April 11. 2003 (Friday) 14:40~15:10

고혈압 관련 측면에서의 alphaENaC, ET-1, cox-2 유전자의 소금에 의한 조절 기전

좌장 : 최종원(경성대학교)

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Sodium Chloride Regulation of αENaC, ET-1, and COX-2 Genes: A Possible Implication of Hypertension

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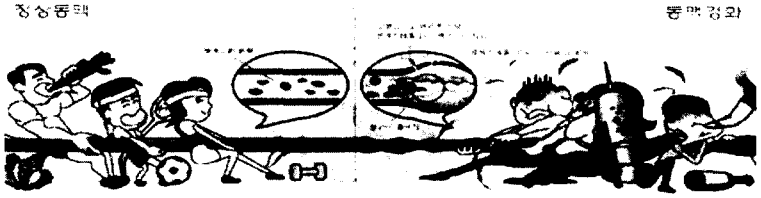
High Blood Pressure, a "Silent Killer."

Blood Pressure (mm Hg)	Optimal	Normal	High Normal	Hypertension
Systolic	<120	<130	130-139	>140
Diastolic	<80	<85	85-89	>90

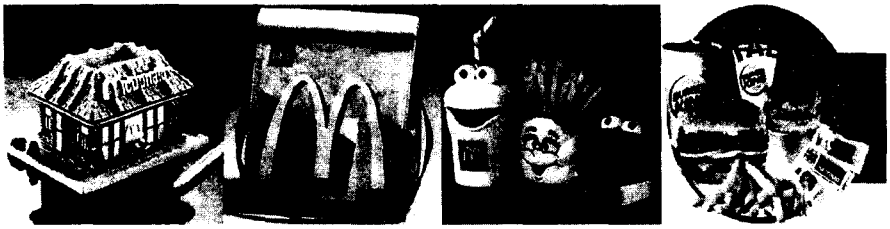
고혈압의 위험인자

- 유전적인 요인
- 고 식염섭취
- 비 만
- 운동부족
- 스트레스,
- 흡연, 과음 등



Healthy American Adults 권장량

2.4g 의 Sodium: 1.25티스푼 소금: 소금 6g
한국인의 1일 섭취량: 15-20g



햄버거 세트 1회 식사: 약 5.8g의 소금

