行政院國家科學委員會補助專題研究計畫成果報告

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※蛋白質激酶對耳蝸毛細胞及神經節鈣離子管道的調控作用 ※
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計畫類別：■個別型計畫 □整合型計畫

計畫編號：NSC89-2320-B-040-071-

執行期間：89 年 08 月 01 日至 90 年 07 月 31 日

計畫主持人：林明忠

共同主持人：李宣佑

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□國際合作研究計畫國外研究報告書一份

執行單位：中山醫學大學生命科學系

中 華 民 國 90 年 10 月 25 日

行政院國家科學委員會專題研究計畫成果報告

國科會專題研究計畫成果報告撰寫格式說明

Preparation of NSC Project Reports

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1. 中文摘要

本篇研究 phorbol esters 在天竺鼠聽神經外毛細胞運動性與鈣離子流動的作用.鈣離子穿透劑 ionomycin (10 μM) 可引起毛細胞的延長作用. 這個延長作用可被前處理蛋白質激酶丙活化劑β-PDBu (0.5 μM) 10 分所對抗.β-PDBu 本身並不會影響毛細胞的運動 而另一種不活性的蛋白質激酶丙立體異構物α-PDBu (1 μM)前處理 15 分則無此作用.因為鈣離子管道抑制劑 nifedipine (10 μM)並不影響 ionomycin 在毛細胞的作用，因此毛細胞的運動性變化與鈣離子管道無關. 蛋白質激酶丙的選擇性抑制劑 BIMI 可以抑制β-PDBu 其對抗 ionomycin 在毛細胞的作用 然而，1 μM 的β-PDBu 並不會影響 ionomycin 引發 Fluo-3 鈣離子影像增加的程度. 這些數據支持蛋白質激酶丙調節運動蛋白而來影響細胞形狀的變化. 這個發現顯示蛋白質激酶丙可能參與毛細胞的運動性，而且與細胞膜上的鈣離子管道無關.

關鍵詞：蛋白質激酶丙，細胞運動性，外毛細胞, Fluo-3, 鈣離子影像分析

Abstract

The effect of phorbol esters on cell motility and intracellular fluorescence imaging of calcium of cochlear outer hair cells isolated from guinea pig were studies. The calcium ionophore ionomycin (10 μM) can to induce length increases of outer hair cells. These length increases can be increases can be inhibited by a 10 min preincubation of the cells with the protein kinase C activator β-phorbol-12,13-dibutyrate (β-PDBu, 0.5 μM). β-PDBu did not affect the cell lengths of outer hair cell itself. No such preventing effect on cell length was detected after 15 min incubation with an inactive isomer of protein kinase C α-PDBu (1 μM) alone. Because of the calium channel blocker nifedipine (10 μM) did not prevent the effect of ionomycin, thus the effect of β-PDBu was not relative with calcium channel. The protein kinase C inhibitor, bisindolylmaleimide I (BIMI) can inhibit the β-PDBu effect on the cell elongation induced by ionomycin. However, 1 μM β-PDBu did not interfere with the ability of ionomycin to elevate fluorescence of the calcium indicator Fluo-3. These data support the hypothesis that protein kinase C regulates motor protein that effect of shape changes of outer hair cells. These findings indicate that protein kinase C pathway may involve in regulating the cell motility of outer hair cell without related with membrane calcium channels.

Keywords: Protein Kinase C, Cell Motility, Outer hair cells; Fluo-3; Calcium imaging

2. Introduction and purpose

The outer hair cells of mammalian cochlea are thought to play a modulatory role in the process of auditory signal transmission (LePage, 1989) as well as determine frequency selectivity. Unlike other sensory receptors, they are capable of operating in a 'reverse transduction' mode whereby electrical energy in converted into mechanical displacement of the cell body (Ashmore, 1987). Electromotility of outer hair cells, also known as 'fast motility', is currently thought to provide a system of

mechanical feedback, the so called cochlear amplifier (Davis, 1983), that sharpens the ability of the inner ear to discriminate acoustic stimuli (Holley, 1996). Phorbol esters are known to capable of activating protein kinase C, which can modulate cellular mechanisms in many tissues, such as calcium channels in neurons or muscle cells. However, it is still not known whether PKC can regulate the cochlear outer hair cells. In intact cell models of hair cells, elevation of intracellular calcium results in longitudinal elongation. This effect can be obtained using calcium ionophore or simply by raising extracellular calcium (Pou et al., 1991). Protein phosphorylation has also been implicated in the regulation of motility of nonmuscle cells including other sensory receptor cells, for example, teleost photoreceptors (Pagh-Roehl et al., 1993)

The purpose of the present study is to investigate the effect of phorbol ester on outer hair cell to determine whether PKC is involved in the modulation of hair cell motility.

