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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

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※　血管收縮素訊息路徑及神經鏈基因表現在人類正常肺細胞及肺癌細胞之研究(1/3)

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計畫主持人：林庭慧

共同主持人：

本成果報告包括以下應繳交之附件：

- □赴國外出差或研習心得報告一份
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- □出席國際學術會議心得報告及發表之論文各一份
- □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　　92 年　　5 月　23　　日

行政院國家科學委員會專題研究計畫成果報告

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中文摘要

關鍵詞: 血管收縮素, 環腺甘酸, 神經鏈, 小細胞肺癌

血管收縮素促進腎臟水份再吸收作用及血管收縮之功能為眾所周知。在最近的報導上，指出了血管收縮素及其受體在小細胞肺癌及乳癌有基因表現的現象。血管收縮素此神經鏈及其受體在癌症病因學上扮演之角色，始終不甚清楚。我們目前利用反轉錄聚合反應，來瞭解正常肺細胞株中(MRC-5)及小細胞肺癌(H-209) 中，血管收縮素此神經鏈及其受體之基因表現。另外，我們探討血管收縮素在正常肺細胞株是否進行正常之訊息傳遞路徑。由我們初步之反轉錄聚合反應結果顯示， 血管收縮素此神經鏈及其第一亞型受體在這兩株肺細胞中並無表現，但血管收縮素第二亞型受體可被偵測。利用環腺甘酸分析組套，分析經過血管收縮素刺激之後，在 MRC-5 細胞中，環腺甘酸之含量是否上升。我們無法偵測出環腺甘酸含量之上升，表示血管收縮素在 MRC-5 細胞中並未進行第二亞型受體驅動之正常訊息傳遞路徑。但在同時，用前列腺素 E2 去刺激 MRC-5 細胞，環腺甘酸之含量卻大幅上升，顯示前列腺素 E2 在 MRC-5 細胞株其下游之訊息路徑是可行的。我們利用反轉錄聚合反應，偵測出第四亞型之前列腺素 E2 受體是存在於 MRC-5 細胞中最主要之前列腺素 E2 受體。我們也同時研究煙草中之致癌物, benzopyrene, 對血管收縮素及相關神經鏈在此二株細胞中之基因表現。我們發現，在 MRC-5 中，全然無血管收縮素及其他神經鏈之基因表現。但在 H-209 中，雖無 AVP 及 V1a receptor 之基因表現，但卻有 V2 receptor, CCK-B 以及 GRP 此二神經鏈之基因表現。然而，使用不同濃度之 benzopyrene, 分別處理不同時間卻看不到 V2 receptor, CCK-B 以及 GRP 在基因表現上有何差異。Benzopyrene 對 V2 receptor, CCK-B 以及 GRP 在 H-209 中之基因表現可能並無直接影響。

英文摘要

Keywords : arginine vasopressin (AVP), cAMP, neuropeptides, small cell lung carcinoma

The roles of arginine vasopressin (AVP) in regulating water retention and vasoconstriction in kidney is well-defined. Despite recent reports on expression of genes coded for AVP and its receptors in small cell lung carcinoma (SCLC) and breast cancer (Ref.1~3), the roles of the neuropeptides and the receptors in cancer etiology are unclear. Therefore, we propose to investigate signaling pathway involving AVP and its receptors in SCLC (H-209) as compared with a normal lung cell line (MRC-5). Using RT-PCR, the gene expression of AVP and its receptors were investigated in these two cell lines. Only V2 receptor was detectable in both cell lines, the AVP and V1a receptors were undetectable. The cAMP signaling in MRC-5 cells triggered by AVP was determined. No detectable cAMP level was increased after AVP stimulation compared with the untreated cells. This indicated the V2 receptor signaling pathway was not function well in MRC-5 cells even the V2 receptor was expressed in MRC-5 cells. On the other hand, when PGE2 was added in MRC-5 cells, the cAMP level was increased beyond 1000 times compared with the basal level of untreated cells. EP4 was the major PGE2 receptor in MRC-5 cells as detected by RT-PCR. The effect of benzopyrene on the

gene expression of AVP and other neuropeptides was investigated in both cell lines. Although V2 receptor was detected in MRC-5 cell, AVP, V1a receptor and other neuropeptides were undetectable. On the other hand, in H-209 cells，although AVP and V1a receptor was undetectable, V2 receptor, CCK-B and GRP were detected. The gene expression of V2 receptor, CCK-B and GRP in H-209 cells were not altered when treated with different doses of benzopyrene with different time. Benzopyrene may not have direct effect on the gene expression of V2 receptor, CCK-B and GRP in H-209 cells.