Specific Aims

Dietary 소금에 의한 분자생물학적 기전 분석

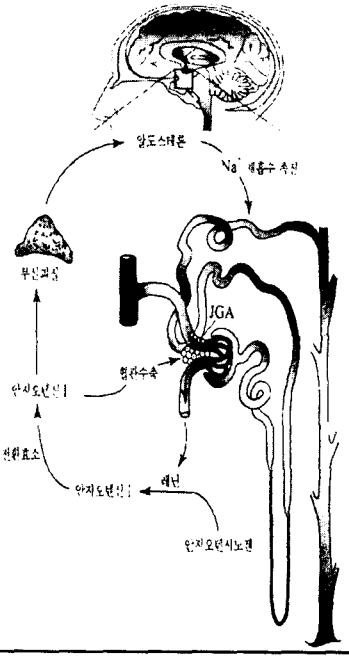
Physiologic Na^+ Regulation

시상하부: 항이뇨호르몬

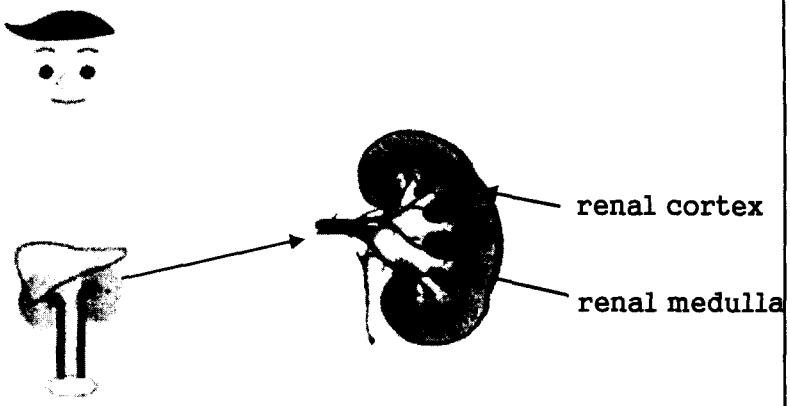
Sodium transport by channels

Renin-Angiotensin system:
vasoconstriction, sodium retention

Aldosterone:
mineralocorticoid receptor



Kidney



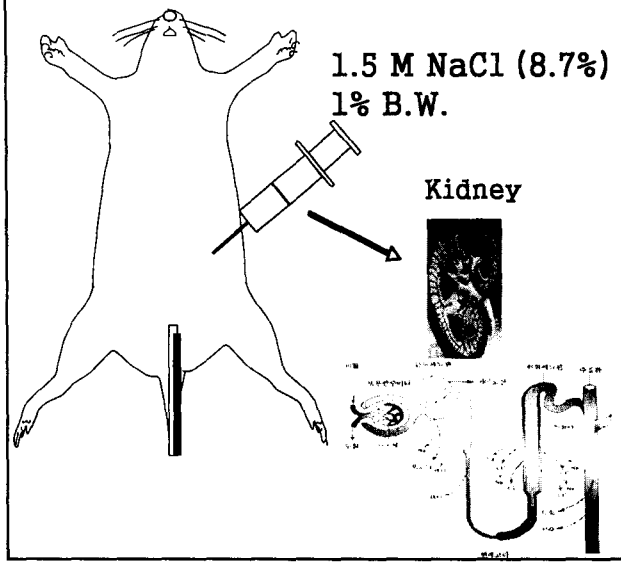
Kidney has a important role in Na^+ homeostasis. There is a unique relationship between the kidney and blood pressure.

현재 이용 가능한 소금의 동물 모델 시스템

- **Dahl salt-sensitive (DS) and salt-resistant (DR) rats**
  다량의 염분에 노출되면 고혈압이 발생.
- **Spontaneously hypertensive rat : SHR**
  Wistar 백서의 변종
  수분저류에 의한 혈장량의 증가
  또는 말초저항의 증가로 고혈압 발생
- **DOCA** 식염 고혈압 백서 **(DOCA salt rat)**