Results

Isolated cochlear and outer hair cell from the guinea pig cochlear were shown in Fig. 1. Outer hair cell motility can be induced by the calcium ionophore ionomycin (10 μM) (Fig. 2), other chemical compound, β-PDBu, α-PDBu or BIMI alone cannot affect the cell lengths of outer hair cell. The cell elongation induced by ionomycin (10 μm) was prevented by pretreatment with β-PDBu but not the α-PDBu or a L-type calcium channel blocker, nifedipine (10 μM) (Fig. 3). The specific protein kinase C inhibitor, BIMI (1 μM) prevent the antagonist effect of β-PDBu on ionomycin-induced cell length elongation (Fig. 4). The possibility that β-PDBu may be acting nonspecifically by blocking calium entry was tested using the fluorescent calcium indicator, Fluo-3 AM. Addition of 1 μM β-PDBu had no effect on the kinetics or magnitude of ionomycin-induced rise of Fluo-3 fluorescence (Fig. 5).

Discussion

The experimental results support the hypothesis that ionomycin-induced motility of outer hair cells is mediated by protein phosphorylation. A voltage-dependent inward Ca^{2+} current flows across the plasma membrane of mammalian cochlear outer hair cell during depolarization and contributes to the motile response (Ikeda et al., 1994)。 Phorbol esters can exert various effects in different smooth muscle systems through activating protein kinase C such as enhancement of the contractile response via modulating calcium channel in smooth muscle (Mironneau et al., 1991; Lin et al., 1998) In this study, β-PDBu but not the nifedipine can inhibit the cell elongation induced by ionomycin. Thus the effect of ionomycin in inducing the cell elongation without relative with the membrane calcium channel. Furthermore, we use the Ca^{2+}-sensitive dye fluo-3, ionomycin produced an increase of $[Ca^{2+}]_i$ in the outer hair cells which can not inhibited by pretreatment with β-PDBu. The effect of β-PDBu is also not direct relative with the intracellular calcium movement. Homeostatic mechanisms, like that proposed in the biochemical model, may modulate hearing in either of two ways. First, activation of calcium-dependent motile systems may amplify or attenuate voltage-dependent responses or may introduce nonlinearities. This general idea was reviewed by Schacht (1995) and in agreement with recent measures of the effects of neurotransmitter substances on the mechanical properties of outer hair cells and on electromotility (Dallos et al., 1997). Second, modulation of calium-dependent motile systems in outer hair cells, as well as in supporting cells of the organ of Corti, could lead to changes in geometrical shape or mechanical impedance of the component cells. Such changes could, in turn, modulate the overall mechanical response of the tissue to stimulation by sound, at levels both within the operating range of the ear as well as at more traumatic sound levels. This idea in consistent with the suggestion by Holley (1996) that slow motility may account for same of the morphological correlates of reversible noise trauma. In conclusion, this study demonstrate

that phorbol ester prevent the cell elongation of outer hair cell induced by ionomycin through a phosphorylation pathway dependent on protein kinase C activation. The evidence obtained suggests that protein kinase C could play an important role in modulating outer hair cell motility of guinea pig cochlear.

Accordingly, further investigations need to be carried out.

Acknowledgment

This study was supported by the National Science Council, Taiwan (NSC 89-2320-B-040-071).

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6. 自評

本計劃原設計蛋白質激酶是否可介由鈣離子管道而來達成調控聽覺毛細胞的運動性. 然而計畫啟始之時, 發現文獻上已報告聽覺毛細胞的運動性可能與鈣管道無關. 雖然如此查相關文獻並無蛋白質激酶丙對於毛細胞本身運動性調控之研究與報告. 幸運地本計畫發現了蛋白質激酶丙可能包含在可調控毛細胞運動性的機制內, 這是之前從未有的發現 (而此與膜鈣管道無關), 此計畫將可望發表在期刊上 (計畫投稿 Hearing Research) 此計畫經這一年的努力很欣慰找到答案, 而更深入的研究將是必須的. 回顧過去一年的研究最大的的缺點可能是不夠深入.這點我將詳加改善. 更希望將來的申請計畫能夠延續, 這是很重要的, 尤其是對一機制的詳加探討與完成, 所以也希望評審委員們能否給予後生支持與鼓勵.

Fig. 1 The cochlear (left) and outer hair cell (right) were isolated from guinea pig. Scale bars: 1 mm (left); 10 μm (right).

