行政院國家科學委員會專題研究計畫　成果報告

腎臟草酸鈣結石的大鼠模式中，kappa 類鴉片受器對腎功能變化的影響。

計畫類別：個別型計畫
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計畫主持人：陳淳
共同主持人：黃鶴翔

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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

Effects of kappa opioid receptor on the renal functional responses in the kidney with calcium oxalate formation of rats

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執行單位：臺大醫學院泌尿部

中　華　民　國　94 年　10 月　20　日

(一)、中文摘要及關鍵詞

根據文獻上也有證據顯示，內生的類鴉片也會作用在活體內的周邊系統來調節心臟血管系統和腎臟功能。但是，目前文獻上所報告的有關類鴉片對腎功能的影響，研究的結果似乎都指出類鴉片是借由腎臟以外的機制來調控腎功能，而非腎臟內的類鴉片受體的作用而來。但是本計劃初步結果卻顯示腎臟內亦有不同類鴉片受體的表現；而且腎臟內的鴉片受體的表現在乙烯甘醇餵食大鼠所誘發草酸鈣腎結石的過程中會改變。

內生的鴉片產生生理作用是經由活化三種不同的鴉片受體，也就是 mu (MOR), delta (DOR) 和 kappa (KOR) 等三種鴉片受體。本實驗發現在腎臟內確實有這三種鴉片受體存在，但是在比較正常大鼠和腎臟有草酸鈣結石的實驗組大鼠的腎臟標本後，我們發現 DOR 在結石的腎臟中的皮層顯著的增加而髓質中的表現則無影響；而 MOR 在結石的腎臟中的表現則無顯著變化。KOR 在結石的腎臟中的表現只有在初期會增加，有趣的是其 mRNA 與蛋白量表現的變化一致，顯示出大鼠餵食乙烯甘醇早期之高草酸尿症與 KOR 之變化有重要的關聯。活化 kappa 類鴉片受體會產生顯著的利尿效果，但是對尿中的電解質卻沒有影響。而由我們先前的實驗已發現，使用乙烯甘醇餵食大鼠以誘發草酸鈣腎結石的過程中，尿流量和尿鈉的排泄在餵食乙烯甘醇 42 天後會有顯著的下降現象。這些結果顯示在結石的大鼠，腎臟的排泄功能可能有受損，而鴉片受體表現的增加可以代償腎功能的改變。此外，鴉片受體在大腦中有神經保護的功能，是否腎臟 DOR 與 KOR 表現的增加可以保護腎臟細胞對高草酸血症的傷害，需進一步的試驗。

關鍵字：κ 類鴉片受體，腎功能，草酸鈣，腎結石，高草酸尿症。

(二)、英文摘要及關鍵詞（keywords）

Based on previously reports, endogenous opioid receptors in peripheral system have been suggested to be involved in regulation of both cardiovascular and renal function. However, the effect of opioid receptors on renal function was suggested as an extrarenal event. In our preliminary study, we found that one of opioid receptor subtype was increased in the rat kidney of experimental nephrolithiasis induced by fed with ethylene glycol. Therefore, we are interested to know whether this change has a significant role on regulation of crystal formation.

Endogenous opiates act on its receptor subtypes, classified as mu (MOR), delta (DOR) and kappa (KOR) opioid receptor, to elicit a physiological effect such as addition and so on. In the present study, we found that three subtypes of opioid receptors were existed in rat kidney. Compared to the normal kidney, the DOR expression in the kidney with calcium oxalate was significantly increased in renal cortex but not in the medulla. However, the MOR expression was unchanged in any part of renal tissues. The KOR expression was increase during the initial phase of crystal formation. Most interesting is that the expressed patterns of KOR mRNA and protein were similar, this indicated that the changes in KOR were participated in EG-induced hyperoxaluria. As previous studies, urinary excretion of electrolytes won't be affected by diuresis elicited by intrarenal KOR activation. Our previous results also showed that urinary output and

sodium excretion were significantly lowered in EG-treated 42 day rats. Together these, we concluded that increased expression of opioid receptor may have an effect on compensation of impaired renal excretory response observed in the nephrolithiatic kidney. In addition, opioid receptor expressed in neurons of brain cortex offered a neuroprotection in respond to detrimental damage. Whether renal opioid receptor show the similar effect on renal cell protection is our further interest.