단점:
고혈압 발생시까지 약 **1-10**주이상 소요
DOCA salt rat은 **1**주일 후부터 혈압 유의하게 증가

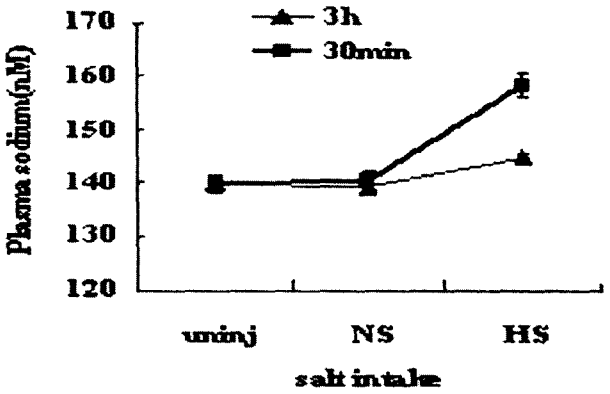
Experimental Model System



① Isolation RNA
⇩
cDNA synthesis
⇩
PCR

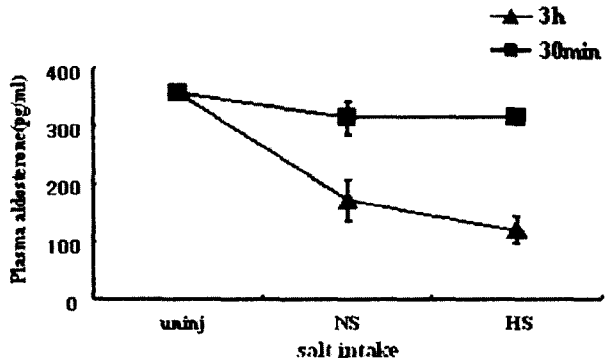
② Isolation Protein
⇩
Western blot

Levels of Plasma Sodium Concentration after IP Injection of Sodium Chloride



방법: ion-selective electrodes 이용 측정

Levels of Plasma Aldosterone Concentration after IP Injection of Sodium Chloride



방법: RIA

Genes Under Investigation

Hypertension Related Genes

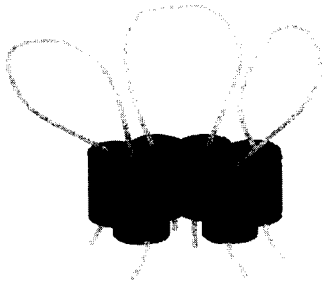
Epithelial Sodium Channel

Endothelin-1

COX-2

Minerlaocorticoid Receptor

Epithelial Sodium Channel (ENaC)



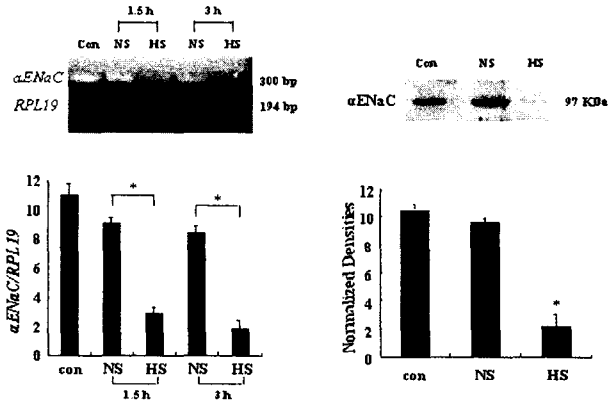
Drug Metabolism and Disposition 2001

.ENaC는 신장, 폐, 대장세포의 **apical membrane**과 다른 내피조직에 존재

.염분과 수분의 항상성유지에 중요한 역할 => **Blood pressure regulat**

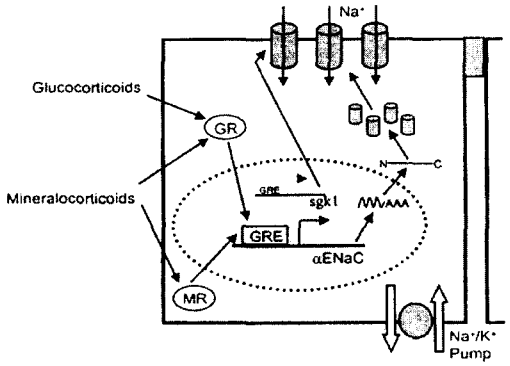
.**Na+ channel**의 β단백 유전자에 변이가 생기면 **channel**은 항상 열려 있는 상태로**(open state)** 있게 되어, **Na+**을 과다하게 재흡수하여 고혈압을 유발**(Liddle's syndrome).**

Expression of αENaC in the Kidney Cortex



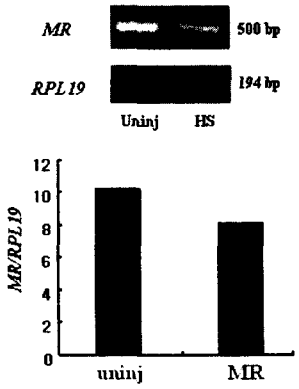
Sodium chloride downregulates αENaC in SD rat kidney cortex at mRNA and protein levels.