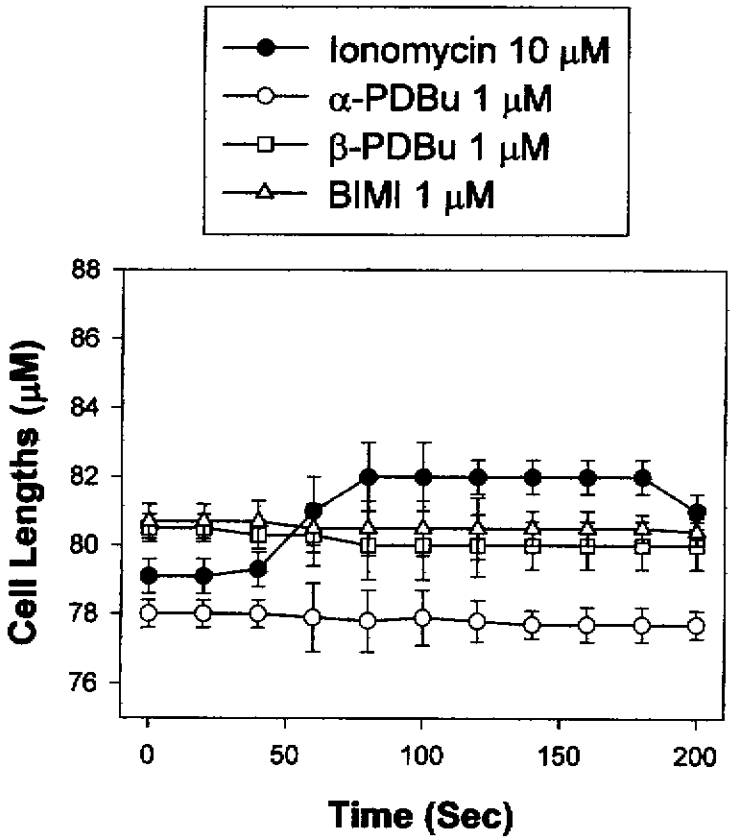

Fig.2. The effect of ionomycin, α-PDBu, β-PDBu or BIMI on the time course of length changes of isolated outer hair cell. Outer hair cell were treated with ionomycin (10 μM; full circle), protein kinase activator (open box), inactive isomer (open circle) or protein kinase inibitor, BIMI (1 μM; open triangl The cell length elongation were induced by ionomycin significantly but, not other chemical compounds each alone (α-PDBu, β-PDBu or BIMI).

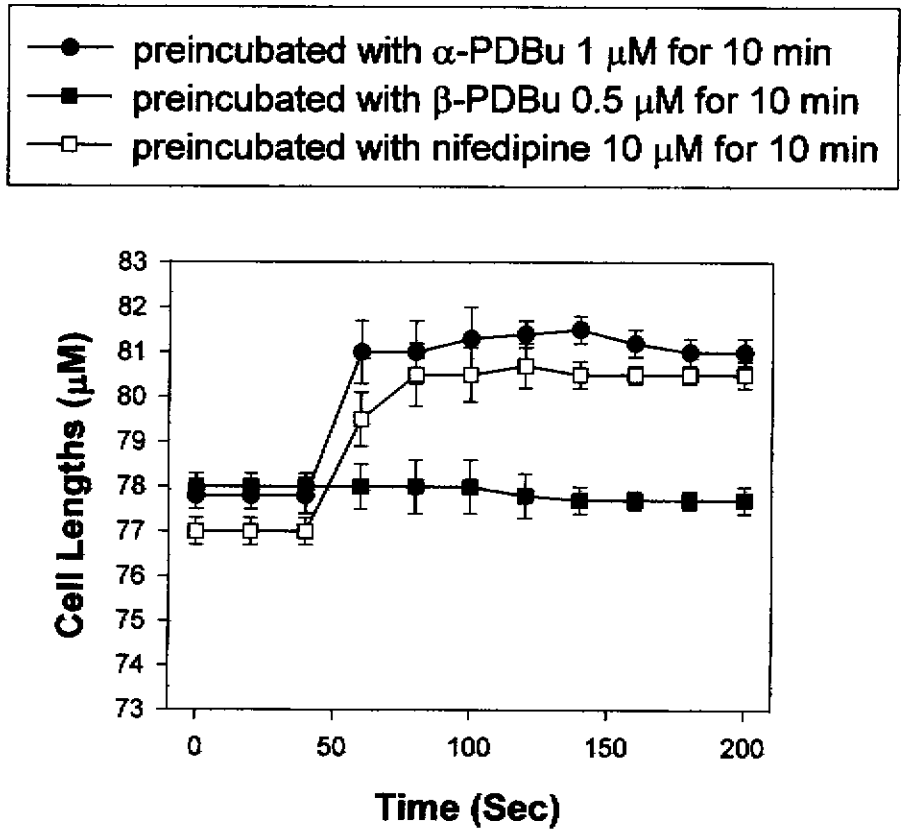

Fig.3. The effect of cell elongation induced by ionomycin was prevented by preincubated with β-PDBu 10 min (full square) but not the inactive from α-PDBu (full circle) or L-type calcium channel, nifedipine (10 μM; open square).

