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A cyclic-AMP-gated conductance in cochlear hair cells

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The patch clamp technique was used to record cAMP-dependent currents of the guinea pig cochlear hair cell plasma membrane. Data obtained indicate that the channels passing this current are moderately selective for monovalent cations and are effectively blocked by L-*cis*-diltiazem and reversibly blocked by 1 mM Mg^{2+} or Ca^{2+}. The single-channel unit conductance estimated in the absence of divalent cations is about 16 pS. The results demonstrate that cyclic nucleotide-dependent channels of cochlear hair cells are virtually identical to the photoreceptor and olfactory ones.

Cochlear hair cell; cAMP-activated channel; Patch clamp technique

1. INTRODUCTION

Hair cells are the mechanoreceptors of the inner ear. Their transducing organelle is the hair bundle, a cluster of interconnected microvilli up to 10 μm in length. Displacement of the hair bundle results in the generation of a transducer current of 10–250 pA on the hair cell plasma membrane [1,2]. Several types of ionic channels have been identified in the hair cell [3]. The prevailing view is that the transducer channel is mechanically gated when the hair bundle is deformed.

Here we report that excised patches of inner and outer hair cell membranes, from the organ of Corti of guinea pig, contain a conductance which is gated directly by cAMP. It is non-selective to monovalent cations and resembles the cyclic nucleotide-dependent conductance of photoreceptors and olfactory cells [4,5], but differs in that it is activated only by cAMP.

2. MATERIALS AND METHODS

Isolated outer and inner hair cells were obtained from the organ of Corti of guinea pigs. Young adult guinea pigs of either sex (weight 200–600 g) were killed by rapid cervical dislocation. Both bullae were quickly removed. One of the bullae was opened, the cochlea was removed and 4 turns of the organ of Corti were dissected from the cochlear spiral in L-15 cell culture medium (Flow laboratories; the major ions in the medium are (mM) Na^+, 135; K^+, 5; Ca^{2+}, 1.26; Mg^{2+}, 2; and amino acids, adjusted to pH 7.4). The isolated coil was transferred to a 100 μM aliquot to which was added 100 μl of L-15 with 1 mg/ml trypsin. The dissection step could be completed within 3 min. After 20 min the droplet was gently triturated to dissociate the cells and diluted with 200 μl L-15. Inner and outer hair cells could be readily identified by their characteristic morphology [6]. Cells could be used for up to 3–4 hours after dissection. The second bulla could be kept, unopened, in moist tissue for subsequent use within about 5 h. Treatment with collagenase (Sigma, 1 mg/ml, 10 min) or pronase (Sigma, 1 mg/ml, 15 min) and mechanical dissociation was also successfully used to obtain isolated cells.

A conventional patch clamp technique was used to obtain gigaseal excised patches and to measure their electrical characteristics [7].

Solution A was changed to solution B just after a gigaseal had been obtained. The patch pipette was usually filled with solution B. The current signal was low-pas filtered (bandwidth 1500 Hz) before being digitized. A computer system was used to generate command voltage ramps and obtain *I–V* relationships. Solutions (mM): (A) NaCl, 150; $CaCl_2$, 1; $MgCl_2$; 10; HEPES, 10; pH 7.5; (B) NaCl, 150; EGTA, 0.5; EDTA, 0.5; HEPES, 10; pH 7.5. In our experiments the cAMP-gated conductance was low when both sides of excised patches were bathed with physiological solution, while in low Ca^{2+}, Mg^{2+} solution with cAMP-gated conductance was much higher. Therefore to increase the cAMP-gated conductance (i.e. to ensure experimental accuracy) patch pipettes were filled with solution B containing less than 0.1 μM Ca^{2+} and Mg^{2+}.

