

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

간암세포 사멸에 미치는 세포막 이온 수송체의 역할

좌장 : 최종원(경성대학교)

이 용 수

(교수, 덕성여자대학교 약학대학)

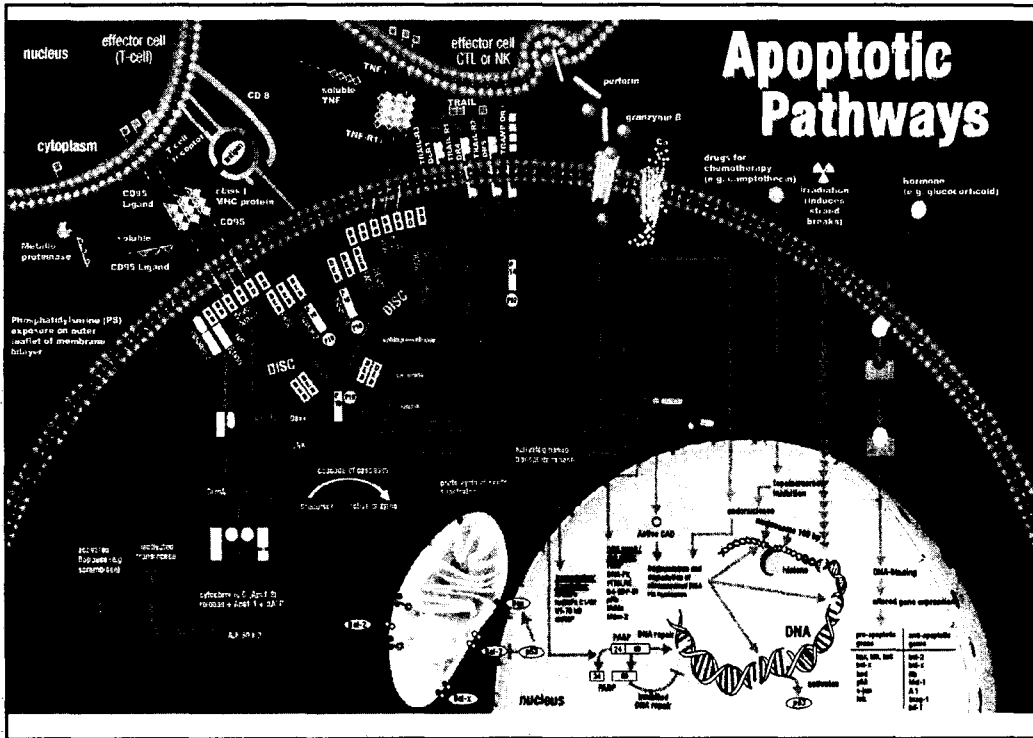
Roles of KCC and NKCC in Hepatoma Cell Apoptosis

Yong Soo Lee, Ph.D.

College of Pharmacy, Duksung Women's University

Differential Features and Significance of Necrosis and Apoptosis

Necrosis	Apoptosis
Morphological features <ul style="list-style-type: none">*Loss of membrane integrity*Swelling of cytoplasm and mitochondria*Total cell lysis	<ul style="list-style-type: none">*Membrane blebbing*Shrinkage of cytoplasm and condensation of nucleus*Fragmentation of cell into apoptotic bodies
Biochemical features <ul style="list-style-type: none">*Loss of regulation of ion homeostasis*No energy requirement*Random digestion of DNA	<ul style="list-style-type: none">*Tightly regulated process*Energy (ATP)-dependent*Fragmentation of DNA*Activation of caspase cascade*Release of cytochrome C*Translocation of phosphatidylserine
Physiological significance <ul style="list-style-type: none">*Affects groups of contiguous cells*Evoked by non-physiological disturbances*Significant inflammatory response	<ul style="list-style-type: none">*Affects individual cells*Induced by physiological stimuli*No inflammatory response

