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蛋白-G 與磷酸化對耳蝸毛細胞鈣離子電流及其運動性調控作用之研究

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行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告 □期中進度報告

（計畫名稱：蛋白-G與磷酸化對耳蝸毛細胞鈣離子電流及其運動性調控作用之研究）

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中文摘要

本篇主要是研究鈣離子阻斷劑對於天竺鼠外毛細胞運動性的研究。外毛細胞前處理太斑毒素(taicatoxin, 0.19μM)可以抑制高鉀(50 mM)引起毛細胞收縮以及ionomycin (10 μM)引起毛細胞延長的作用。太斑毒素的這些作用可以被無鈣的緩衝液所模擬。其餘的選擇性鈣離子管道抑制劑, Nifedipine (L-型), ω-conotoxin GVIA (N-型), ω-agatoxin IVA (P-型) 以及鉀離子管道阻斷劑 tetraethylammonium chloride (TEA), 或 3,4-diaminopyridine (3,4-DAP) 皆無法影響高鉀以及ionomycin 引起的毛細胞運動性。再鈣離子螢光影像分析的實驗顯示, 太斑毒素不會影響高鉀所引起的細胞內鈣上昇作用。此結果顯示, 太斑毒素的作用是經由干擾鈣離子與細胞膜表面的交互作用而不是經由鈣離子管道或是鉀離子管道的作用而來。太斑毒素毒素可以提供一個有力的工具用來研究鈣離子依賴性的毛細胞運動性的分子機制。(已投稿至 Hearing Research) 。

關鍵詞: 鈣離子管道阻斷劑、太斑毒素、外毛細胞、運動性、鈣離子。

Abstract

The effects of calcium channel blockers on the slow motility of isolated outer hair cells of guinea-pig were studied in the experiments. Pretreatment with taicatoxin (0.19 μM) was able to prevent the cell shortening induced by high K^+ (50 mM) medium and the cell elongation induced by ionomycin (10 μM), respectively. These effects of taicatoxin can be mimicked by cells treatment with Ca^{2+}-free medium on the slow motility in response to ionomycin or high K^+ medium. Neither the pretreatment of calcium channel blockers, nifedipine (L-type blocker), ω-conotoxin GVIA (N-type blocker), ω-agatoxin IVA (P-type blocker) nor potassium channel blockers, tetraethylammonium chloride (TEA), or 3,4-diaminopyridine (3,4-DAP) can antagonize the cell shortening effect induced by high K^+ medium and cell elongation induced by ionomycin. The calcium-imaging experiment also indicated that taicatoxin does not affect the increase of intracellular Ca^{2+} level induced by high K^+ medium. Our data indicate that the effect of taicatoxin (a membrane impermeable chemical) was to interfere the calcium to act on membrane surface of cell without relative with its properties of calcium channel blocker and calcium-activated potassium blocker. The taicatoxin may provide a powerful tool for the studies on the precise molecular mechanism of calcium-dependent motility of outer hair cell.

Key words: Calcium channel blocker, Taicatoxin, Outer hair cell; Motility; Calcium

1. Introduction (前言、研究目的、文獻探討)

Taicatoxin isolated from the venom of the Australian taipan snake (*Oxyuranus*

scutellatus), has been previously regarded as a specific blocker of high threshold calcium channel (Possani et al., 1992) and further reconsidered as a blocker of small conductance Ca^{2+}-activated K^+ channels (Doorty, et al., 1997).

Mechanosensory hair cells of the vertebrate inner ear contribute to acoustic tuning through feedback processes involving Ca^{2+} and Ca^{2+}-activated K^+ channels in the basolateral membrane and mechanotransduction channels in the apical hair bundle (Fettiplace and Fuchs, 1999). Electrical tuning in hair cells of vertebrates is derived chiefly from the kinetic interplay between basolateral voltage-gated Ca^{2+} channels and nearby large-conductance Ca^{2+}-activated K^+ (BK) channels, as modelled by Hudspeth & Lewis (1988) for the frog sacculus and Wu *et al.* (1995) for the turtle cochlea. Voltage-gated Ca^{2+} channels have been characterized in hair cells of several different species (Fuchs et al., 1990; Zidanic and Fuchs, 1995). A recent study revealed that hair cells of the chick basilar papilla predominantly expressed in these the $\alpha 1_D$ subunit (Kollmar et al., 1997), suggesting a role for class D-LTCCs (Voltage-gated L-type Ca^{2+} channels) for afferent synaptic transmission. Hair cells employ Ca^{2+} ions to mediate sharp electrical tuning and neurotransmitter release.

