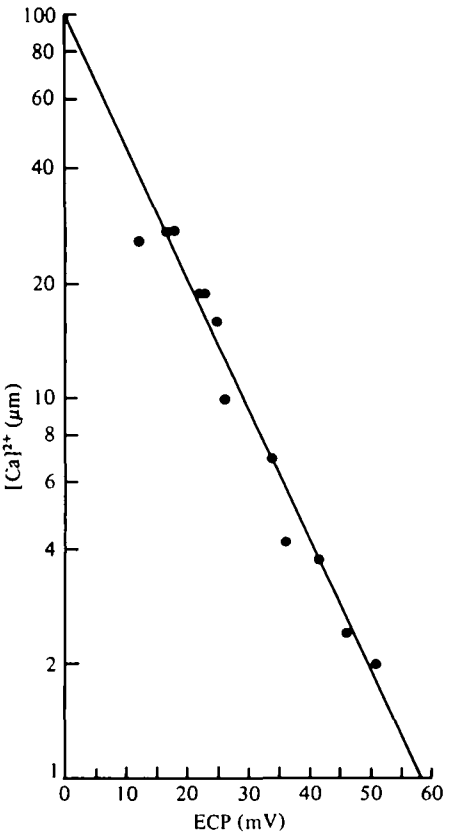

Fig. 2. The relationship between endocupular potential (ECP) and Ca^{2+} concentration of the cupula in a single animal which was made progressively anoxic to reduce the endocupular potential. Each point is recorded from a different cupula, and the bath solution contained 0·1 mM-$CaCl_2$ as reference. The line shows the expected relation if Ca^{2+} is passively distributed in the cupula.

(Fig. 3*b*). La^{3+} is the most potent of the tested blocking agents for the lateral line organs in *Necturus*, and less than 1 μM-La^{3+} completely inhibits the mechanosensitivity. However, 100 μm-La^{3+} had no effect on the endocupular potential or the endocupular K^+ in *Xenopus* (Fig. 3*c*).

DISCUSSION

We have succeeded in demonstrating that Ca^{2+} is passively distributed in the lateral line cupulae of *Xenopus laevis* according to the free concentration of the ion in the aquatic environment and the endocupular potential. The role of the endocupular potential in relation to the distribution of Ca^{2+} is somewhat ambivalent. It tends to increase the driving force for Ca^{2+} across the apical membrane of the hair cell, but tends to exclude it from the cupulae. Thus the normal free concentration of this ion in the lateral line cupulae of a healthy animal is about 10–100 μM, and it is probable that under normal, physiological conditions potassium and not calcium carries the receptor current in the lateral line hair cells of *Xenopus*.