二、緣由與目的

The present study is designed to understand the gene expression and signaling pathway of AVP and its receptors in both a normal lung cells (MRC-5) and SCLC (H-209). Although the roles of AVP and its receptors in kidney was well-defined, their roles in lung, especially in SCLC were poorly understood. By understanding the signaling pathway of AVP in both lung cell lines, the physiological roles of AVP and its receptors in lung will be elucidated.

三、Results (結果)

Detection of V2 receptor but not AVP, V1a receptor in lung fibroblast MRC-5 cells, the expression of V2 receptor was not altered after treatment of cells with different concentration of benzopyrene with different time.

As seen in fig.1, the expression of AVP , AVP receptors (V1a and V2) and neuropeptides such as CCK-B and GRP was investigated in MRC-5 cells using RT-PCR.(Fig.1 A~D). MRC-5 cells were treated with different doses of benzopyrene (0.1, 0.5, 2.5, 5 μM) for 2 hours and 24 hours. Specifically designed primers for each mentioned genes was shown in table 1,only V2 receptor was detected in MRC-5 cells, the expression of V2 receptor was not altered after treatment cells with different concentration of benzopyrene with different time (Fig.1 E). AVP, V1areceptor, neuropeptides (CCK-B and GRP) were not induced by benzopyrene in our experimental condition in MRC-5 cells.

Fig.1A

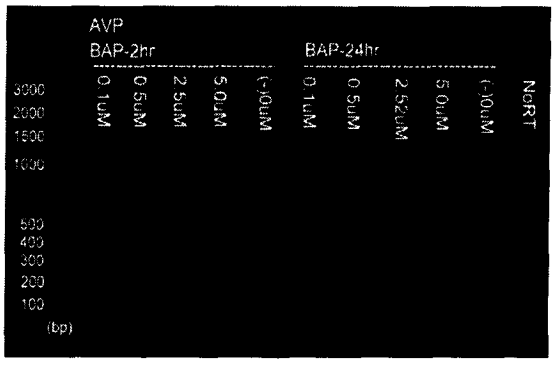


Fig. 1B.

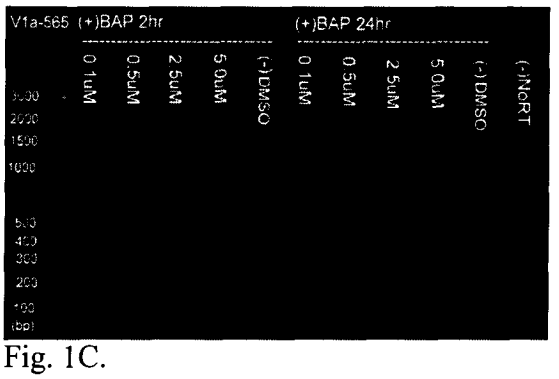


Fig. 1C.

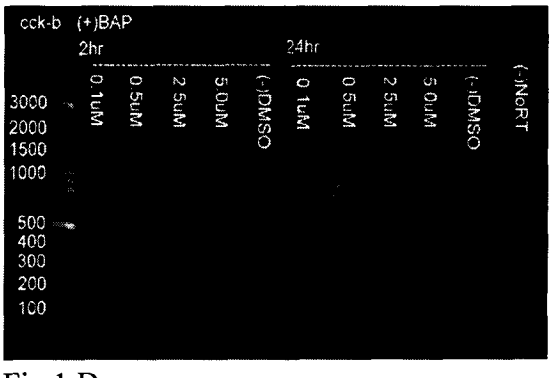
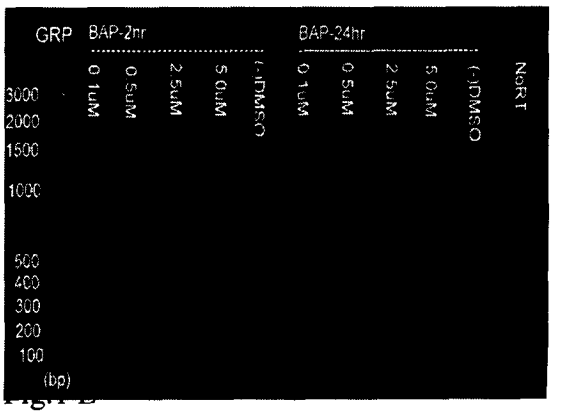


Fig 1.D.