Therefore, the expectation on the cell motility would be affected by the blockade of the Ca^{2+} or Ca^{2+}-activated K^+ channels. In the present studies, we have tested the effect of taicatoxin on the slow motility of mammalian outer hair cells. Unexpectedly, the effect of taicatoxin on the slow motility is to interfere the extracellular Ca^{2+} to act on the target of external membrane surface of outer hair cell without relative with its properties of Ca^{2+} or K^+ channels blocker.

2. Materials and Methods (研究方法)

2.1. Single outer hair cell isolation

Outer hair cells were isolated from guinea pig (Hartley, National Laboratory Animal Breeding and Research Center, Taiwan) after enzymatic and mechanic dissociation as described previously (Tan et al., 2001; Lin et al., 2003). Adult guinea pig (250~400g) were suffocated with carbon dioxide and decapitated according to *National Institutes of Health Guidelines for Animal Use*. The temporal bones quickly removed and bulla and the bony walls of cochlea were immediately opened and the organ of Corti dissected. Tissues and dissociated cells were handled in a Leibovitz cell culture medium (L-15) containing the following inorganic salts (in mM): 137 NaCl, 5.4 KCl, 1.3 $CaCl_2$, 1.0 $MgCl_2$, 1.0 Na_2HPO_4, 0.44 KH_2PO_4, and 0.81 $MgSO_4$, pH 7.4 (was adjusted with 1 N HCl). The first two apical turns of organ of Corti were collected and transferred into collagenase (type IV, Sigma) solution (0.5 mg/ml in L-15 medium). After 10~15 min digestion the pieces of organ of Corti were placed in a cover-slip chamber and the outer hair cell were isolated by gentle titration for 3~5

times with a 20 µl pipette under stereomicroscope at room temperature. The cells were hold on the coverslip surface it was pre-coated with adhesive Cell-Tak (20 µg/coverslip; Collaborative Biomedical Products, Bedford, MA) or poly-l-lysine (50-80 µg/ml; Sigma).

2.2. Cell slow motility measurement

Slow cell motility (contraction and elongation) was recorded for 10 min using 40 X or 100 X oil immersion objective on an inverted Zeiss microscope (Axiovert 25, Germany) fitted with a digital camera Axiocam (Zeiss, Germany). The image of cells were captured in 30 s intervals and recorded in the computer. Analysis of length change was performed by the software AxioVision (Zeiss, Germany) or the image-processing software National Institutes of Health Image (National Institutes of Health, Bethesda, MD). Cells were selected for measurement without obvious sign of damage, swelling, nucleus dislocation or granulation. Cell lengths were measured from the distance between the cuticular plate and the synaptic ending of the cell. Hair cells were immersed in 0.5 ml cover-slip chamber containing standard extracellular solution (Hank's balanced salt solution) consisting of 137 mM NaCl, 5.4 mM KCl, 1.25 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 0.33 mM Na_2HPO_4, 10 mM D-glucose and 5 mM HEPES. High-K^+ medium (50 mM) was prepared by isosmotically substituting KCl for NaCl. In some experiments Ca^{2+} free medium was used, which consisting of 137 mM NaCl, 5.4 mM KCl, 0.5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 0.33 mM Na_2HPO_4, EGTA 1 mM, 10 mM D-glucose and 5 mM HEPES. The pH was adjusted to 7.35~7.40 with 1 N NaOH and the Osmolality to 316 ± 3 mosm. Osmolality (osm) of the medium was controlled before and after each experiment at room temperature (24~27 ℃). The length of isolated apical outer hair cells ranged from 60~80 µm were used for this experiment.