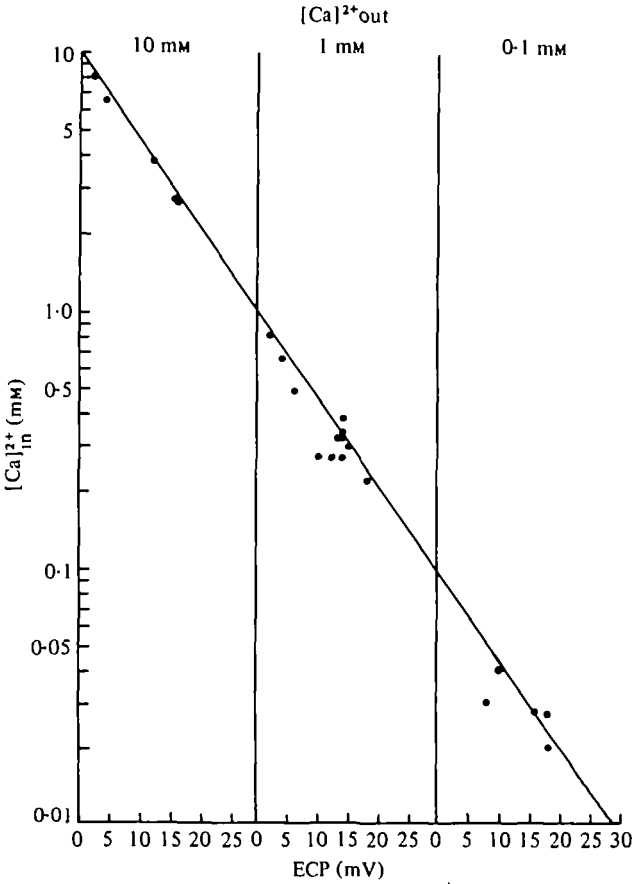

Fig. 3. The relationship between endocupular potential (ECP), endocupular Ca^{2+} and the Ca^{2+} concentration of the bath solution. These are the pooled results from six animals and each point represents a reading from a different cupula. Only those cupulae with endocupular potentials below 29 mV were chosen for convenience of plotting. The line shows the expected relation if Ca^{2+} is passively distributed in the cupula.

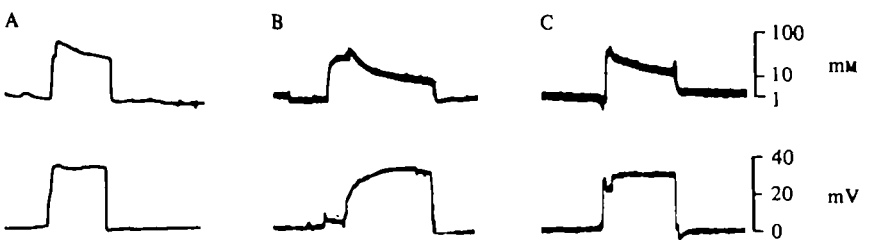

Fig. 4. Responses from double-barrel K^+ electrodes during insertion into a single lateral line cupula. (A) When bathed in the control solution 1 mM-KCl. (B) In the presence of 1 mM EGTA. (C) In the presence of 100 μM-La^{3+}. The cupula was washed in the control bathing solution for at least 10 min between records. In each record upper trace: response from K^+ electrode (E_{BT}), lower trace: potential record.

The very powerful influence of calcium blocking and chelating agents on the mechano-sensitivity of lateral line receptors observed by Sand (1975) is not due to their action on the electrogenic potassium pump. Neither the endocupular potential nor the K^+ concentration was influenced by EGTA or La^{3+} in concentrations well above those known to block the mechano-sensitivity of the receptors. Thus calcium must have some other role, possibly more directly concerned with the transducer process.

The distribution of calcium and potassium in the cupulae of *Xenopus* lateral line organs is remarkably similar to their distribution in cochlear endolymph (Sellick & Johnstone, 1975; Bosher & Warren, 1978), but differs from the composition of the canal jelly to which the embryologically related electrosensory ampullary receptors cells of fishes are exposed (Okitsu, Umekita & Obara, 1978). The jelly is rich in K^+ (40–60 mM) but in addition contains about 6–8 mM-Ca^{2+}. It is tempting to speculate that the presence of high Ca^{2+} may be responsible for the electrosensitivity of these receptors. Ca^{2+} is known to carry the electro-receptor current in the Organs of Lorenzini and these electro-receptors generate Ca^{2+} spikes in the presence of TEA (Clusin & Bennet, 1973). Similarly the normally electrically inexcitable hair cells of the lateral line system (Suga, 1967; Flock *et al.* 1973) become electrically excitable and produce spikes when exposed to high Ca^{2+}, low K^+ solutions (Hudspeth & Corey, 1977). Thus to some extent the ionic environment to which acoustico lateralis hair cells are exposed may influence their electrophysiological properties and consequently their sensory modality.

We thank Professor W. Simon for his generous gift of ion exchanger, Dr P. M. Sellick for helpful comments, and Miss W. Randall for technical assistance. This work was supported by a grant from the S.R.C. and the Johan Peter Ebbel and Wife's Foundation.