Current Model of Sodium Transport by Alpha Epithelial Sodium Channel

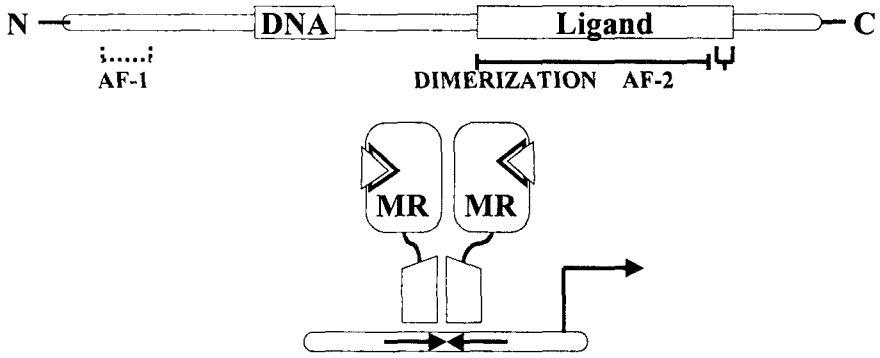


Mol Endo. 2001

No Changes in Mineralocorticoid Receptor mRNA after Sodium Chloride Treatment

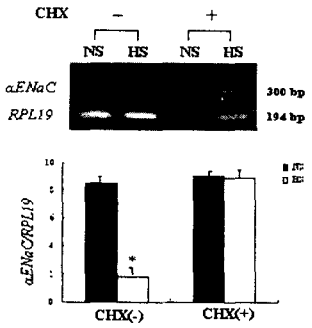


Nuclear Receptor Superfamily



MR mineralocorticoid
GR glucocorticoid
AR androgen
ER estrogen

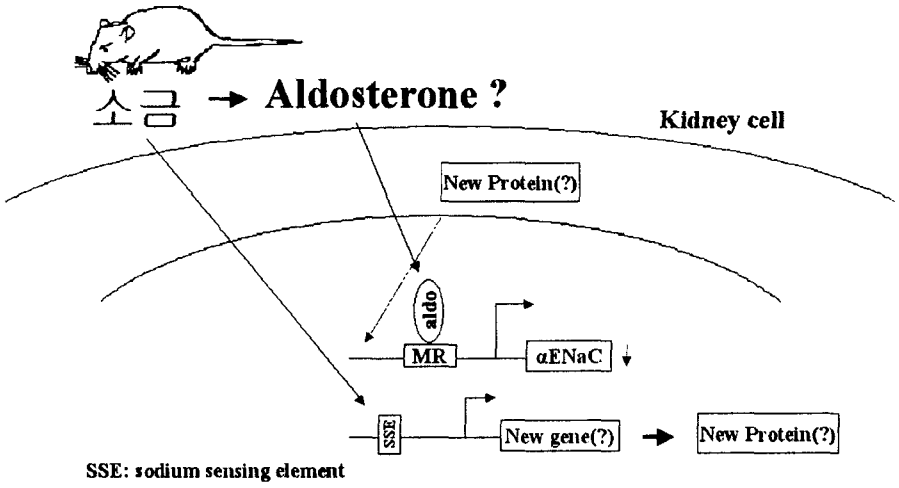
Expression of αENaC in the Kidney Cortex



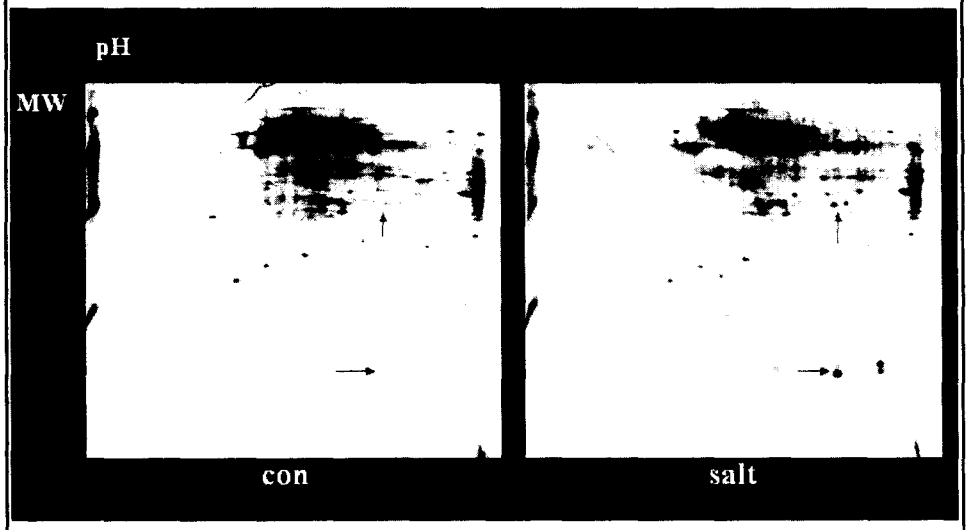
Coinjection of CHX(1.5 mg/kg of body mass) blocked the sodium-induced αENaC down-regulation at 3 h of treatment.