2.3. Measurement of cell intracellular calcium

The changes of intracellular Ca^{2+} concentration in outer hair cells were determined using the fluorescent Ca^{2+} indicator fluo-3/AM. We simultaneously visualized hair cells loaded fluo-3 via epifluorescence with a Zeiss LSM 410 confocal microscope equipped with 100 X, oil-immersion objective lens of numerical aperture 1.3. The scanned pictured was recorded in the computer by LSM 410 software at 10 s intervals. Fluorescent images were converted to gray scale for analysis using Scion (NIH) software. A stock solution of Fluo-3/AM 1 mM (Molecular Probes, Eugene, USA) was dissolved in DMSO and diluted to final 3 µM with Hanks' balanced salt solution loaded into outer hair cells on cover slip for 40 ~ 60 min at room temperature in the dark. Cells loaded with fluo-3 were observed with an inverted microscope (Zeiss Axiovert 135M, Germany), with excitation at 488nm and emission at 525nm. The illumination intensity was attenuated with a neutral density filter to avoid

phototoxicity by reducing dye-bleaching rates to < 0.2%. The changes of intracellular calcium levels of outer hair cells were indicated by the relative Fluo-3 fluorescence intensity. The intracellular calcium increase of cells can be elicited by the addition of high K^+ medium as following: 78 mM NaCl, 50 mM KCl, 10 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 0.33 mM Na_2HPO_4, 10 mM D-glucose and 5 mM HEPES.

2.4. Mediums Osmolality

The Osmolality of mediums for used in these experiments were controlled to be 316 ± 3 mOsm/Kg, using a vapor pressure osmometer (model 5004, Precision System Inc., Mass, USA).

Chemicals and Chemical preparation

Nifedipine, tetraethylammonium chloride (TEA), 3,4-diaminopyridine (3,4-DAP), ionomycin were obtained from Sigma (St Louis, MO, USA). Taicatoxin, ω-conotoxin GVIA and ω-agatoxin IVA were obtained from Alomone labs (Jerusalem, Israel). Flou-3 AM was obtained from Fluka (Buchs, Switzerland). All drugs were dissolved in Hank's balanced salt solution (normal Ca^{2+}, Ca^{2+}-free or high K^+ medium) except Fluo-3 AM, which were dissolved in dimethyl sulphoxide (DMSO). The final total DMSO concentration did not exceed 0.5 % in the bath medium. Each day of use, we made a fresh stock solution for all chemicals.

Statistics

The values given are means ± SEM. The significance of differences was evaluated by the Student's *t*-test. When more than one group was compared with one control, significance was evaluated according to one-way analysis of variance (ANOVA). Probability values of < 0.05 were considered to be significant.

3. Results (結果)

3.1. Taicatoxin block the effect of cell shortening induced by high K^+

The size of outer hair cell between from 60~ 80 μm were used through the experiments. Outer hair cells were soaked in 0.5 ml chamber containing Hanks' balanced salt solution and ready for the experiments. The isolated outer hair cell shortening (slow motility) can be induced by the addition of isotonic high K^+ (50 mM) medium (n = 12). The effect of cell shortening (contraction) with high K^+-treated reached a maximum of about 90% of the before treatment. The pretreatment of taicatoxin (0.19 μM; n = 9) or EGTA (3 mM, n = 6) for 10 min, can significantly prevent the effect of cell shortening induced by high K^+ medium (Fig. 1).

3.2. Effect of various calcium channel blockers on the outer hair cell shortening induced by high K^+.

Three selective calcium channel blockers were tested in the experiment. Nifedipine, ω-conotoxin GVIA and ω-agatoxin IVA have been known as a selective

L-, N-, or P type Ca^{2+} channel blocker, respectively. The pretreatment of nifedipine (50 μM) or ω-conotoxin GVIA (5 μM) or ω-agatoxin IVA (0.5 μM) for 10 min, did not prevent the effect of cell shortening induced by high K^+ medium. Nifedipine, ω-conotoxin GVIA or ω-agatoxin IVA was 90.7 ± 0.8 % (*n* = 10), 90.1 ± 0.5 % (*n* = 7) or 92.7 ± 0.6 % (*n* = 10) of control, respectively, there are no significant difference between selective Ca^{2+} channel blocker and high K^+ alone (90.3 ± 0.7 % of control; *n* = 12) (Fig. 2).