REFERENCES

Bosher, S. K. & Warren, R. L. (1978). Very low calcium content of cochlear endolymph, an extracellular fluid. *Nature, Lond.* **273**, 377–379.

Clusin, W. T. & Bennett, M. V. L. (1973). Calcium electrogenesis in skate electroreceptors. *Biol. Bull. mar. biol. Lab., Woods Hole* **145**, 429.

Clusin, W., Spray, D. C. & Bennett, M. V. L. (1975). Activation of a voltage insensitive conductance by inward calcium current. *Nature, Lond.* **256**, 425–427.

Davis, H. (1965). A model for transducer action in the cochlea. *Cold Spr. Harb. Symp. Quant. Biol.* **30**, 181–190.

Flock, Å., Jørgensen, J. M. & Russell, I. J. (1973). The physiology of individual hair cells and their synapses. In *Basic Mechanisms in Hearing* (ed. A. Møller), pp. 273–306. New York: Academic Press.

Furukawa, T. & Ishii, Y. (1967). Neurophysiological studies on hearing in goldfish. *J. Neurophysiol.* **30**, 1377–1403.

Harris, G. G., Frischkopf, L. S. & Flock, Å. (1970). Receptor potentials from hair cells of the lateral line. *Science, N. Y.* **167**, 76–79.

Hudspeth, A. J. & Corey, D. P. (1977). Sensitivity, polarity and conductance change in the response of vertebrate hair cells to controlled mechanical stimuli. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2407–2411.

Johnstone, B. M. & Sellick, P. M. (1972). The peripheral auditory apparatus. *Q. Rev. Biophy.* **5**, 1–57.

Meech, R. W. & Standen, N. B. (1975). Potassium activation in *Helix aspersa* neurones under voltage clamp: a component mediated by calcium influx. *J. Physiol., Lond.* **249**, 211–240.

Mulroy, M. J., Altmann, D. W., Weiss, T. F. & Peak, W. T. (1974). Intracellular responses to sound in a vertebrate cochlea. *Nature, Lond.* **249**, 482–485.

[illegible]ne, M., Kessler, M. & Simon, W. (1976). Neutral carrier Ca^{2+} microelectrode. *Chimia* **30**, 204–206.

Okitsu, S., Umekita, S. & Obarra, S. (1978). Ionic composition of the media across the sensory epithelium in the ampullae of lorenzini of the marine catfish *Plotosus*. *J. comp. Physiol.* **126**, 115–121.

RUSSELL, I. J. & SELLICK, P. M. (1976). Measurement of potassium and chloride ion concentrations in the cupulae of the lateral lines in *Xenopus laevis*. *J. Physiol., Lond.* **257**, 245–255.

RUSSELL, I. J. & SELLICK, P. M. (1977). Turning properties of cochlear hair cells. *Nature, Lond.* **267**, 858–860.

SAND, O. (1975). Effects of different ionic environments on the mechano-sensitivity of lateral line organs in the mudpuppy. *J. comp. Physiol.* **102**, 27–42.

SAND, O., OZAWA, S. & HAGIWARA, S. (1975). Electrical and mechanical stimulation of hair cells in the mudpuppy. *J. comp. Physiol.* A **102**, 13–26.

SELLICK, P. M. & JOHNSTONE, B. M. (1975). Production and role of inner ear fluid. *Prog. Neurobiol.* 5, 337–362.

SUGA, N. (1967). Electrosensitivity of specialised and ordinary lateral line organs of the electric fish *Gymnotus caraper*. In *Lateral Line Detectors* (ed. P. H. Cahn), pp. 395–409. Bloomington: Indiana University Press.

WALKER, J. L. (1977). Liquid ion-exchanger microelectrodes for Ca^{2+}, Cl and K^{+}. In *Ion-Selective Microelectrodes* (ed. W. C. Herbert and R. N. Khun). New York: Dekker.

WERBLIN, F. (1975). Regenerative hyperpolarization in rods. *J. Physiol., Lond.* **244**, 53–82.