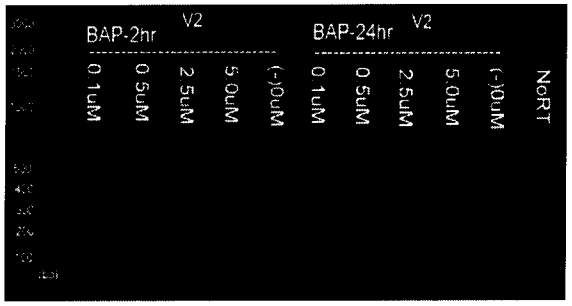


Fig 1.(A~D) The expression of AVP , V1a receptor and neuropeptides (CCK-B and GRP) was not induced after treatment of MRC-5 cells with different doses of benzopyrene with different times. (E) V2 receptor was expressed in MRC-5 without benzopyrene treatment. No alternation of V2 receptor gene expression was detected after treatment of MRC-5 cells with different doses of benzopyrene with different times.

Distribution of V2 receptor, CCK-b and GRP but not AVP , V1a receptors in H-209. Tthe expression of V2 receptor, CCK-B and GRP was not altered after treatment cells with different concentration of benzopyrene with different time.

As seen in Fig.2, the expression of V2 receptor, CCK-B and GRP was detected in H-209 cells (Fig.2 A~B).

Fig.2 A

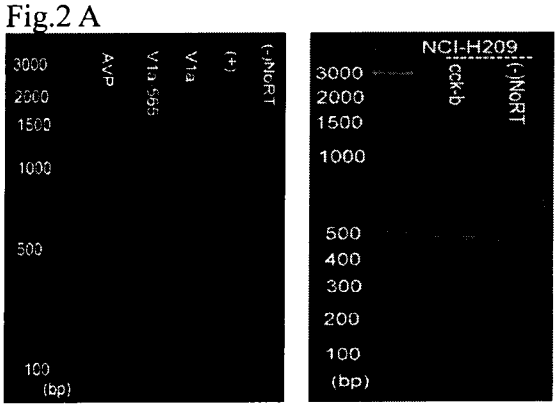
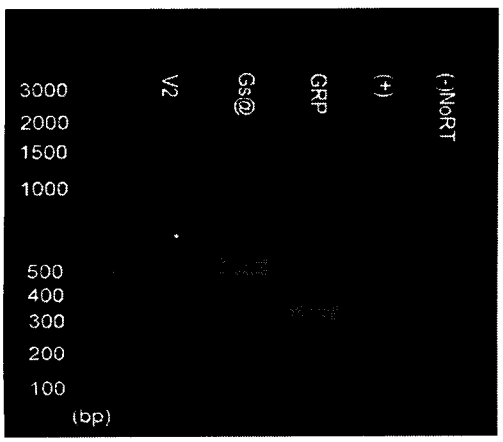


Fig.2 B.



H-209 cells were treated with different doses of benzopyrene (0.1, 0.5, 2.5, 5 μM) for 2 hours and 24 hours. No alternation of V2 receptor and CCK-B and GRP gene expression was detected after treatment of MRC-5 cells with different doses of benzopyrene with different times (data not shown)

No change in cAMP level after V2 receptor activation but around 1000 folds increase of cAMP level was observed after PGE2 treatment in MRC-5 cell.

As shown in fig 3, when the MRC-5 cells was stimulated with 10^{-6}M AVP, no detection of cAMP level was observed compared with the untreated cells. This indicated the V2 receptor signaling pathway was not function well in MRC-5 cells even the V2 receptor was expressed in MRC-5 cells. On the other hand, when 10^{-6}M PGE2 was added to MRC-5 cells, around 1000 folds increase of cAMP level was observed compared with the untreated cells. Further RT-PCR data indicated the EP4 was the major PGE2 receptor in MRC-5 cells. Using the 11-deoxy PGE1, a specific agonist of EP4 receptor to evoke the cAMP pathway in MRC-5 cells, around 200 folds increase in cAMP level was observed.

