

ATP- γ -S shifts the operating point of outer hair cell transduction towards scala tympani

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Received 22 November 2004; accepted 16 February 2005

Available online 22 March 2005

Abstract

ATP receptor agonists and antagonists alter cochlear mechanics as measured by changes in distortion product otoacoustic emissions (DPOAE). Some of the effects on DPOAEs are consistent with the hypothesis that ATP affects mechano-electrical transduction and the operating point of the outer hair cells (OHCs). This hypothesis was tested by monitoring the effect of ATP- γ -S on the operating point of the OHCs. Guinea pigs anesthetized with urethane and with sectioned middle ear muscles were used. The cochlear microphonic (CM) was recorded differentially (scala vestibuli referenced to scala tympani) across the basal turn before and after perfusion (20 min) of the perilymph compartment with artificial perilymph (AP) and ATP- γ -S dissolved in AP. The operating point was derived from the cochlear microphonics (CM) recorded in response low frequency (200 Hz) tones at high level (106, 112 and 118 dB SPL). The analysis procedure used a Boltzmann function to simulate the CM waveform and the Boltzmann parameters were adjusted to best-fit the calculated waveform to the CM. Compared to the initial perfusion with AP, ATP- γ -S (333 μ M) enhanced peak clipping of the positive peak of the CM (that occurs during organ of Corti displacements towards scala tympani), which was in keeping with ATP-induced displacement of the transducer towards scala tympani. CM waveform analysis quantified the degree of displacement and showed that the changes were consistent with the stimulus being centered on a different region of the transducer curve. The change of operating point meant that the stimulus was applied to a region of the transducer curve where there was greater saturation of the output on excursions towards scala tympani and less saturation towards scala vestibuli. A significant degree of recovery of the operating point was observed after washing with AP. Dose response curves generated by perfusing ATP- γ -S (333 μ M) in a cumulative manner yielded an EC₅₀ of 19.8 μ M. The ATP antagonist PPADS (0.1 mM) failed to block the effect of ATP- γ -S on operating point, suggesting the response was due to activation of metabotropic and not ionotropic ATP receptors. Multiple perfusions of AP had no significant effect (118 and 112 dB) or moved the operating point slightly (106 dB) in the direction opposite of ATP- γ -S. Results are consistent with an ATP- γ -S induced transducer change comparable to a static movement of the organ of Corti or reticular lamina towards scala tympani.

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Keywords: ATP receptor; ATP- γ -S; Operating point; Cochlear microphonic; Basilar membrane mechanics

Abbreviations: AP, artificial perilymph; ATP, adenosine triphosphate; ATP- γ -S, Adenosine 5'-O-(3-thiotriphosphate); CAP, compound action potential of the auditory nerve; CM, cochlear microphonic; DPOAE, distortion product otoacoustic emissions; OHC, outer hair cell; PPADS, pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid

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1. Introduction

Assigning physiological roles to extracellular ATP and ATP receptors in the cochlea has eluded investigators to date. There are receptors for ATP on many cell types in the cochlea including outer hair cells (OHCs)

and supporting cells (see review: LePrell et al., 2001). ATP receptor agonists and antagonists placed in perilymph have large effects on cochlear potentials including the compound action potential of the auditory nerve (CAP; Bobbin and Thompson, 1978; Kujawa et al., 1994; Skellett et al., 1997; Chen et al., 1998) and on the activity of single auditory nerve fibers (Sueta et al., 2003). One of the largest effects of the ATP antagonist PPADS and the ATP receptor agonist ATP- γ -S is on the magnitude of distortion product otoacoustic emissions (DPOAE; Kujawa et al., 1994; Chen et al., 1998; Bobbin et al., 2000). This suggests that endogenous ATP is affecting the generator of DPOAEs. Although there is a great deal of uncertainty regarding the generation of DPOAEs, there is no doubt that DPOAEs are acoustic events that reflect the mechanical motion of the cochlear partition (Kemp, 1998; Mills, 1998). Therefore, these results led to the speculation that one physiological role for endogenous ATP may be to alter cochlear mechanics during sound exposure (Chen et al., 1998; Parker et al., 2003; Bobbin et al., 2000).

A change in the cochlear mechanics can cause a change in mechano-electrical transduction and changes in the operating point of the OHCs (Kirk et al., 1997; Frank and Kossl, 1996; Sirjani et al., 2004). Therefore, we carried out experiments to measure the effect of ATP- γ -S on cochlear transducer characteristics, as revealed by analysis of the CM waveform. We utilized ATP- γ -S rather than ATP because ATP- γ -S is resistant to metabolism by ectonucleotidases present in the perilymph compartment (Valajkovic et al., 1996) and is therefore more effective than ATP when placed in perilymph (Kujawa et al., 1994). To measure the operating point of the OHCs we used the technique of deriving the operating point from the cochlear microphonic (CM) recorded from the basal turn of the cochlea in response to an intense low frequency tone (Kirk et al., 1997; Frank and Kossl, 1996; Sirjani et al., 2004).