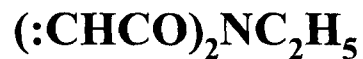


Role of K^+ , Cl^- -Cotransport (KCC) in Hepatoma Cell Apoptosis

K⁺, Cl⁻-Cotransporter (KCC)

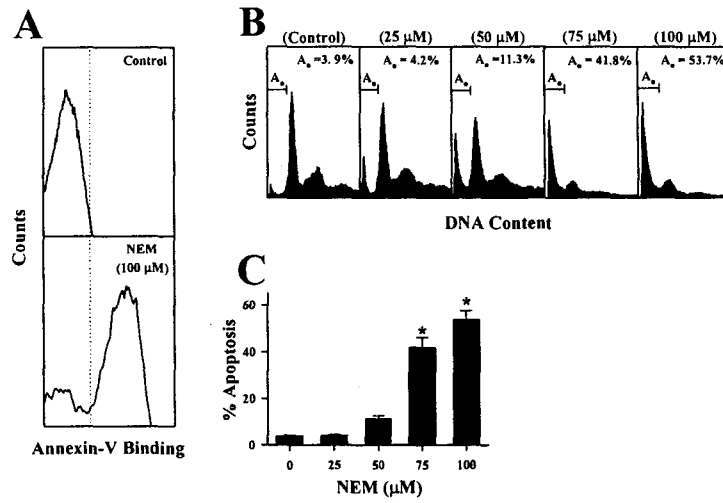
- **Expressed in a wide variety of cell types**
- **At least four isoforms of the KCC protein:**
 - ***KCC1 – in erythroid cell types**
 - ***KCC2 – in only neurons within CNS**
 - ***KCC3 & 4 – not much characterized**
- **Regulatory volume decrease (RVD)**
- **Regulation of transmembrane Cl⁻ gradient**
- **No report on the role of KCC in apoptosis**

N-Ethylmaleimide (NEM)

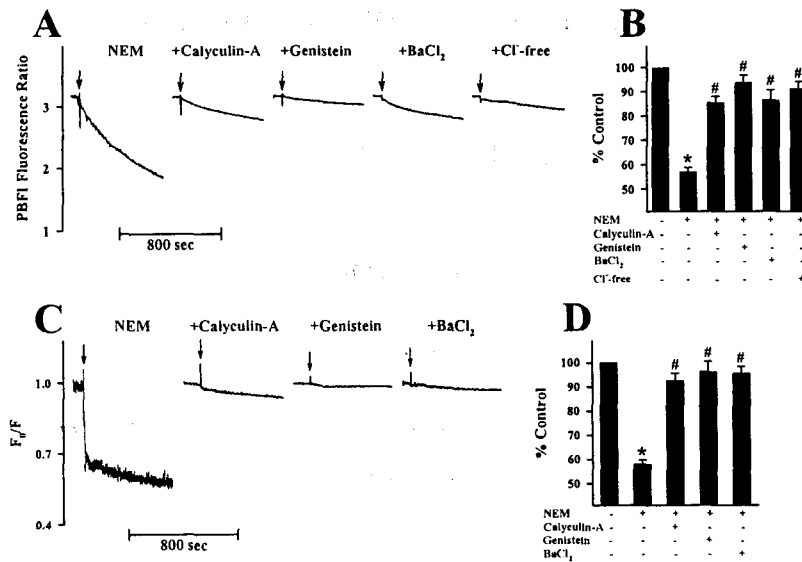


- **A thiol-alkylating agent**
- **An KCC activator:**
 - †**phosphorylation-dephosphorylation of the regulatory protein**