3. RESULTS

We have examined the conductance of the isolated hair cell plasma membrane using the patch-clamp technique [7]. After gigaseal formation, the membrane patch was excised in the 'inside-out' configuration so the cytoplasmic surface was exposed to the bath solution. The application of cAMP resulted in a reversible increase in patch conductance (Fig. 2) which occurred in the absence of nucleotide triphosphates and thus cannot have resulted from cAMP-stimulated protein phosphorylation. This was observed in 18 out of 37 outer hair cell patches and 9 out of 22 inner hair cell patches. The subsequent addition of 1 mM Mg^{2+} or Ca^{2+} ions in the presence of cAMP reversibly blocked the conductance

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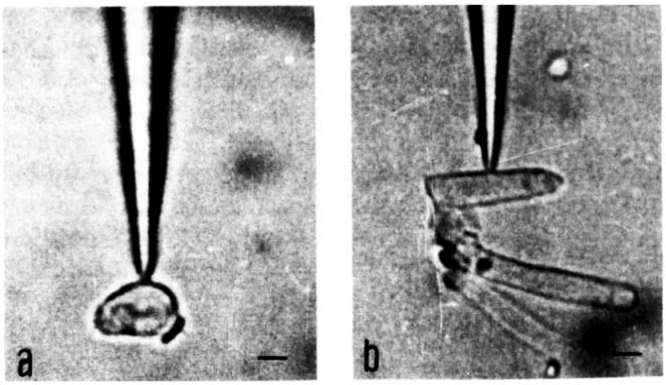

Fig. 1. Photomicrograph of dissociated inner (a) and outer (b) hair cell showing the position of the patch pipette during gigaseal formation. The photomicrograph was taken 3 hours after isolation of the cell. (Scale bar = 10 μm)

(Fig. 2). Further, l-*cis*-diltiazem, which effectively blocks the cyclic nucleotide gated conductance in photoreceptor and olfactory receptor cells [4,5], in 0.5 mM concentration blocked the cAMP-gated conductance of hair cells as well (not shown in the figure).

The outer segments of retinal rods and cones are known to be highly specific for cGMP, while olfactory cell conductance is activated by both cAMP and cGMP. We therefore investigated the sensitivity of the hair cell conductance to cGMP. In our experiments cGMP at 1 mM concentration did not affect the conductance (Fig. 2) although 30 μM of cAMP application was sufficient to significantly increase conductance in the same patch (Fig. 2).

The conductance of the excised patch varied with the concentration of agonist (Fig. 3a). We characterized conductance by measuring the current–voltage relationships of the patches. In Fig. 3b, the normalized conductance of a patch from an inner hair cell is plotted against cAMP concentration. The conductance shows a secondary decrease at a high concentration of agonist, similar to the conductance of photoreceptor and olfactory cells [4,5]. Similar results were obtained in an identical experiment on an outer hair cell.

The selectivity of the cAMP-gated channels was characterized by the shift of the reversal potential of the cAMP-dependent current seen upon equimolar substitution of NaCl by other monovalent metal chlorides in the bathing solution. Two patches, in which the conductance was sufficiently high and stable to provide appropriate accuracy of measurements yielded the following reversal potential shifts (mV): -0.3 ± 0.5; 5.4 ± 0.8; 7.3 ± 1, for Cs^+, Li^+ and Rb^+, respectively. This suggests the following order of permeabilities:

$$Cs^+ > Na^+ > Li^+ > Rb^+$$

The shift induced by substituting NaCl for KCl was not measured exactly because of the high density of potassium channels which are not gated by cAMP in the cell. But this value did not exceed 10 mV and therefore the K^+ permeability of the cAMP-gated channels seems to be of the same order as for other monovalent cations.

We failed in our attempts to record the single channel current. Therefore, the amplitude and kinetic parameters of the channels were estimated by analysis of the fluctuations of the cAMP-gated current. The power spectrum for the current, fitted with two least-square Lorenzians (Fig. 4), indicated cut-off frequencies of 170 Hz and 1000 kHz, corresponding to an intrinsic time of channel fluctuation in the order of 1 ms. The single-