2. Methods

2.1. Animal preparation

Experiments were performed on pigmented guinea pigs of either sex weighing between 300 and 500 g. The animals were anesthetized (urethane, Sigma; 1.5 g/kg, i.p.), their trachea cannulated, and they were allowed to breathe unassisted. The electrocardiogram was monitored throughout each experiment, and rectal temperature was maintained at 38 ± 1 °C by a heating pad. Additional urethane was administered as required to maintain an adequate depth of anesthesia as monitored by lack of withdrawal reflex in response to deep pressure applied to the paw. In all animals, the right auditory bulla was exposed using a ventrolateral ap-

proach, and tendons of the right middle ear muscles were sectioned.

2.2. Cochlear perfusions and drug testing

The ATP- γ -S (Sigma), PPADS (Research Biochemicals International) and salicylate (Sigma) were applied to the guinea pig cochlea by perfusion of the perilymph compartment using an artificial perilymph (AP) having a composition of (in mM): NaCl, 137; KCl, 5; CaCl₂, 2; NaH₂PO₄, 1; MgCl₂, 1; glucose, 11; NaHCO₃, 12. The pH of all solutions was 7.4, with an osmolality of 303 mOsm/kg water. The drugs were dissolved in the AP on the day of use. Perfusates were introduced into the cochlear perilymph at room temperature (24 °C) at a rate of 2.5 μ l/min through a hole made in basal turn scala tympani and were allowed to flow from the cochlea through an effluent hole placed in basal turn scala vestibuli. Effluent was absorbed within the bulla using small cotton wicks. All perfusions were of 20 min duration and recordings were obtained within 3 min of the termination of the perfusions. Approximately 10 min elapsed between perfusions. In 5 drug-treated animals, the protocol involved CM measurements before perfusion, after perfusion of AP, after perfusion of AP containing 333 μ M ATP- γ -S and then following 3 further perfusions of AP. In 5 control animals, CM measurements were made before perfusion and then following up to 6 AP perfusions. In an additional 5 animals a dose response curve was established with a protocol in which CM was measured before perfusion, after perfusion with AP, then after perfusion sequentially with 1, 3.3, 10, 33, 100 and 333 μ M ATP- γ -S.

2.3. Cochlear microphonic recording

The acoustic stimulus (200 Hz) was generated by a waveform generator (Systron Donner, Datapulse 410), attenuated manually (HP 360 D), amplified (McIntosh 250), transduced by a speaker (RadioShack #40-1289) and delivered through a hard wall tube to a hollow ear bar fitted tightly to the external ear canal of each animal. Tones were applied at intensities of 106, 112 and 118 dB SPL. Acoustic distortion was more than 50 dB below the stimulus frequency. The low frequency CM (200 Hz) was recorded by an electrode in scala vestibuli (G1) of the basal turn relative to a reference electrode in scala tympani (G2) of the basal turn with ground in the neck muscle and amplified (Grass, P15, gain 100; filters 0.1 Hz–50 kHz). Within five seconds of presenting the tone to the animal's ear two periods of the CM were captured on an oscilloscope (Tektronix TDS 360), averaged (16 samples of identical phase) and the digitized waveforms stored on the computer disk for offline analysis.

2.4. Operating point analysis of cochlear microphonic

The hair cells which represent the cochlear transducer have nonlinear, saturating characteristics that can be approximated by a Boltzmann function (see discussion by Sirjani et al., 2004). With such a transducer, the output signal depends on where the input signal is “centered” on the transducer curve. The point on the transducer curve at the zero crossings of the applied stimulus is called the operating point, and is expressed as an equivalent pressure (in Pa) at the external canal. Operating point was derived from the low frequency CM using methods identical to those described by Sirjani et al. (2004). First, a simulated microphonic waveform was calculated using a Boltzmann equation to define the relationship between input pressure (P) and output voltage (V). The equation used was

$$V = V_o - V_{\text{sat}} + 2 * V_{\text{sat}} / (1 + \exp(-2 * S / V_{\text{sat}} * (P + P_o))), \quad (1)$$

where V is the output signal; P is the sine wave input pressure to the transducer (Pascals); V_{sat} is the saturation voltage of the transducer; P_o is the operating point (a constant pressure, in Pascals, that defines the transducer output when the input stimulus is at zero); S is the slope of the transducer curve (V/Pa) and V_o is an offset voltage. A curve-fitting procedure was then used in which the four parameters of the Boltzmann curve (V_{sat} , S , P_o , and V_o) together with parameters for frequency and phase of the applied stimulus were simultaneously varied until the calculated waveform represented a best fit to the measured microphonic. V_o , the offset voltage, was simply a DC value to compensate for the fact that CM recordings are typically AC coupled to the recording amplifier so that DC shifts are excluded from the waveform. Changing V_o permits the calculated waveform to be DC shifted towards zero so that the calculated waveform can be aligned with the AC-coupled CM waveform. The best fit between the two waveforms was established as the parameter set that minimized the sum of squares of differences between the calculated and measured waveforms. The parameters S and V_{sat} were highly dependent on the amplitude of the cochlear microphonic while P_o was highly dependent on the microphonic waveform shape, especially with respect to the degree of asymmetry of positive and negative waveform components.