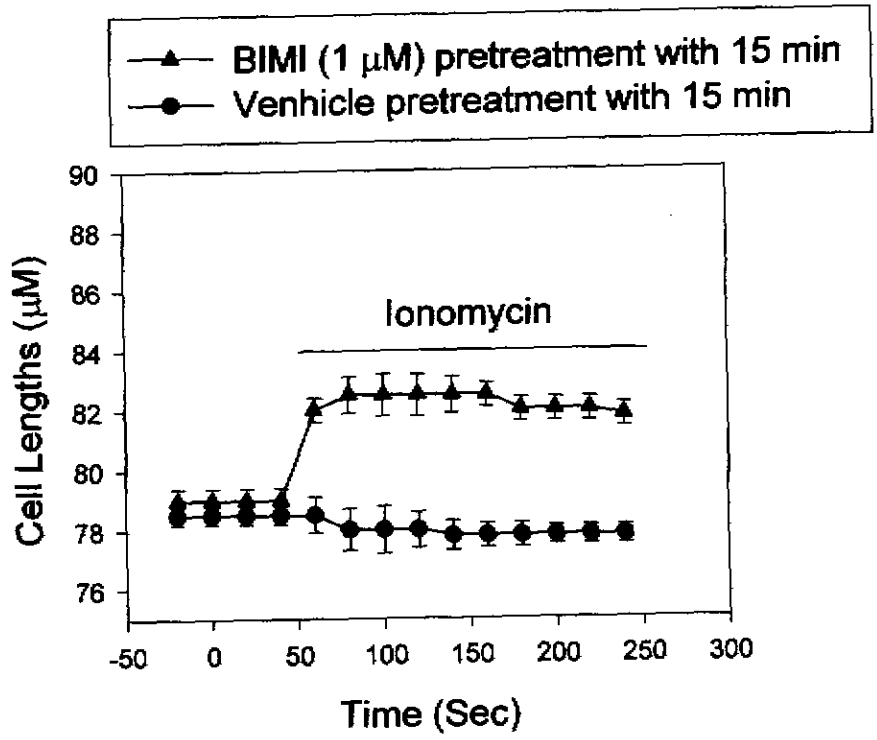

Fig.4 The protein kinase C inhibitor, BIMI can interfere the antagonist effect of β-BDBu on ionomycin in the induction of cell length elongation.

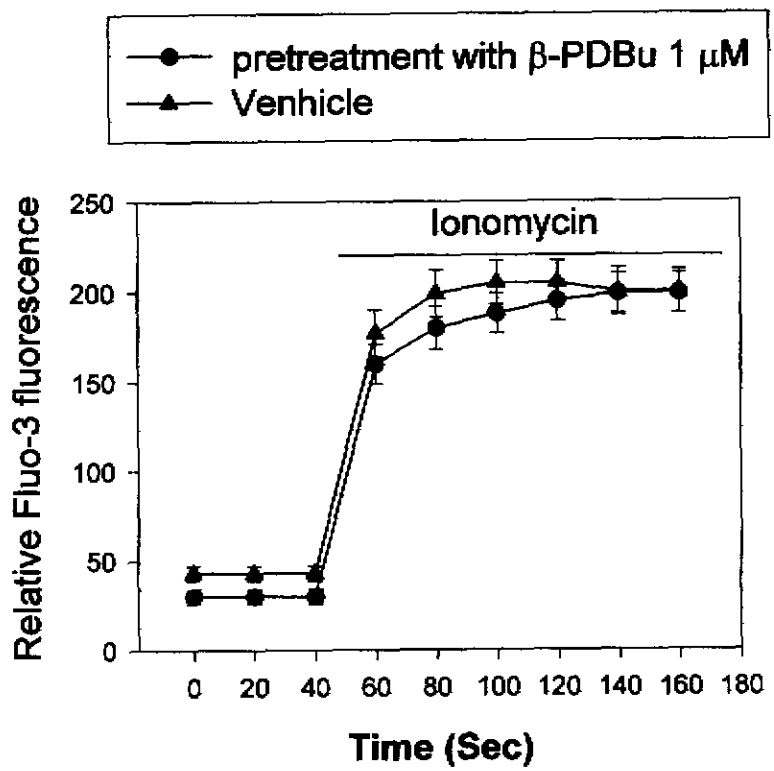

Fig.5 β-PDBu does not impair ionophore-induced calcium entry. Isolated hair cells were loaded with Fluo-3 AM. Cells were pretreated for 15 min with either 1 μM β-PDBu (full triangle) or venhicle (full circle).