3.3 Effect of cobalt chloride on the cell length changes induced by ionomycin or high K^+

Cobalt chloride (Co^{2+}), a non-selective calcium channel blocker, was to compete the Ca^{2+} binding site of plasma membrane. Pretreatment with Co^{2+} (1 mM) for 10 min was able to significantly prevent the cell shortening induced by high K^+ (Co^{2+} pretreatment 97.8 ± 0.5 %, *n* = 12; high K^+ alone 90.3 ± 1.1 %, *n* = 6). However, Co^{2+} slightly inhibit the effect of cell elongation induced by ionomycin (Co^{2+} pretreatment 104.2 ± 0.6 %, *n* = 6; ionomycin alone 106.5 ± 0.7 %, *n* = 14).

3.4. Ca^{2+}-free medium mimicked the effect of taicatoxin on the slow motility in response to ionomycin.

The comparison between effect of ionomycin in normal extracellular Ca^{2+} and Ca^{2+} free medium on hair cells, ionomycin produce the effect of cell shortening but not the elongation in Ca^{2+} free medium. Thus the extracellular Ca^{2+} is required for the effect of cell elongation induced by ionomycin. The maximal cell elongation induced by ionomycin (10 μM) in normal medium was 106.5 ± 0.7 % of the before-treatment (control group), the cell shortening but not elongation induced by ionomycin in Ca^{2+} free medium. (maximal value was 92.4 ± 1.2 % of the before-treatment, $P < 0.001$ as compared with control). The effect of taicatoxin under standard Ca^{2+} medium can be mimicked by the Ca^{2+}-free medium on the slow motility in response to ionomycin. The taicatoxin-treated group was 91.7 ± 1.7 % of before the treatment ($P < 0.001$ as compared with control).

3.5. Effect of taicatoxin or cobalt chloride on intracellular Ca^{2+} mobilization induced by the high potassium.

To determine whether taicatoxin would affect the Ca^{2+} channel and prevent the intracellular Ca^{2+} increase in response to high K^+, the fluorescence imaging of Ca^{2+} signals were applied in the experiment. Changes in $[Ca^{2+}]_i$ was measured using a fluorescent indicator, fluo-3/AM. Outer hair cells were loaded with fluorescent Ca^{2+} indicator 3 μM fluo-3/AM for 40-60 min before the experiment. The intracellular Ca^{2+} increase of outer hair cells can be observed by exposure to high K^+ medium. Pretreatment with cobalt chloride (1 mM) significant inhibit the intracellular calcium increase of outer hair cells induced by high K^+ medium. However, taicatoxin was not

able to affect the intracellular Ca^{2+} increase of outer hair cells induced by high K^+ medium (Fig. 5).

3.6 Potassium channel blockers did not affect on the cell length changes in response to ionomycin or high K^+.

Potassium channel blockers, tetraethylammonium chloride (TEA) or 3,4-diaminopryridine (3,4-DAP) has been characterized as inhibitors of various types of voltage-dependent K^+ channels. Either pretreatment with TEA (5 mM) or 3,4-DAP (2 mM) was unable affect the cell slow motility in response to high K^+ (Fig. 6B) or ionomycin (Fig. 6A), respectively.

4. Discussion (討論)

Taicatoxin have been considered as a Ca^{2+} channel blocker (Bkaily et al., 1992), and was further reconsidered as a Ca^{2+}-activated K^+ channel blocker (Doorty et al., 1997). In the present studied taicatoxin was able to prevent the cell shortening induced by high K^+ and cell elongation induced by ionomycin. These effects of taicatoxin can be mimicked by Ca^{2+}-free medium (see Fig 1 and 4), therefore, the action of taicatoxin on the slow motility of outer hair cell is to interfere the extracellular Ca^{2+} to act on the outer membrane surface and independent of its effect on Ca^{2+} and Ca^{2+} activate K^+ channel blockers (see Fig 2 and 6). Although it has been known that prestin play an important role in the hair cell motility (Liberman et al., 2002), we do not whether the activity of prestin is modulated by Ca^{2+}. There is a need for further studies to explore the interaction between taicatoxin and prestin.