Synthesis of new, uncharacterized protein(s) is required for sodium chloride-mediated inhibition of αENaC gene transcription.

Our Working Model



2-DE map of protein from kidney cortex



Cyclooxygenase-2 (COX-2)

. **Prostaglandin** 합성효소

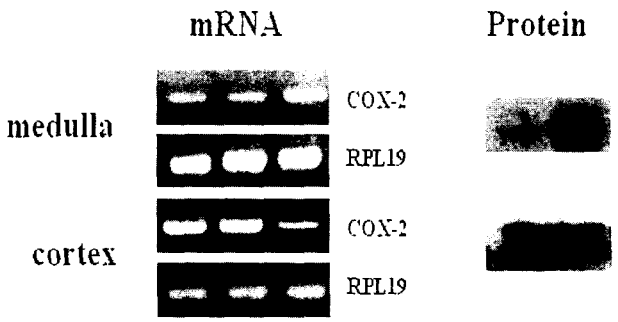
.신장에서 신혈류와 물/소금의 항상성을 조절

.**cox-2**발현은 **volume**상태의 변화에 따른 반응에 의해 조절

.수질: **volume overload**상태에서 소금과 물의 배출을 촉진

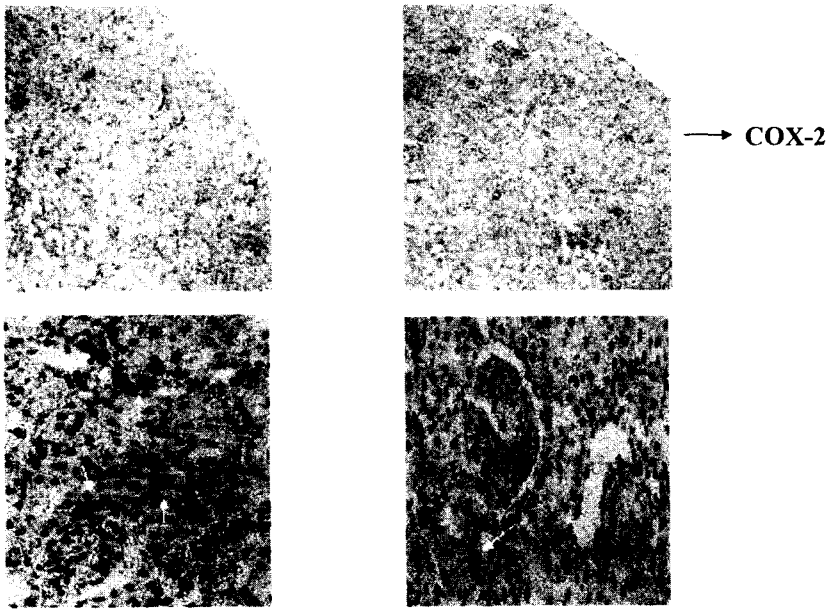
.피질: **volume depletion**상태에서 혈관수축효과를 약화시켜 사구체순환을 보호