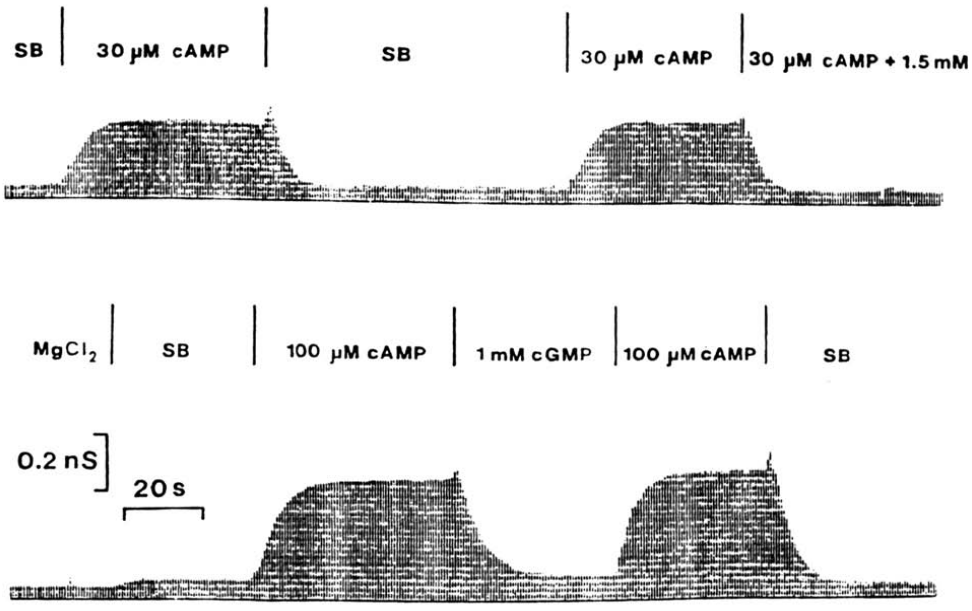

Fig. 2. Chart recordinf of the action of cAMP, Mg^{2+} ions and cGMP on the conductance of an isolated patch of the hair cell plasma membrane. The bars indicate changes in bathing solution. Mg^{2+}, cGMP and cAMP were added to solution B (SB). Mg^{2+} strongly suppressed the cAMP-gated conductance. The same results were obtained with Ca^{2+} (not shown). 10 mV, 15 Hz pulses were applied to measure the patch conductance.

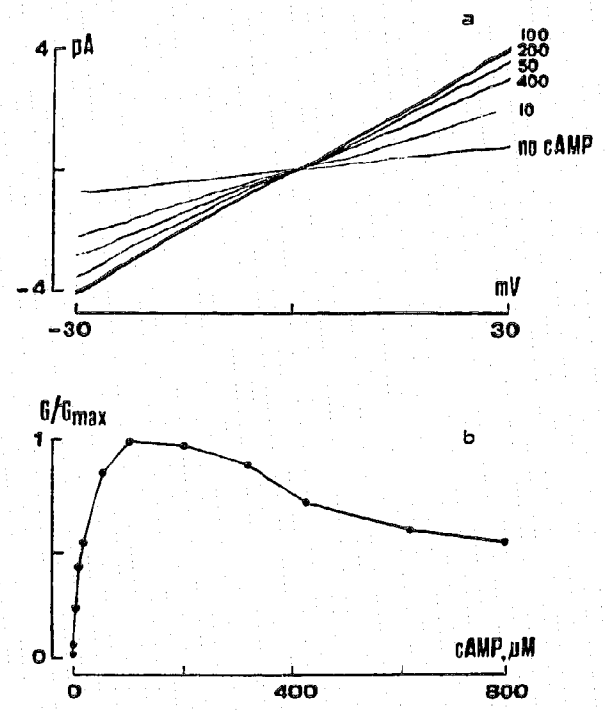

Fig. 3. Concentration dependence of the cAMP-gated conductance measured with divalent-action-free solution on both sides of the membrane: a I–V curves measured in the presence of different concentrations of cAMP (concentration (μM) indicated to the right of each trace); b, normalized conductance plotted as a function of cAMP concentration. The Hill coefficient estimated from the linear part of the curve (0–100 μM) is 1.2.

channel unit conductance estimated in the absence of divalent cations from the variance-to-mean ratio of the cAMP-gated current [8], was 16 pS (Ca^{2+}, Mg^{2+} free solution, holding potential −40 mV). Divalent cations decreased the cAMP-gated conductance by more than an order of magnitude. Suggesting that the blockage