2.5. Statistics

Effects of the treatments were statistically evaluated using repeated measures analysis of variance (ANOVA; StatView, SAS Institute, Inc.) and Student-Neuman-Kuels multiple range test with a $P < 0.01$ considered significant.

The care and use of the animals reported on in this study were approved by LSUHSC’s Institutional Animal Care and Use Committee.

3. Results

Compared to pre-perfusion measures, a perfusion of AP induced slight alterations in the CM and the operating point. Therefore, for each animal, responses measured following this control perfusion served as the new baseline to which all subsequent perfusions were compared.

3.1. Effect of ATP- γ -S (333 μM)

To test whether ATP- γ -S would affect the operating point we first examined a large concentration (333 μM) of the drug in order to ensure a maximal effect. The concentration chosen was based on the dose response curves for this compound reported by Kujawa et al. (1994) for the effect of ATP- γ -S on CAP and cubic DPOAEs. Fig. 1A and 1B show examples of the low frequency CM in response to a 112 dB SPL tone recorded from the basal turn of the guinea pig cochlea after perfusion of AP (Fig. 1A) and after a perfusion of 333 μM ATP- γ -S (Fig. 1B). The waveforms calculated by the Boltzmann fitting procedure are also shown in Fig. 1A and B demonstrating the close fit as the CM waveforms (red) overly the calculated waveforms with only minor areas of deviation. It is established that positive voltages in scala vestibuli occur during displacements of the organ of Corti towards scala tympani (Patuzzi et al., 1989; Patuzzi and Rajan, 1990). ATP- γ -S induced a reduction in the magnitude of the positive peak of the CM (5/5 experiments) accompanied by enhanced peak clipping. It also decreased clipping during negative peaks, corresponding to displacements of the organ of Corti towards scala vestibuli (5/5 experiments) at all three intensities.

Fig. 1C and D show the transducer curves calculated from the CM through the Boltzmann fitting procedure. Fig. 1C shows the transducer curve after perfusion of AP and Fig. 1D after 333 μM ATP- γ -S. The circle indicates the operating point value, around which the stimulus produces equal positive and negative displacements. It is readily apparent that the reduction in the positive peak of the CM together with the peak clipping following 333 μM ATP- γ -S was due to a movement of the operating point along the transducer curve towards scala tympani into a region where there was greater saturation during displacements towards scala tympani and less saturation during displacements towards scala vestibuli.

At the three intensities tested, ATP- γ -S (333 μM) induced a significant shift of the operating point in a