Expression of COX-2 in SD rat kidney

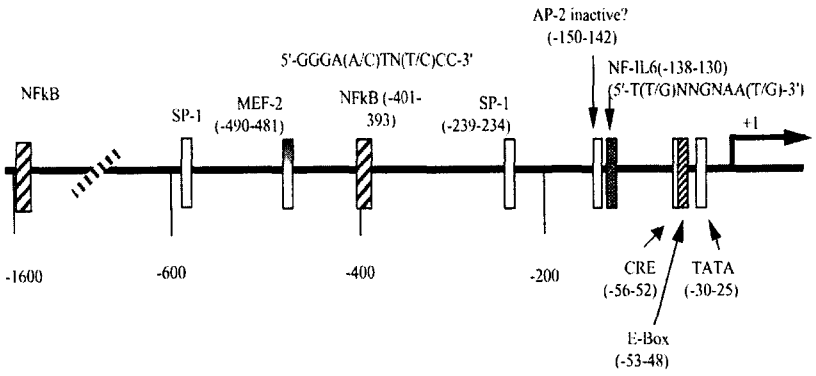


Expression of COX-2 showed tissue specific regulation.

Expression of COX-2 in SD rat kidney

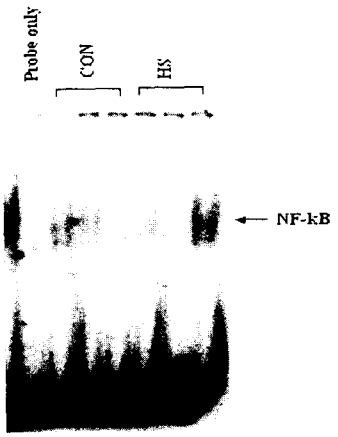


Transcriptional regulation of COX-2



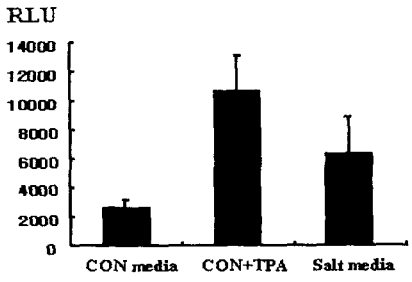
Multiple response elements in the COX-2 promoter region.

Is NF-KB binding responsible for COX-2 regulation by salt?



Activation of NF-KB is recognized to be critical for regulating the induction of COX-2.

Effect of hypertonic NaCl on full-length (-3400) COX-2 Promoter activity in transfected COS+ER cells.



Which region is crucial for salt-induced COX-2 promoter activity?

Endothelin 1 (ET-1)

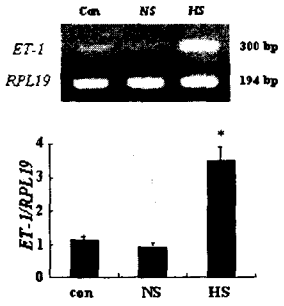
.돼지의 대동맥내피세포의 배양액에서 발견한 21개의 아미노산으로 구성된 혈관 반응 물질로서 강력한 혈관 수축 작용

.인접한 내피세포나 평활근 세포에서 **ETa, ETb receptor**를 통해 **autocrine** 또는 **paracrine** 작용

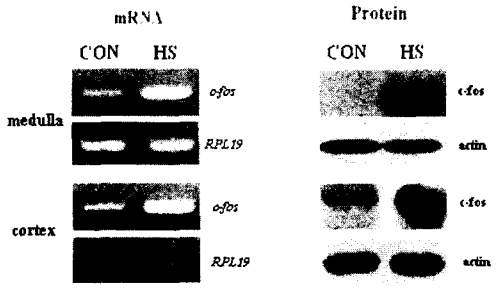
.신장혈관에서의 강력한 혈관수축작용으로사구체 여과율과 나트륨배설을 감소

.ETa, ETb receptor의 선택적인 길항제에 의해 혈압을 낮출 수 있다.

Sodium Chloride Dramatically Upregulates Endothelin-1 in SD Rat Kidney Medulla at mRNA Level



Expression of c-fos in kidney medulla and cortex



Sodium chloride stimulates c-fos gene expression in SD rat kidney.

SUMMARYS

1. We have established a model system to study sodium chloride, an environmental factor, induced gene regulations.

2. αENaC, cox-2, and c-fos genes are regulated by sodium chloride at mRNA level as well as at protein level .

3. Regulation of αENaC requires syntheses of new protein(s).

4. COX-2 may have a important role for homeostasis in hypertonic situation.

Acknowledgements

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