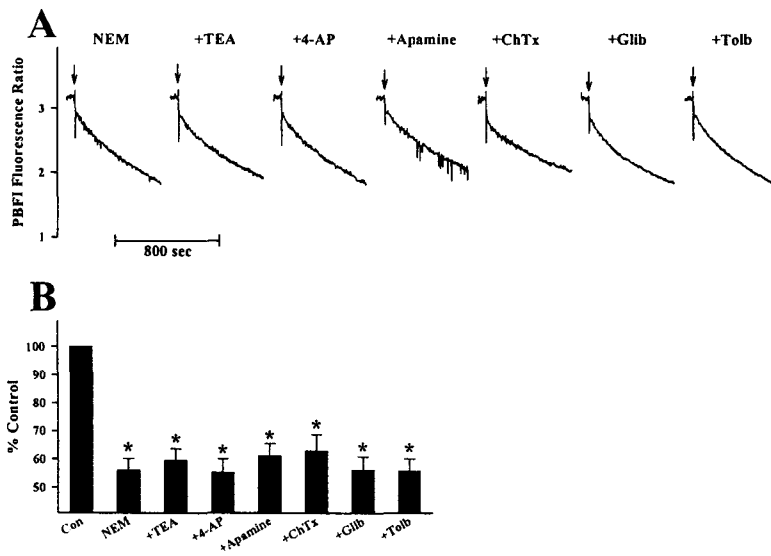
Induction of Apoptosis by NEM in HepG2 Cells



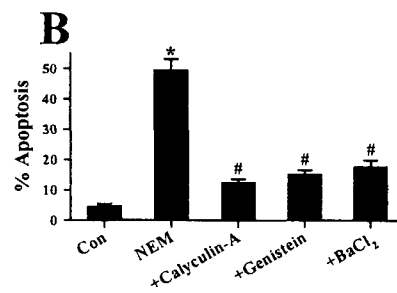
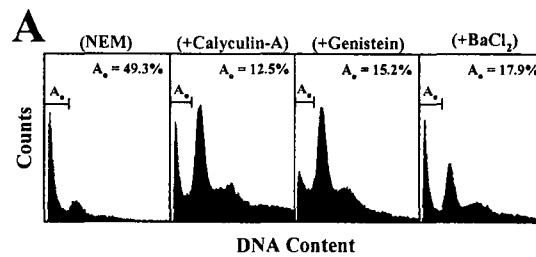
NEM Activates KCC in HepG2 Cells



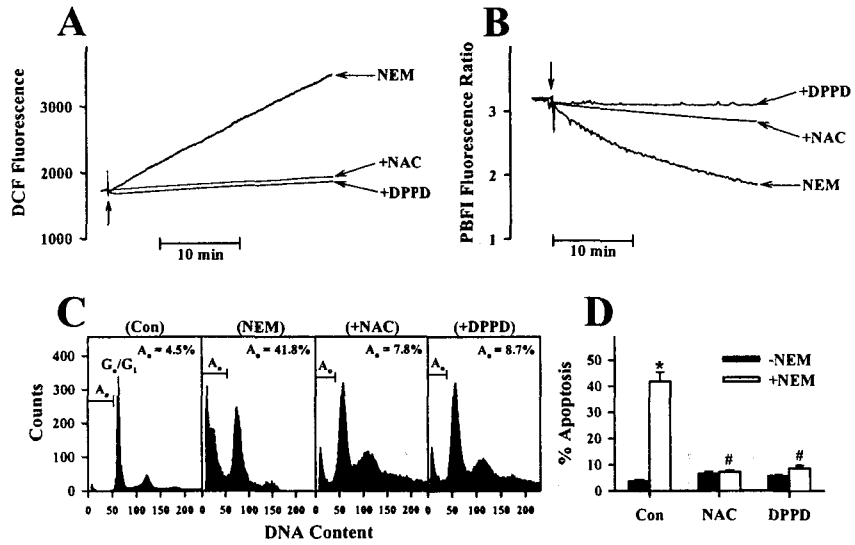
No Involvement of K^+ Channels in the NEM-Induced $[K^+]_i$ Decrease in HepG2 Cells