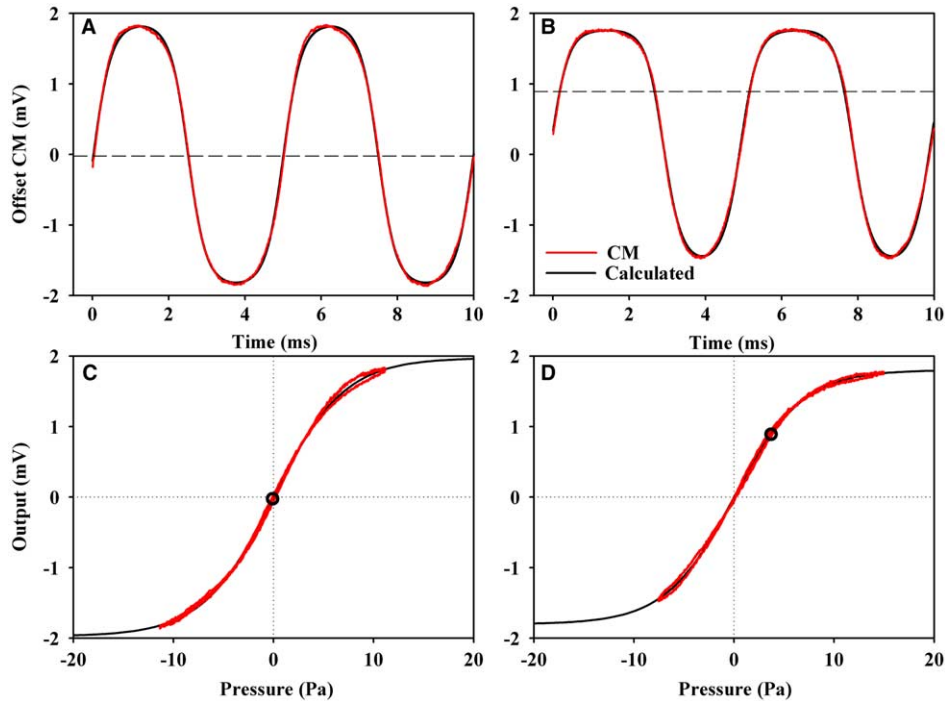


Fig. 1. An example of the effect of perfusion with artificial perilymph (AP) and ATP- γ -S (333 μ M) on the cochlear microphonics (A, B) and on the transducer curves derived from the CM (C, D). The measured cochlear microphonic (red) closely overlies the waveform calculated from a Boltzmann function (black) with only minor regions of deviation. In A and B the dashed line represents the voltage at the zero-crossings of the applied stimulus. There is markedly enhanced peak clipping of the positive deflections in B compared to A and increased amplitude with less clipping of negative deflections. Panels C and D show the microphonic voltage plotted as a function of the combined pressure applied ($P + P_o$). The plots show while the underlying transducer curves are very similar, the location of the operating point (P_o , indicated by the circles) on the curve is shifted towards a positive pressure after ATP- γ -S treatment. The red lines in C and D indicate the instantaneous CM voltages (from the waveforms shown in panels A and B respectively) plotted against the input pressure on a cycle-by-cycle basis.

positive, scala tympani direction (Table 1; Fig. 2; $n = 5$). The magnitude of the induced shift was larger when measured with higher level tones, which is only partially offset by the increasingly positive operating point values following the first AP perfusion in the control experiments (mean operating point in Pa \pm SD: 106 dB SPL,

-0.48 ± 0.47 ; 112 dB SPL, 0.07 ± 1.05 ; 118 dB SPL, 0.53 ± 1.78 ; $n = 5$).

The magnitude of operating point changes induced by ATP- γ -S and by control perfusions are summarized in Fig. 2. There was significant recovery upon washing with AP, although the operating point remained

Table 1
Effect of ATP- γ -S (333 μ M) on various parameters in the Boltzmann fitting procedure^a

Parameter	AP	ATP- γ -S 333 μ M	Wash 1	Wash 2	Wash 3
P_o 118 dB	1.476 ± 1.837	$7.276 \pm 0.902^*$	$4.765 \pm 1.222^*$	$4.175 \pm 1.119^*$	$3.979 \pm 1.199^*$
P_o 112 dB	-0.045 ± 0.861	$4.068 \pm 0.378^*$	$2.139 \pm 0.396^*$	$1.994 \pm 0.599^*$	$1.892 \pm 0.594^*$
P_o 106 dB	-0.523 ± 0.655	$2.119 \pm 2.119^*$	$0.878 \pm 0.878^*$	$0.797 \pm 0.797^*$	$0.902 \pm 0.902^*$
V_o 118 dB	-0.087 ± 0.147	$-0.449 \pm 0.084^*$	$-0.308 \pm 0.098^*$	$-0.250 \pm 0.088^*$	$-0.236 \pm 0.101^*$
V_o 112 dB	0.026 ± 0.133	$-0.478 \pm 0.077^*$	$-0.251 \pm 0.080^*$	$-0.220 \pm 0.096^*$	$-0.187 \pm 0.074^*$
V_o 106 dB	0.141 ± 0.147	$-0.417 \pm 0.078^*$	$-0.157 \pm 0.104^*$	$-0.124 \pm 0.120^*$	$-0.114 \pm 0.090^*$
z 118 dB	0.255 ± 0.054	0.250 ± 0.053	0.227 ± 0.038	0.217 ± 0.046	$0.199 \pm 0.050^*$
z 112 dB	0.341 ± 0.095	0.331 ± 0.106	0.299 ± 0.076	0.286 ± 0.092	$0.261 \pm 0.093^*$
z 106 dB	0.404 ± 0.167	0.417 ± 0.181	0.353 ± 0.128	0.341 ± 0.158	$0.301 \pm 0.143^*$
V_{sat} 118 dB	2.432 ± 0.256	$2.124 \pm 0.220^*$	2.264 ± 0.217	$2.160 \pm 0.183^*$	$2.100 \pm 0.128^*$
V_{sat} 112 dB	2.213 ± 0.273	$1.929 \pm 0.171^*$	2.053 ± 0.187	$1.931 \pm 0.173^*$	$1.874 \pm 0.136^*$
V_{sat} 106 dB	1.900 ± 0.242	1.737 ± 0.149	1.736 ± 0.178	$1.653 \pm 0.200^*$	$1.614 \pm 0.164^*$

^a Shown are means \pm SD.

* Indicates significantly different from the AP value ($P < 0.01$). Parameters shown are those from Eq. 1 (see Section 2) in which P_o is the operating point, V_o is the DC voltage offset, z is transducer slope and V_{sat} is the saturation voltage of the transducer.