Taicatoxin and calcium channel blockers

Ca^{2+} channels play a role on the transmission of hair cell responses to afferent nerves at the presynaptic terminal (Hudspeth, 1989; Fuchs, 1996), electrical tuning (Armstrong and Roberts, 1998) and provide the conduct through which Ca^{2+} ions can be transported from the perilymphatic to endolymphatic space (Yamoah et al., 1998). High K^+ medium depolarize the cell membrane of outer hair cell, allowing the Ca^{2+} ion entry into intracellular space through the Ca^{2+} channel. The rate of $[Ca^{2+}]_i$ increase induced by high K^+ in isolated outer hair cells is slower than the rate of KCl concentration increase. The relatively slow rate of $[Ca^{2+}]$ increase might be partially attributed to regulation of Ca^{2+} homeostasis by Na^+/Ca^{2+} exchanger (Surin et al., 2000). In addition, the Ca^{2+}-induced contractions of permeabilized isolated outer hair cells suggest a physiology actin-dependent mechanism (Zenner, 1986). Recently, the experiments of α1D knockout mice suggest that hair cells express both dihydropyridine-sensitive and insensitive Ca^{2+} current (Platzer et al., 2000). In the present experiments, we have tested various Ca^{2+} channel blockers on the slow motility of outer hair cells. Ca^{2+} channel blockers, nifedipine (L-type blocker),

ω-agatoxin (P-type blocker) and ω-conotoxin GVIA (N-type blocker) were not able to prevent the cell shortening induced by high K^+, these data demonstrate that Ca^{2+} channels might not play an important role on the slow motility of outer hair cell. However, cobalt chloride, a non-selective Ca^{2+} channel blocker and taicatoxin did prevent the cell shortening induced by high K^+. Since the cobalt chloride but not the taicatoxin was able to prevent the intracellular Ca^{2+} increase induced by high K^+ (see Fig. 5), the action of taicatoxin should be different from that of cobalt chloride. We also cannot rule out whether the action of cobalt chloride is on the Ca^{2+} channel or compete with Ca^{2+} ion on another site of plasma membrane. Due to taicatoxin cannot prevent the intracellular Ca^{2+} increase induced by the application of high K^+ medium, we can preclude the action of taicatoxin on cell slow motility is through the blockade of Ca^{2+} channel on plasma membrane.

Taicatoxin and potassium channel blockers

Large conductance Ca^{2+} activate K^+ (BK) channels may play an important role on the outer hair cell motility and tuning (Fettiplace and Fuchs, 1999). Taicatoxin has been reconsidered as a small conductance Ca^{2+} activated K^+ (SK) channels blocker. However, there was no evidence to show that the SK channels play a role on hair cell motility. Whether the effect of taicatoxin on the slow motility of outer hair cell is arising from the blockade of the SK channels? We have tested the non-selective K^+ channel blockers, tetraethylammonium chloride (TEA) and 3,4-aminopyride (3,4-DAP) in the experiments. TEA is well recognized to block Ca^{2+}-activated K^+ channels (Bond et al., 1999) and aminopyridines block the slow Ca^{2+}-activated K^+ current (Andreasen and Lambert, 2001). In the present studies, TEA or 3,4 –DAP was not able to prevent the effect of cell shortening induced by high K^+ or cell elongation in response to ionomycin (see Fig. 6). Taicatoxin can effectly prevent the cell shortening or elongation induced by high K^+ or ionomycin respectively, the action of taicatoxin on cell slow motility seem not relative with its effect on the Ca^{2+}-activated K^+ channel.

Taicatoxin and the role of extracellular calcium

It has been reported that EGTA or a Ca^{2+} concentration below 1-3 x 10^{-4} M prevented relaxation of the outer hair cell to the original cell length (Zenner et al., 1985; Dulon et. al., 1990). It is has been suggested that the decrease in length by lowering the extracellular Ca^{2+} is due to relaxation of a circumferential contractile mechanism (Slepecky, 1989; Dulon et. al., 1990; Pou et. al., 1991). Therefore, extracellular Ca^{2+} was required for relaxation of the outer hair cell. In the present experiments, either preincubation of extracellular EGTA with low Ca^{2+} buffer or extracellular normal Ca^{2+} with taicatoxin (0.19 μM) was to prevent the cell elongation induced by ionomycin. Due to the hydrophilic property of taicatoxin, it was considered as

membrane impermeable chemical. Although the mechanism of role of extracellular Ca^{2+} on relaxation of outer hair cell is still unclear, the action of taicatoxin may interfere the interaction of extracellular Ca^{2+} on the outer membrane of cells.