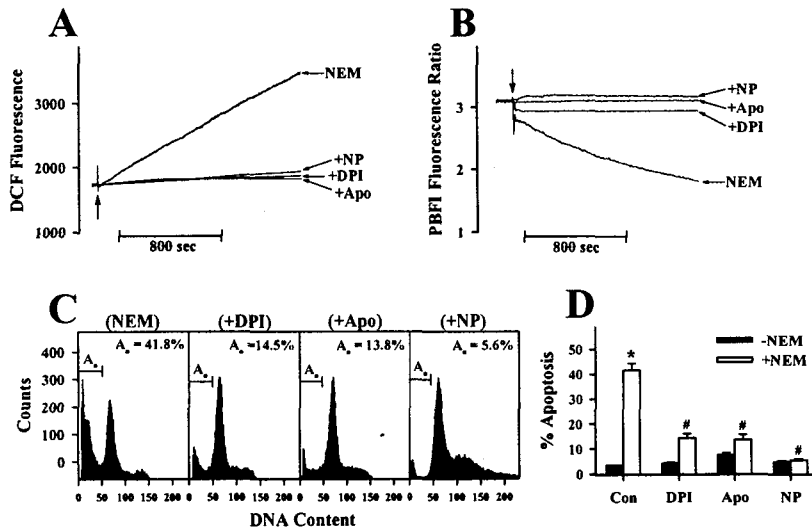
KCC Inhibitors Suppress the NEM-Induced Apoptosis in HepG2 Cells



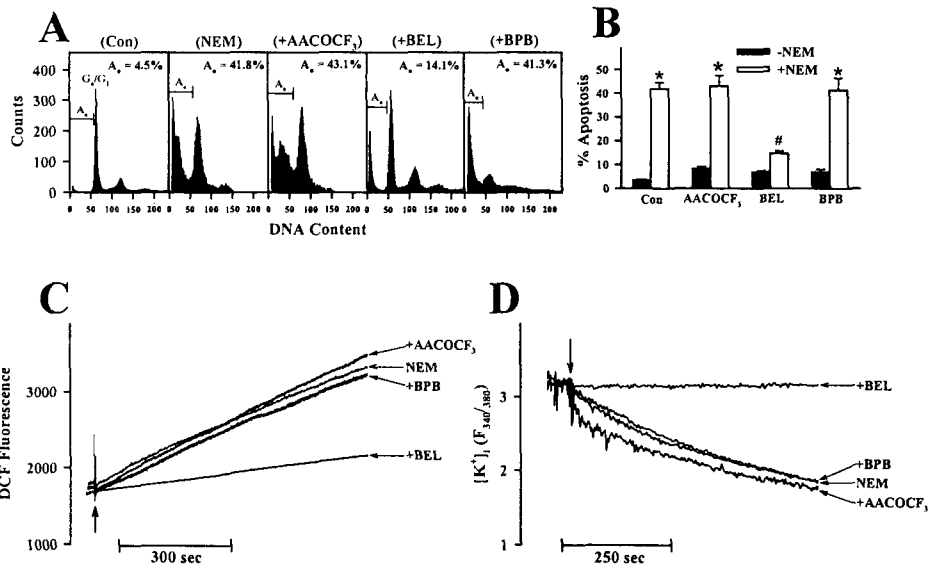
Roles of Reactive Oxygen Species in the NEM-Induced KCC Activation and Apoptosis in HepG2 Cells



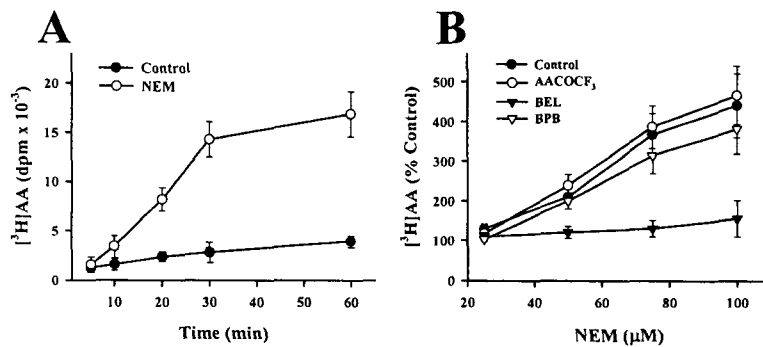
Roles of NADPH Oxidase in the NEM-Induced KCC Activation and Apoptosis in HepG2 Cells