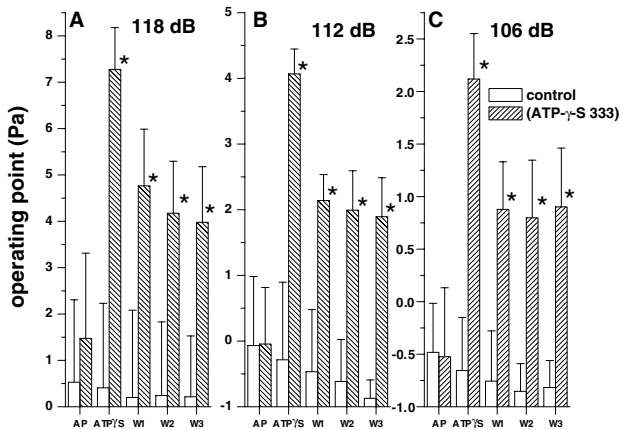


Fig. 2. Effect of ATP- γ -S (333 μ M) on the operating point. Shown are the data evoked by tones of 118 dB (A), 112 dB (B) and 106 dB (C) after perfusion with control artificial perilymph (AP), ATP- γ -S (333 μ M), and artificial perilymph washes (W1–W3). For comparison, the data obtained following multiple perfusions with artificial perilymph are also shown (control). Data are displayed as means \pm SD across five animals for both the drug and control groups. The asterisk indicates values significantly ($P < 0.01$) different from the first artificial perilymph perfusion (AP).

significantly different from the initial AP value. Multiple control perfusions of AP did not significantly alter the operating point at 118 and 112 dB ($n = 5$) although at 106 dB there was a small significant movement of the operating point towards negative values after the 4th and 5th perfusions of AP compared to the first AP value. The induced operating point increases were highly significant at all three stimulus levels tested.

Changes in the values of other Boltzmann parameters were also observed (Table 1). ATP- γ -S (333 μ M) induced a significantly greater negative voltage offset that paralleled the changes in the operating point. The drug induced small but significant decreases in V_{sat} at 118 and 112 dB with some recovery occurring following wash 1 but additional changes with subsequent washes. The slope of the transducer curve was not significantly altered by ATP- γ -S (333 μ M) but slope did decline significantly following later washes. This lack of effect of ATP on the transducer slope suggests that the drug did not alter the ability of the hair cells to transduce input stimuli. None of the parameters were significantly altered by multiple perfusions of AP except for the changes operating point following the 4th and 5th perfusions noted above.

3.2. Effect of salicylate 10 mM

Salicylate has been shown to change the asymmetry of the transduction curve (Frank and Kossl, 1996) and to shift the operating point towards scala tympani (Bian and Chertoff, 1998; Patuzzi and Moleirinho, 1998). Therefore we tested the effects of salicylate on the oper-

ating point to compare with the effects of ATP- γ -S. Following multiple perfusions with AP in the control animals salicylate (10 mM) was perfused and the operating point measured ($n = 5$ animals). Consistent with the data of others (Bian and Chertoff, 1998; Patuzzi and Moleirinho, 1998), salicylate shifted the operating point towards positive values (towards scala tympani) when compared to the AP immediately preceding the perfusion with salicylate (mean \pm SD; 118 dB: AP = 0.14 ± 1.28 ; salicylate = 1.51 ± 1.07 ; 112 dB: AP = -0.82 ± 0.33 ; salicylate = 0.58 ± 0.63 ; 106 dB: AP = -0.80 ± 0.35 ; salicylate = 0.51 ± 0.40). The effect of salicylate on the operating point was much smaller than the effect of ATP- γ -S and only the salicylate effect at 106 dB was statistically significant from AP.

3.3. Effect of increasing concentrations of ATP- γ -S

Upon obtaining significant effects with 333 μ M ATP- γ -S, the dose responsiveness of the effect on operating point was examined. For this purpose the perilymph compartment was perfused first with AP and then with increasing concentrations (1, 3.3, 10, 33, 100 and 333 μ M) of ATP- γ -S in a cumulative manner (Figs. 3 and 4; $n = 5$ experiments). As illustrated for the 112 dB tone in Fig. 3 with increasing concentrations of the drug the positive peak of the CM decreased and the peak clipping on the positive, scala tympani phase became larger. The increase in the magnitude of the negative peak of the CM in response to 100 μ M ATP- γ -S shown in Fig. 3 occurred in all five experiments and varied with intensity (118 dB SPL = 3 increase, 2 no change; 112 dB SPL = 4 increase, 1 no change; 106 dB = 5 increase). As expected given the changes in the CM, at the three intensities tested increasing concentrations of ATP- γ -S induced an increasing shift of the operating point in a positive, scala tympani direction (Figs. 3 and 4). The operating point value for 1 μ M was not different from the preceding AP value and therefore was considered equal to baseline. To obtain an EC_{50} the responses at the three intensities were normalized so that the operating point value for 1 μ M was equal to zero and the maximal effect was equal to one and the resultant values were fit with a Hill equation (Origin, Microcal Software, Inc.; Fig. 5). This procedure gave an EC_{50} of 19.8 ± 1.9 μ M with a Hill coefficient of 1.26 ± 0.12 .