Fig.3

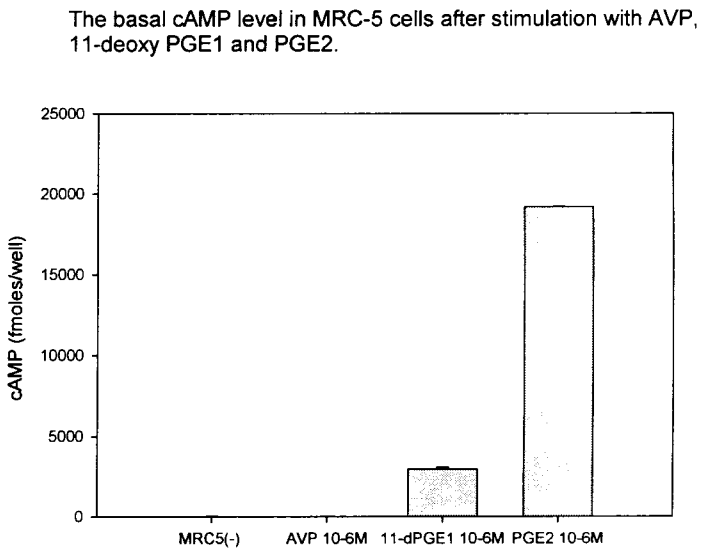


Table 1. Primers use to detect AVP, AVP receptors, neuropeptides and PGE2 receptors in MRC-5 cells and H-209 cells.

AVP receptor	Sequences	Size
AVP	5'-TGCATACGGGGTCCACCTGT-3'	**271 bp**
	5'-TAGTTCTCCTCCTGGCAGC-3'	
V1a	5'-GCTCAAGACTCTGCAACAGCC	**365 bp**
	5'-GGTTCCTTGAATAGCTGCTGTA-3'	
V2	5'-CCATGGTTCTGCAAATCGGG-3'	**625 bp**
	5'-TAGGTCATCATCAACCACCCCA-3'	
GRP	5'-TGCTGACCAAGATGTACCCG-3'	**323 bp**
	5'-CTTCACGTTGAGAACCTGGA-3'	
CCK-B		
	5'-CTGAGGACTGTCACCAATGC-3'	**486 bp**
	5'-AGAGCTCGCGAGAGATAAG-3'	
EP1-F	5'-CGCAGGGTTCACGCACACGA-3	**336 bp**
EP1-R	5'-CACTGTGCCGGGAACATACGC-3'	
EP2-F	5'-AGGACTTCGATGGCAGAGGAGAC-3'	**401bp**
EP2-R	5'-CAGCCCCTTACACTTCTCCAATG-3'	
EP3-F	5'-CCGGGCACGTGGTGCTTCAT-3'	**437 bp**
EP3-R	5'-TAGCAGCAGATAAACCCAGG-3'	
EP4-F	5'-TTCCGCTCGTGGTGCGAGTGTTC-3'	**423 bp**
EP4-R	5'-GAGGTGGTGTCTGCTTGGGTCAG –3	

四、討論 (Discussion)

Our preliminary RT-PCR data indicated only V2 receptor, but not AVP, V1a receptor was detected in lung fibroblast MRC-5 cells. When the MRC-5 cells was stimulated with 10^{-6}M AVP, no detection of cAMP level was observed compared with the untreated cells. This indicated the V2 receptor signaling pathway was not function well in MRC-5 cells even the V2 receptor was expressed in MRC-5 cells. On the other hand, the PGE2 receptor signaling pathway could be detected in MRC-5 cells and EP4 might be the major PGE2 receptor trigger the cAMP signaling pathway in MRC-5 cells. With these results, we will switch our research orientation toward PGE2 signaling pathway instead of AVP signaling pathway in MRC-5 cells. AC III, an AC isozyme inhibited by calcium through calmodulin kinase II, was detected in MRC-5 cells (data not shown). The interaction of EP4 receptor and ACIII will soon be determined in MRC-5 cells.

We have compared the effect of benzopyrene on the gene expression of AVP and its receptors, neuropeptides such as CCK-B and GRP in both MRC-5 and H-209 cell. No significant difference was observed between the BAP treated and untreated group in both cell lines. RT-PCR reveals the gene expression of V2 receptor and EP4 receptors in H-209. Whether the cAMP pathway triggered by AVP and PGE2 function well in H-209 cells will be determined.

五、參考文獻 (References)

1. Marta Ocejo-Garcia, Samreen I. Ahmed, Judy M. Coulson, Penella J. Woll (2001) *Lung Cancer 33, 1-9.*

2. Michael J. fay, Andrew S. Friedmann, Xiao-ming Yu, William G. North (1994) *Cancer Letters 82, 167-174.*

3. William G. North *Experimental Physiology(2000)* **85S**, 27S-40S.