Conclusion

Taicatoxin, at nanomolar concentration, can affect the slow motility of outer hair cells induced by high K^+ or ionomycin. The action of taicatoxin is independent of its Ca^{2+} or Ca^{2+} activated K^+ channel blocking properties. Toxicologically, there would be reasonable expectation that patient is bitten by Australian *Taipan* snake will resulted in the impairment of hearing function. Physiologically, taicatoxin can provide as a powerful tool to reveal the detail molecular mechanism of Ca^{2+}-dependent cell slow motility in outer hair cells as previously suggested (Dulon et al., 1990; Pou et al., 1991).

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自評部份: 本實驗皆大部分與研究計畫相符合, 除了有關 G-蛋白對於鈣離子管道調節的部分, 因 G-蛋白對於鈣離子管道無調節作用而未列. 其餘對於鈣離子管道抑制劑對於外毛細胞運動性之調控已充分研究, 發現鈣離子管道抑制劑除了 taicatoxin 有作用外, 其餘皆無作用, 而 taicatoxin 在非常低的濃度既對於外毛細胞運動性有顯著的影響, taicatoxin 可以特異的影響鈣離子依賴性的毛細胞運動性, 此可以提供一個顯著有效的工具進一步去了解鈣離子依賴型運動性的分子機制, 此結果已投稿於 Hearing Research.

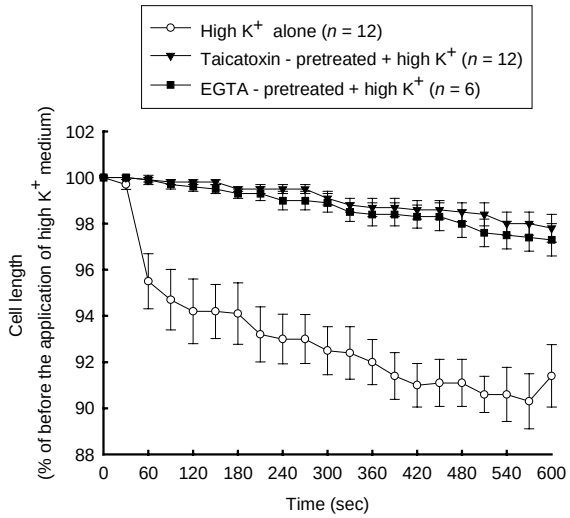

Fig. 1. Taicatoxin prevent the effect of cell shortening induced by high potassium (50 mM) medium. Isolated outer hair cells were preincubated in 0.19 μM taicatoxin (n = 10, -▼-) or 3 mM EGTA (n = 3, -■-) for 10 min prior to the addition of high K^+ medium. The cell shortening can be induced by high K^+ medium (50 mM) alone (-◯-). Length changes had shown as percent change from cell length before the addition of high K^+ medium. Either the pretreatment of taicatoxin or EGTA was able to significantly prevent the effect of cell shortening induced by high K^+ medium. Values are means ± S.E.M. * $P < 0.001$ as compared with high K^+ alone.

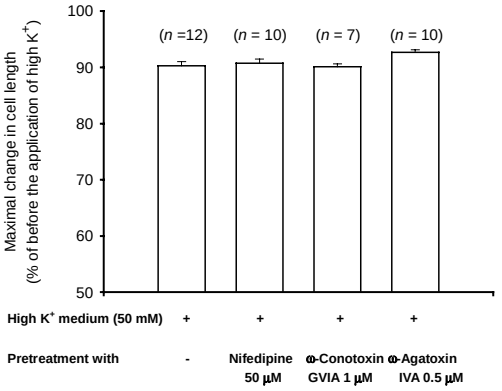

Fig 2. Lin et al.

Fig.2 Calcium channel blockers do not affect the cell shortening induced by high K^+ (50 mM) medium. The selective calcium channel blockers, nifedipine (L-type blocker), ω-conotoxin GVIA (N-type blocker) or ω-agatoxin IVA (P-type blocker) was unable to prevent the cell shortening induced by high K^+ medium (K^+ medium alone, n =12). Values are means ± S.E.M.