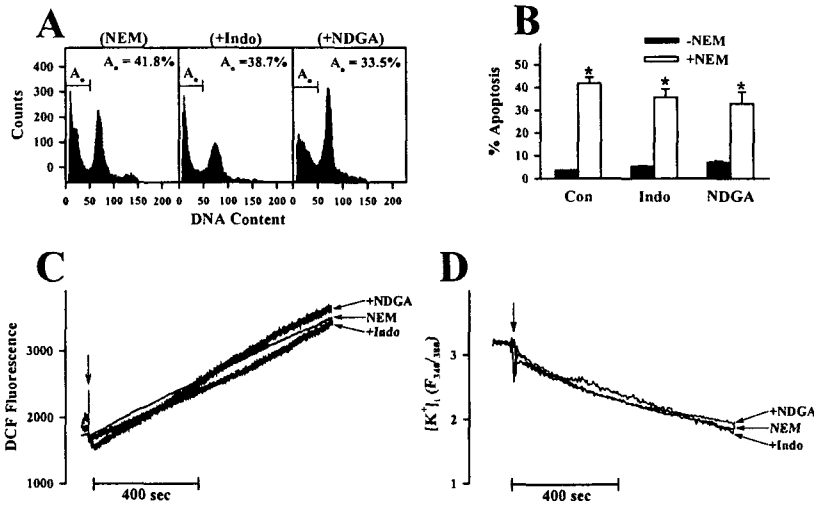
Roles of iPLA₂ in the NEM-Induced Effects in HepG2 Cells



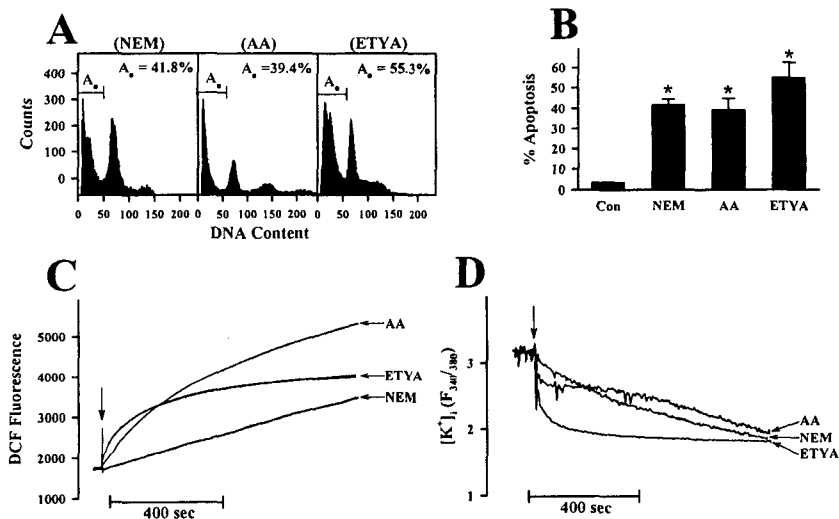
Production of Arachidonic Acid (AA) by NEM in HepG2 Cells



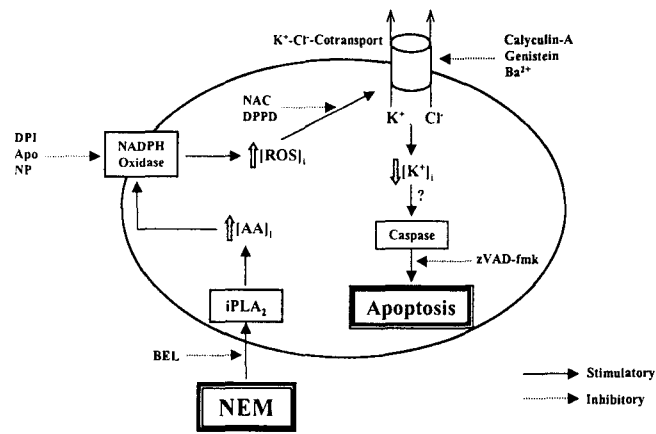
No Involvement of COX and LOX in the NEM-Induced Effects in HepG2 Cells