3.4. Effect of PPADS an ATP receptor antagonist

PPADS is an effective blocker of the actions of ATP on ionotropic receptors on OHCs, Deiters' cells, Hensen's cells and pillar cells (Chen et al., 1998). To test if ionotropic receptors contributed to the actions of ATP- γ -S on the operating point we carried out experiments similar to those used to construct the dose

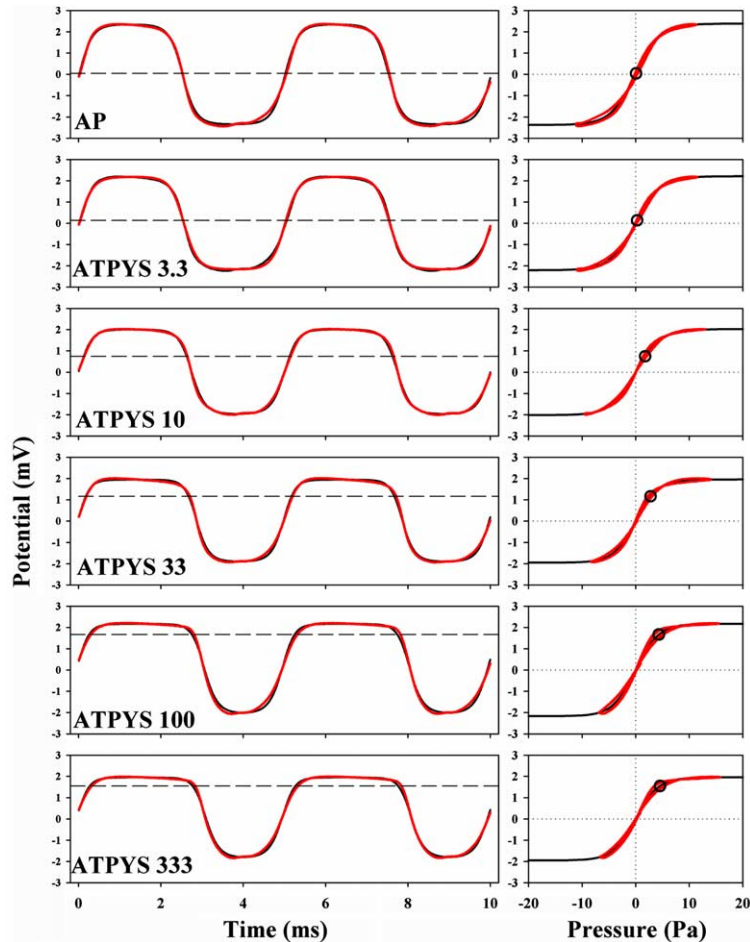


Fig. 3. An example of the effect of perfusion with artificial perilymph (AP) and cumulative increasing concentrations of ATP- γ -S (3.3, 10, 33, 100, 333 μ M) on the CM evoked by 200 Hz tones at 112 dB. The inferred changes in transducer properties are shown in the panels at the right. Measured cochlear microphonics (red) closely overlies waveforms calculated from a Boltzmann function (black) with only minor regions of deviation. The dose-dependent ATP- γ -S induced changes of the microphonic waveform are completely accounted for by a change in the operating point on which the stimulus is centered on the transducer curve. For additional information see legend for Fig. 1.

response curves in the presence of PPADS. The scala was perfused in a cumulative manner with concentrations of ATP- γ -S ranging from 1 to 333 μ M in the presence of 100 μ M PPADS ($n = 2$). The presence of PPADS did not suppress the effects of ATP- γ -S on the operating point and instead appeared to slightly enhance the effects of low concentrations (1, 3 and 10 μ M) of the agonist (data not shown).

4. Discussion

The present study tested the hypothesis that ATP receptor activation with the agonist ATP- γ -S affects mechano-electrical transduction and the operating point of the OHCs. The hypothesis was suggested by previous studies showing activation and blockade of ATP receptors affected the mechanical motion of the cochlear partition as indicated by changes in DPOAEs (Bobbin et al., 2000; Chen et al., 1998; Kujawa et al., 1994).

We found that in the presence of ATP- γ -S, the CM demonstrated a very large increase in peak clipping on the positive, scala tympani phase with a decrease in peak clipping on the negative, scala vestibuli phase. A Boltzmann fitting procedure indicated a movement of the stimulus along the transducer curve towards scala tympani with an accompanied movement of the operating point also towards scala tympani. This movement was into a region of the transducer curve where there was greater saturation of the output on excursions towards scala tympani and less saturation towards scala vestibuli. As discussed by others (Kirk et al., 1997; Frank and Kossl, 1996; Sirjani et al., 2004) this type of change in the operating point is consistent with an ATP- γ -S induced static movement of the transducer towards scala tympani. The precise origins of this displacement remain uncertain and it could be mediated through anatomic changes at the stereocilia, the hair cells, supporting cells, the position of the organ of Corti, or even through endolymph volume changes.