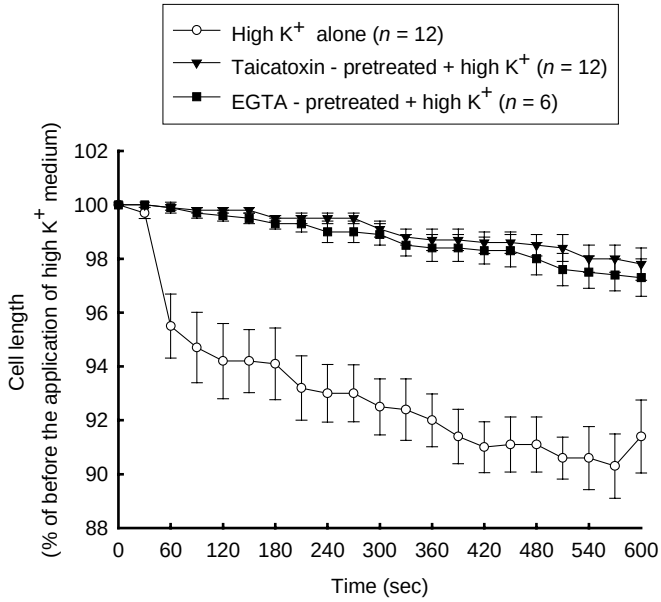

Fig.3 Effect of cobalt chloride (Co^{2+}) on the cell slow motility in response to high K^+ or ionomycin. Co^{2+}, an inorganic non-selective calcium channel blocker, was able to significantly prevent the cell shortening induced by high K^+ medium (gray bars) but not the cell elongation induced by ionomycin (blank bars). Values are means ± S.E.M. * $P < 0.001$ as compared with high K^+ alone.

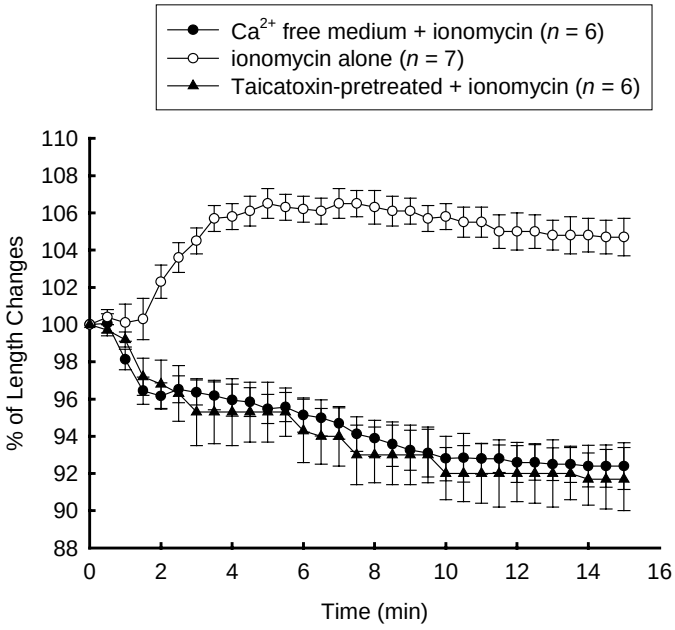

Fig. 4. The Ca^{2+} free buffer was able to mimic the effect of taicatoxin-pretreated in the cell length changes induced by ionomycin. The Ca^{2+} free medium was prepared by the low calcium medium plus 1 mM EGTA (see Materials and Methods). The experiment of taicatoxin treatment was under the normal Ca^{2+} Hank's buffer. Either preincubation of hair cells with taicatoxin (0.19 μM; -▲-) for 10 min or hair cells immersed in Ca^{2+} free buffer (-●-), prevent significantly the length elongation in response to ionomycin (10 μM). Values are means ± S.E.M. * $P < 0.001$ as compared with ionomycin alone (-○-).