AA and ETYA, a Non-metabolizable AA Analogue, Mimic the Effects of NEM in HepG2 Cells



Mechanism of the NEM-Induced Apoptosis in HepG2 Cells

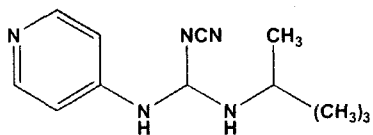


Role of Na⁺, K⁺, Cl⁻-Cotransport (NKCC) in Hepatoma Cell Apoptosis

Na⁺, K⁺, Cl⁻-Cotransporter (NKCC)

- Expressed in nearly every animal cell type
- Two isoforms of the NKCC protein:
 - *~120-130 kDa, unglycosylated
 - *NKCC1 – in all cell types
 - *NKCC2 – only in the kidney
- Maintenance of intracellular Cl⁻ concentration
- Control of cell volume
- Regulation of cell cycle

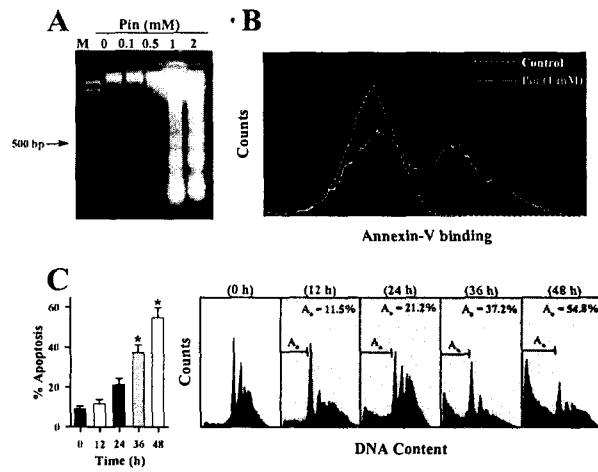
Pinacidil



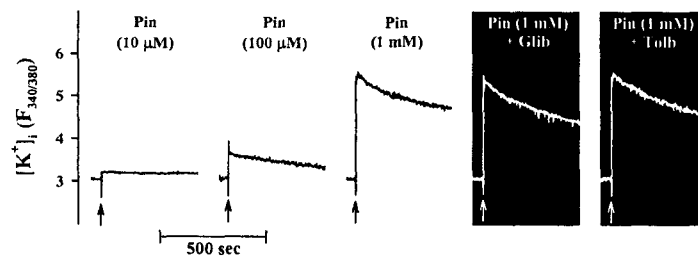
N-Cyano-N'-(4-pyridinyl)-
N''(1,2,2-trimethylpropyl)guanidine

- K_{ATP} Channel Activator
- Antihypertensive Activity
- No Reports on Apoptosis NKCC Activity

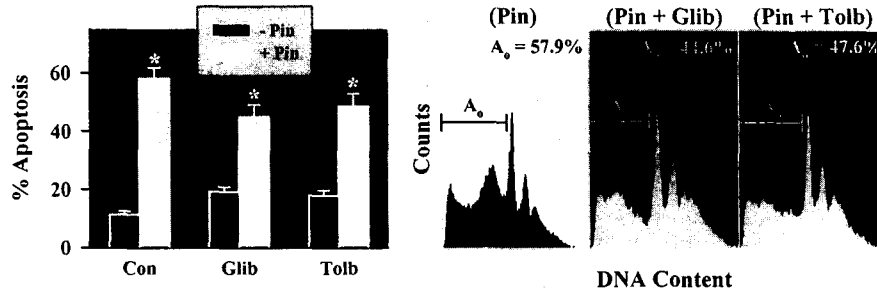
Induction of Apoptosis by Pinacidil in HepG2 Cells