Key Words: Opioid receptor, calcium oxalate, kidney crystal

(三)、前言

我們研究團隊曾於民國 89 年提出國科會研究計畫:"以乙烯甘醇誘發大白鼠產生腎結石的模式中一氧化氮所扮演的角色 (NSC89-2314-B-002-298)"，發現經由腎動脈導管直接給予一氧化氮合成酵素的受質灌注腎臟 — 即 L-arginine 的灌注，我們發現在腎結石的實驗組大鼠其腎臟利尿和利鈉的反應與正常組的大鼠相比會顯著的降低。利用 volume expansion 的方法來測試腎臟內 NOS 的活性，結果一樣發現在腎結石的大鼠腎臟內，利鈉和利尿的反應與正常大鼠相比顯著的降低。腎臟內一氧化氮系統是一很重要調控利鈉利尿反應的物質，相對於腎臟內鴉片受體系統，也是一很重要調控排泄反應的物質。所以本計劃探討是否在結石的腎臟，其鴉片系統的變化會參與在結石形成的過程中。

(四)、研究目的

本計畫首先觀察在結石的腎臟內鴉片受體 mRNA 和蛋白表現的時間性的變化，我們檢測:

1. 三種不同鴉片受體 MOR、DOR 和 KOR 在實驗性腎臟草酸鈣腎結石的大鼠中的變化情形。
2. 利用 RT-PCR 偵測鴉片受體 mRNA 量的變化。
3. 利用 immunoblot 偵測鴉片受體蛋白量的變化。

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（六）、研究方法

Induction of Experimental Nephrolithiasis and Animal Grouping.

Male Wistar rats (200–250 g) were housed under a constant temperature and a light/dark cycle (light from 07:00 to 18:00). The animal care and experimental protocol were in accord with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The rats were divided into control group and group rats received 0.75% ethylene glycol (EG) in drinking water. There were six rats in each group, fed for 7, 14, 21, and 42 days. During the experimental period, all groups had free access to ground regular rat chow.

Semi-quantitative RT-PCR to Detect opioid receptor mRNA Expression.

Total cellular RNA in the cultured neurons was extracted by using the TRIzol reagent (Gibco) as the manufacturer's protocol. The RNA pellet was washed twice with 75% ethanol, dried, and resuspended in the diechypyrocarbonate-treated water. Complementary DNA (cDNA) was synthesized from 5 μg of the total RNA sample at 42^{o}C for 47 min using 0.5 μg of poly dT_{15} oligonucleotide primer (Invitrogen, Carlsbad, CA) and 200 units of reverse transcriptase (M-MLV, Promega, Madison, WI) in a final volume of 20 μl. Opioid receptor and glyceraldehyde 3-phosphate dehydrogenase cDNA were amplified using specific primers designed as previously described (55). The sequences for GAPDH were 5'-TTA GCA CCC CTG GCC AAG G-3' and 5'-CTT ACT CCT TGG AGG CCA TG-3' (535 bases). The reverse transcription product (2.5 μl) was amplified with a mixture of primers and 2.5 units of REDTag DNA polymerase (Sigma) for 30 cycles under the following conditions: 1 min at 95^{o}C for denaturation, 50 sec at 58^{o}C for annealing, and 1 min at 72^{o}C for extension. Reaction products were electrophoresed through a 2% agarose gel and visualized by ethidium bromide staining. The density of appreciate band was determined using an image analytic system (Alpha Innotech, San Leandro, CA). Expression of opioid receptor mRNA was calculated as a ratio of opioid receptor and glyceraldehyde 3-phosphate dehydrogenase PCR products.

Immunoblotting of opioid receptor.

The abundance of KOR, MOR, DOR and actin were examined by Western blots as described previously (Ma et al., 2005). Kidneys (divided into cortex and medulla) were sampled to prepare total proteins, a part of hepatic tissue was served as a positive control. The same amount of protein from each preparation (150 μg for opioid receptor) was separated on a 7% SDS-PAGE under denaturing, and electrophoretically transferred to nitrocellulose (Amersham, Buckingham, England, UK). Membrane was incubated with monoclonal anti-MOR antibody (1:500; BD Biosciences), anti-DOR (1:250; BD Biosciences), anti-KOR (1:500; BD Biosciences), and anti-actin (1:1000, Sigma), respectively, overnight at 4℃, washed, and then incubated with goat biotinylated anti-mouse IgG conjugated to horseradish peroxidase (Leinco Technologies, Saint Louis, Missouri, USA) for one hour at room temperature. The samples were washed and the bound antibody was visualized using a commercial DAB (3, 3'-diaminobenzidine) substrate kit (Vector, Burlingame, CA, USA) for peroxidase, following the manufacturer's protocol. The density of the acquired band in each lane was semi-quantitatively determined by densitometer with an image analytic system (Alpha Innotech, San Leandro, CA, USA). The data in each group was presented as the mean ± standard error for the integrated digital value (IDV) divided by corresponding actin of each lane.

（七）、結果與討論

Figure 1. Representative Western blot of DOR, KOR, and MOR in the specimen of renal cortex and medulla from the controls and ethylene glycol (EG)-treated rats with different time-points.