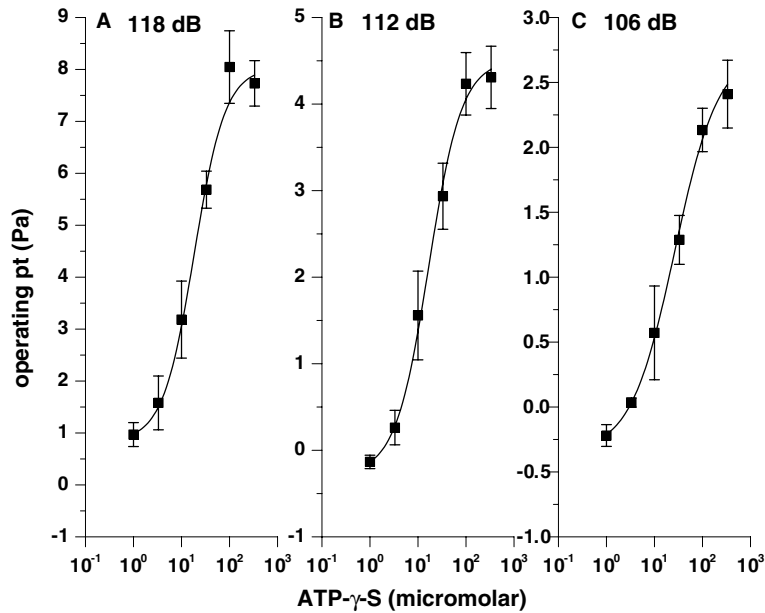


Fig. 4. Effects of cumulative increasing concentrations of ATP- γ -S on the operating point obtained in response to 118 dB (A), 112 dB (B) and 106 dB (C) tones. Data are displayed as means \pm SD across five animals. The curves represent sigmoidal fits of the data (Origin, Microcal Software, Inc.).

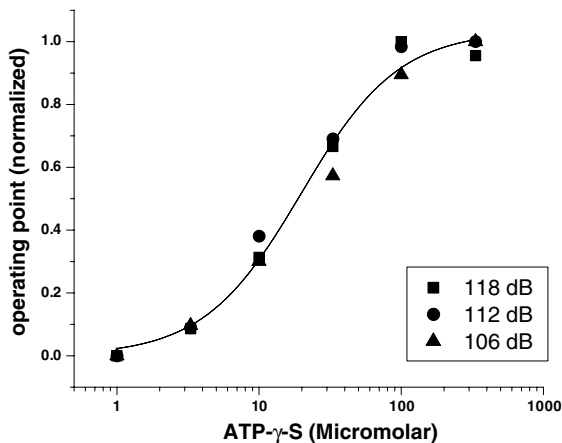


Fig. 5. Dose response curve of the effect of ATP- γ -S on the operating point. The operating point values for each intensity from Fig. 4 were normalized by setting the operating point value for 1 μ M to zero and the maximal operating point value to one yielding three values (one for each intensity) for each concentration of drug. These were then fit to a Hill equation to generate the dose response curve and yielded an EC_{50} of $19.8 \pm 1.9 \mu$ M and a Hill coefficient of 1.26 ± 0.12 (Origin, Microcal Software, Inc.).

The failure to detect a reduction in the response to the agonist in the presence of PPADS suggests that the actions of ATP- γ -S are due to activation of metabotropic ATP receptors and not ionotropic receptors. From the perilymph compartment ATP- γ -S had access to several cells that apparently have metabotropic ATP receptors (LePrell et al., 2001; Parker et al., 2003). We assume that the ATP- γ -S being a charged hydrophilic compound had little access to receptors exposed to endolymph.

The ATP- γ -S may have acted directly on metabotropic receptors on the OHCs. The ionotropic receptors on the OHCs appear to be located on the endolymph surface of the OHCs (Housley et al., 1992) but the location of metabotropic receptors may be on the perilymph surface and accessible to the drug (Ashmore and Ohmori, 1990; Parker et al., 2003). In invertebrate hair cells, it is known that transduction is regulated by levels of calcium in the stereocilia (Assad et al., 1989; Crawford et al., 1989; Ricci and Fettiplace, 1997, 1998). Elevation of calcium in the stereocilia increases adaptation and shifts the current-displacement relationship of the transducer to the right (Wu et al., 1999). This shift changes the set point of the transducer channels that reduces the amount of current on at rest and makes larger displacements necessary to obtain the same amount of current (Wu et al., 1999). Although such an effect could explain our results, it is not known to what degree mammalian OHC show comparable adaptation. In addition it is very unlikely that ATP applied to perilymph and acting on OHCs to release calcium from stores can alter calcium levels in the stereocilia given the barriers to intracellular calcium diffusion and the various calcium regulatory processes in these cells. Motility of OHCs may have played very little role in altering the operating point since previous studies failed to observe a movement of isolated OHCs in response to the application of ATP (Kujawa et al., 1994). In addition, salicylate, a drug thought to block the OHC motor protein (Zheng et al., 2000), had only a very slight effect on the operating point.