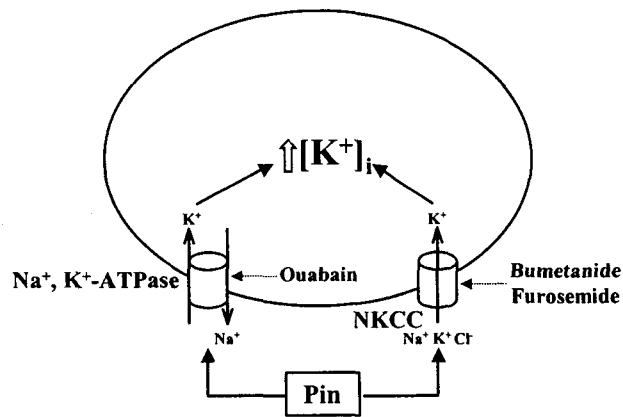
Pinacidil Increases $[K^+]_i$ in HepG2 Cells



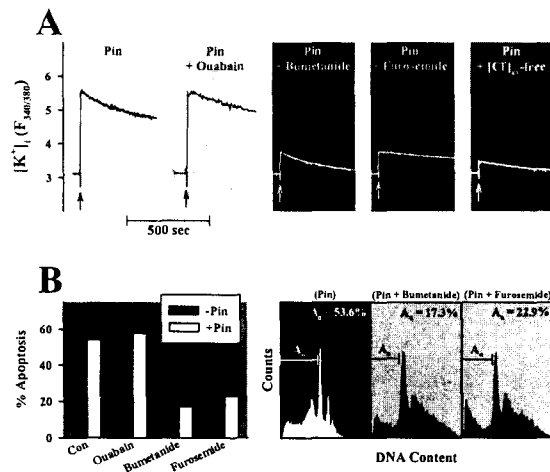
No Involvement of K_{ATP} Channels in the Pinacidil-Induced Apoptosis in HepG2 Cells



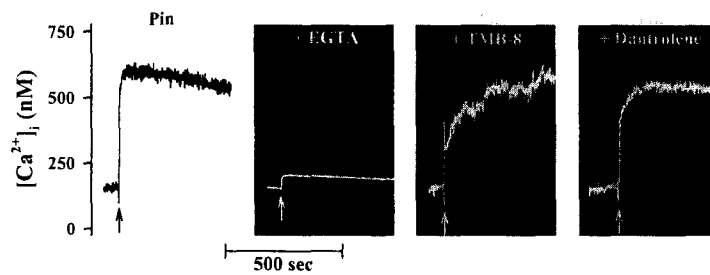
Possible Ways to Increase Intracellular K^+ Concentration



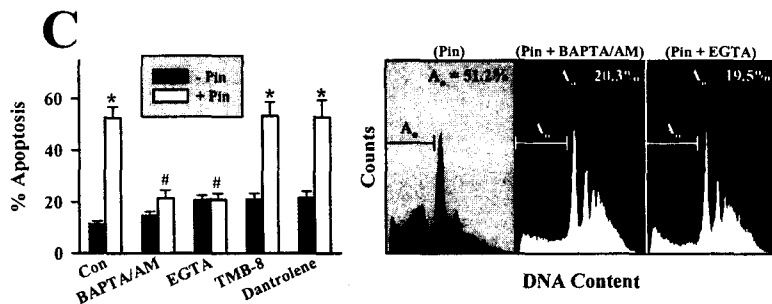
Roles of NKCC in the Pinacidil-Induced $[K^+]_i$ Increase and Apoptosis in HepG2 Cells