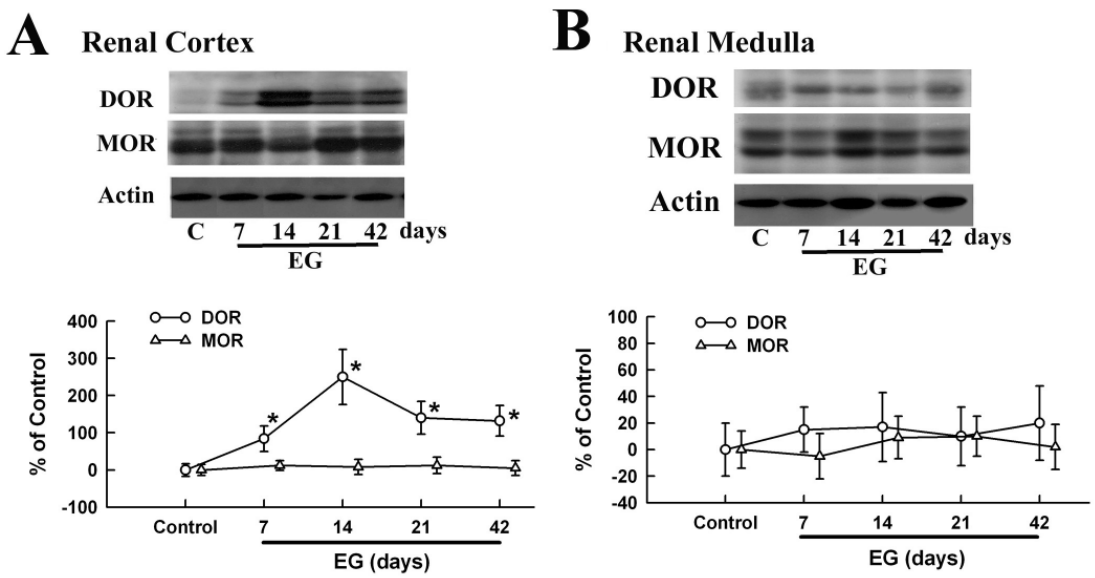
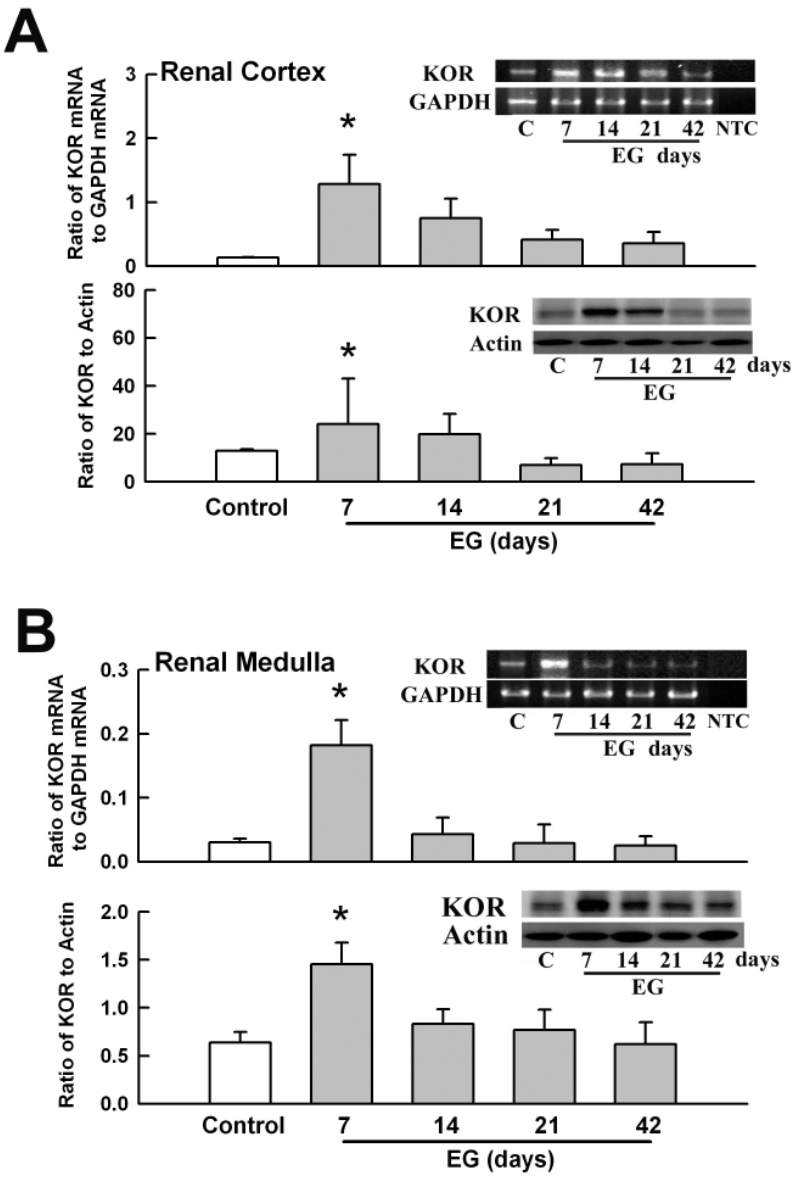


Figure 2. Results of immunoblot and PCR of KOR in the specimen of renal cortex and medulla from the controls and ethylene glycol (EG)-treated rats with different time-points. Insects showed the representative pictures.



（八）、計畫成果自評部份

本計劃進行相當順利，預計在本年度下半年中即可將所得之結果彙理並投稿於腎臟與泌尿學相關學門之國際雜誌。