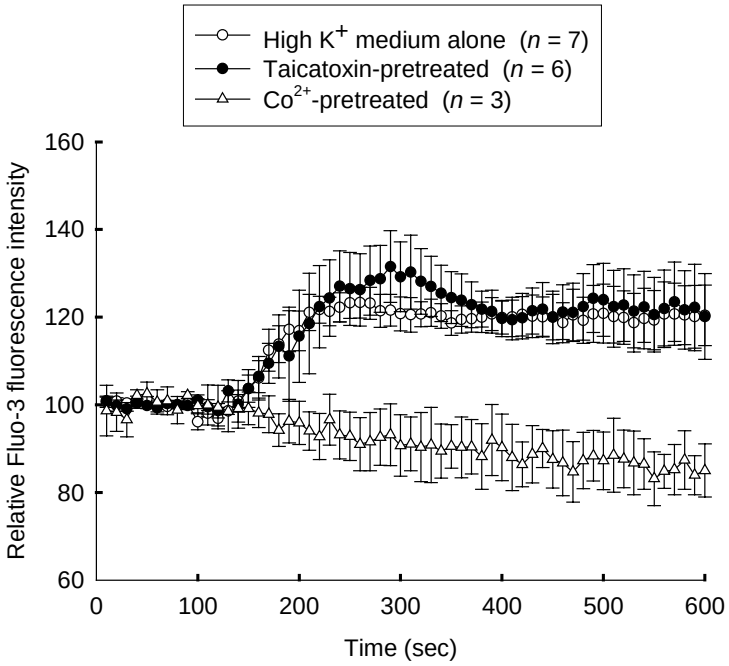

Fig. 5. Taicatoxin was not able to change the intracellular calcium level induced by high K^+ medium. Isolated outer hair cells were loaded with the fluorescent calcium indicator Fluo-3/AM. Application of high K^+ to extracellular compartment produced a significant increase the concentration of intracellular calcium (-○-). Pretreatment with taicatoxin (0.19 μM) for 10 min was unable to alter the level of intracellular calcium induced by high K^+ medium (-●-). However, the pretreatment of cobalt chloride (1 mM) for 10 min was able to abolish the increased effect of intracellular calcium level induced by high K^+ medium (-△-). Values are means ± S.E.M.* $P < 0.001$ as compared with high- K^+ medium alone. There is no significance difference between the taicatoxin-treated and control group (high K^+ alone)

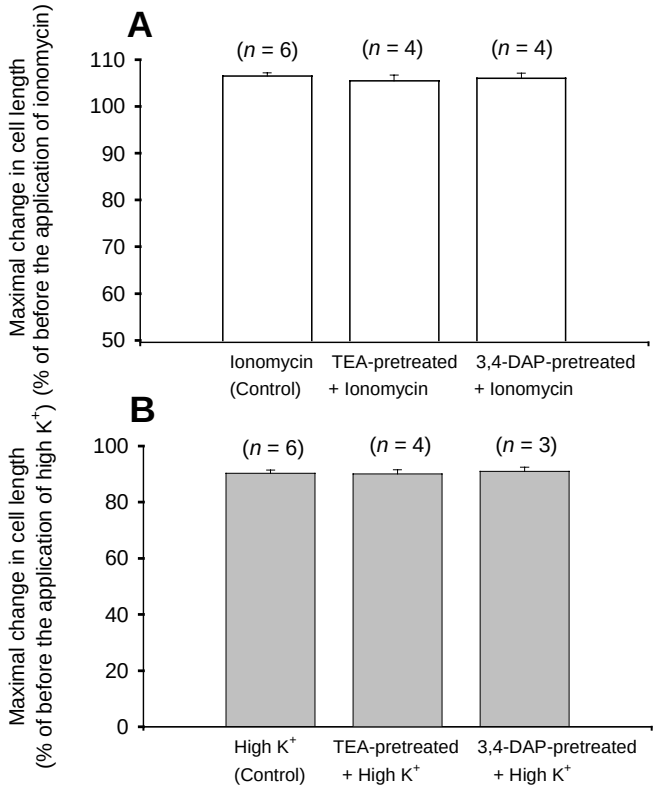

Fig 6. Lin et al.

Fig. 6 Potassium channel blockers had no effect on the cell length change induced by ionomycin (blank bars) or high K^+ (gray bars). Pretreatment with potassium channel blockers, tetraethylammonium chloride (TEA, 5 mM), or 3,4-diaminopyridine (3,4-DAP, 2 mM) for 10 min was unable to change the cell slow motility induced by ionomycin (10 μM, A) and high K^+ (50 mM, B). Values are means ± S.E.M.