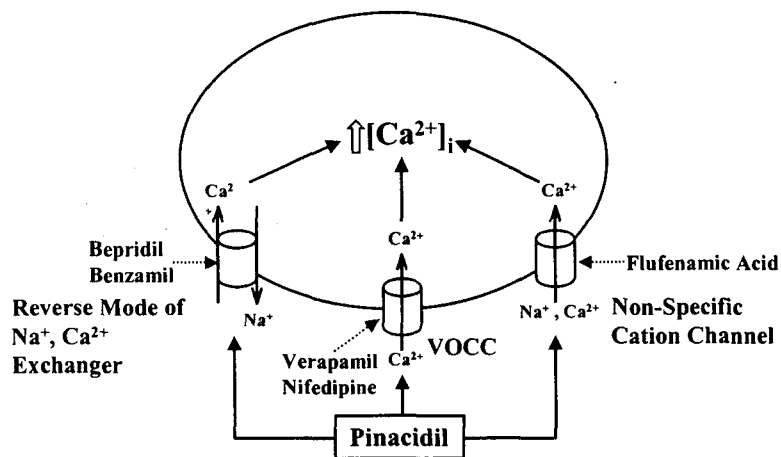
Pinacidil Induced Ca^{2+} Influx in HepG2 Cells



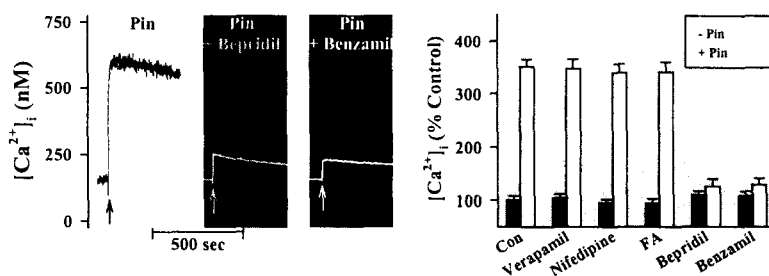
Role of Ca^{2+} Influx in the Pinacidil-Induced Apoptosis in HepG2 Cells



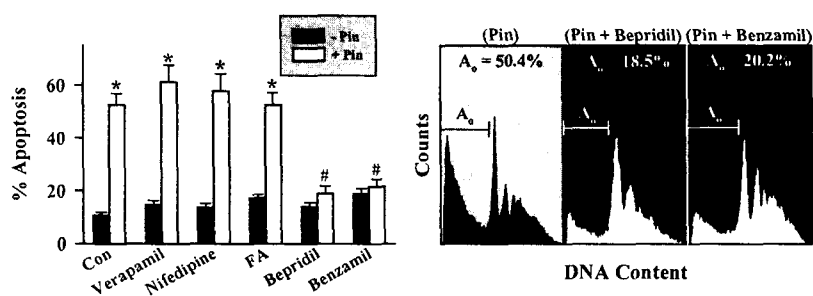
Possible Ways to Induce Ca^{2+} Influx



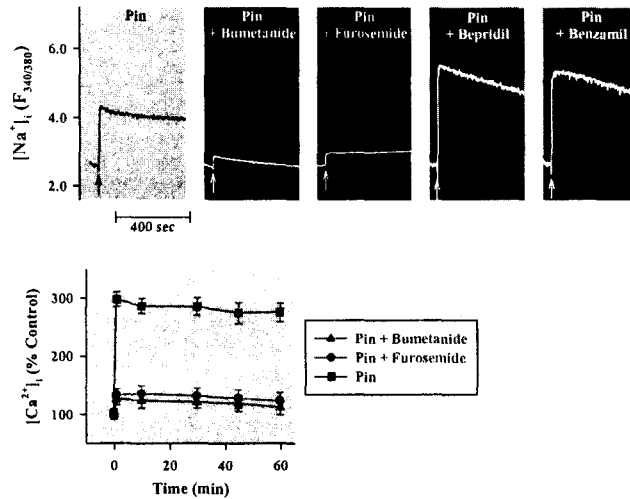
Role of Na⁺, Ca²⁺ Exchanger in the Pinacidil-Induced Ca²⁺ Influx in HepG2 Cells



Role of Na⁺, Ca²⁺ Exchanger in the Pinacidil-Induced Apoptosis in HepG2 Cells



NKCC Mediates Activation of Reverse-Mode of Na^+ , Ca^{2+} Exchanger Induced by Pinacidil in HepG2 Cells



Mechanism of Apoptosis Induced by Pinacidil in HepG2 Cells