There are other cells in the organ of Corti other than OHCs that ATP- γ -S may have acted on to alter the

mechanics of the organ, including pillar cells, Deiters' cells, and Hensen's cells that also appear to have metabotropic ATP receptors (Bobbin et al., 2001; Chen and Bobbin, 1998; Dulon, 1995; Lagostena et al., 2001; LePorell et al., 2001; Parker et al., 2003). An ATP induced motion of Deiters' cells, or an alteration in their stiffness has been hypothesized as one of the mechanisms that underlies the ATP induced changes in cochlear mechanics (Bobbin, 2001; Bobbin et al., 2000, 2001). Anatomically, Deiters' cells appear to be positioned to directly affect the operating point. The cell body of Deiters' cells attaches to the base of the OHCs, and the long stalk or phalangeal process of Deiters' cells attaches to the apex of the OHCs (Slepecky, 1996). A movement of the stalk towards scala tympani could shift the transducer and the operating point towards scala tympani (Kirk et al., 1997; Frank and Kossel, 1996; Sirjani et al., 2004). Dulon et al. (1994) has demonstrated that Deiters' cells will change their stiffness in response to a change in intracellular calcium levels. Bobbin (2001) has demonstrated that the stalk of an isolated Deiters' cell will move in response to the application of ATP. Thus there is some evidence that Deiters' cells may be involved in the ATP- γ -S induced shift in the operating point.

An increase in the volume of scala media may have also contributed to the effects of ATP- γ -S since this would act to displace the organ of Corti towards scala tympani and shift the operating point towards scala tympani (Salt, 2004). One mechanism for this increase in volume may be found in the experiments by Flock et al. (1999). Utilizing an *in vivo* preparation, they demonstrated a reversible motion of the Deiters' cells and Hensen's cells towards "the center of the cochlear turn" in response to an intense low frequency sound. Flock et al. (1999) attribute the motion to the outer row of Deiters' cells. The outer row of Deiters' cells is the row we reported stained most intensely for P2Y₄ receptors (Parker et al., 2003). Others have demonstrated that a low frequency sound similar to the one used by Flock et al. (1999) induces a movement of the operating point towards scala tympani (Kirk et al., 1997; Kirk and Patuzzi, 1997; Sirjani et al., 2004), increases the second harmonic in the CM (Sirjani et al., 2004), and increases the volume of scala media (Salt, 2004). Future experiments will determine if there is a relationship between these physiological responses and ATP receptors.

The physiological significance of the results is speculative. As reported here and by others (Bian and Chertoff, 1998; Patuzzi and Moleirinho, 1998) as intensity of the tone increased the operating point moved in the scala tympani direction and toward a region of the transducer curve where there was greater saturation. Activation of ATP receptors moves the operating point in the same direction as increasing the intensity of sound. This correlation suggests that activation of ATP receptors may play a role in the shift of the oper-

ating point with sound intensity. The future availability of metabotropic ATP receptor antagonists will allow the testing of this and other possible hypothesis.

A displacement of the transducer may explain the increase in endocochlear potential observed with ATP agonists reported first by Kujawa et al. (1994) and later confirmed by others (Sueta et al., 2003). It is generally thought that excursions of the cochlear partition towards scala tympani results in the closing of transduction channels, while excursions of the partition towards scala vestibuli opens transduction channels (Patuzzi et al., 1989). Thus an ATP- γ -S induced static displacement of the organ of Corti or reticular lamina towards scala tympani would result in the closing of additional transduction channels at rest compared to the pre-drug condition (Kirk et al., 1997). The closing of the transduction channels, the accompanied increase in resistance and decrease in current flow through the transduction channels may in turn result in an increase the endocochlear potential (as discussed by Kirk and Patuzzi, 1997).

In summary, this study reports that activation of ATP receptors with ATP- γ -S results in changes in low frequency CM that are consistent with an alteration in the mechanical electrical transduction of the OHCs and a movement of the operating point towards scala tympani. In addition, these results are consistent with the interpretation that the drug induced a static movement of the organ of Corti or reticular lamina towards scala tympani. The cellular mechanisms underlying this movement remain to be elucidated by future studies.

Acknowledgements

This work was supported by Grants DC04994 (RPB) and DC01368 (ANS) from the National Institute of Deafness and Other Communication Disorders. Thanks to Megan E. Fleming and Anthony Ricci, Ph.D. for help.

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