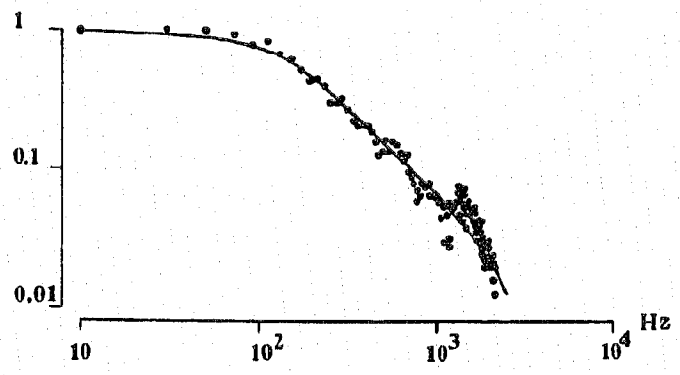

Fig. 4. Power density spectrum of the cAMP-gated fluctuations from a patch of outer hair cell plasma membrane at holding potential −40 mV. The curve is a Lorenzian function

$$S(f) = \frac{0.35}{1 + (f/170)^2} + \frac{0.05}{1 + (f/1000)^2}$$

fitted by the least-squares method. The difference spectrum of patch current fluctuations in the presence and absence of cAMP was calculated to obtain the cAMP-gated current spectrum.

results in a decrease of the unit event amplitude, the single channel conductance in the normal divalent cation concentration seems to be not higher than 1 pS.

4. DISCUSSION

Our data indicate that the properties of cyclic nucleotide-activated channels in hair cell, olfactory cell and photoreceptor cell patches are very similar in respect to conductance, ion selectivity, blockage by Ca^{2+} and Mg^{2+} and sensitivity to diltiazem. Likewise, the cAMP-conductance of hair cell decreased at high agonist concentrations as has been observed in photoreceptor and olfactory patches [4,5]. In rod and cone photoreceptors, photoresponses are known to be generated by the closure of channels directly gated by cGMP [9,10]. Direct gating of conductance by cyclic nucleotides has also been observed in the plasma membrane of olfactory cells [4,11] and has been suggested to play an important role in the olfactory transduction.

The demonstration of a cyclic nucleotide-gated conductance in outer and inner hair cells raises the possibility that mechanotransduction might be mediated by cyclic nucleotides in a similar manner as phototransduction and olfactory transduction. To what extent do these characteristics agree with those which are thought to control the hair cell transducer current? At least two characteristics are available for comparison: (1) selectivity, and (2) unit conductance. (1) The reversal potential of the transducer current of isolated hair cell is close to zero [1,12]. It means that the channels are non-selective which is consistent with the properties of the cAMP-gated conductance described here. (2) The single-channel conductance estimated in ref. [13] from the variance of the transducer current in the presence of divalent cations is 12 pS. This value does not agree with the very small unit conductance of our cAMP-gated channel in a physiological concentration of divalent cations. On the other hand, the estimation in ref. [13] is in principle susceptible to several sources of error. It was based on whole-cell recording, and thus it was necessary to block other conductances by some inhibitors; e.g., if the blockage of Ca^{2+}-activated potassium channels by Cs^+ ions was incomplete, their contribution to the current variance could be substantial.

It might be argued that if the cAMP-gated channels are really connected with transduction, they should be located in the microvilli rather than in the cell body. Our attempts to obtain gigaseal recordings from the microvilli were unfortunately unsuccessful. However in olfactory cells, where the cilia are considered to be the site of transduction [14,15] the cyclic nucleotide-gated conductance is found also in the dendrite and cell body [4].

A role in transduction would require a mechanism by which mechanical movement could change the concentration of cAMP. But there is presently no evidence for the existence of an adenylate cyclase or phosphodies-

terase stimulated by hair bundle displacement. It is also possible that there are several parallel molecular mechanisms providing the transducer current and cAMP-gated channels are involved only in one of them. Thus the involvement of cyclic nucleotides in mechanotransduction would not exclude the possibility that the displacement of microvilli could also directly gate channels in the plasma membrane [3].

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