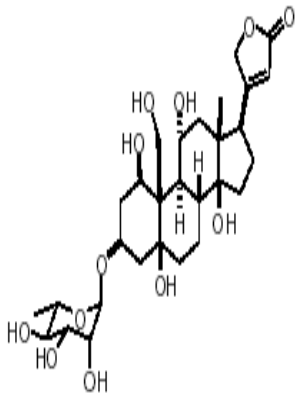
Οι σηματοδοτικές οδοί της ενδογενούς ουαμπαίνης

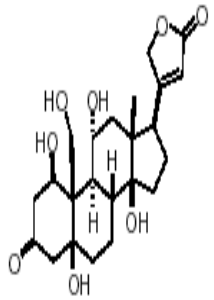
Ιωάννης Γριβέας

Νεφρολογικό Τμήμα 401 ΓΣΝΑ

Background



Ouabain



Ouabagenin

Acokanthera oblongifolia

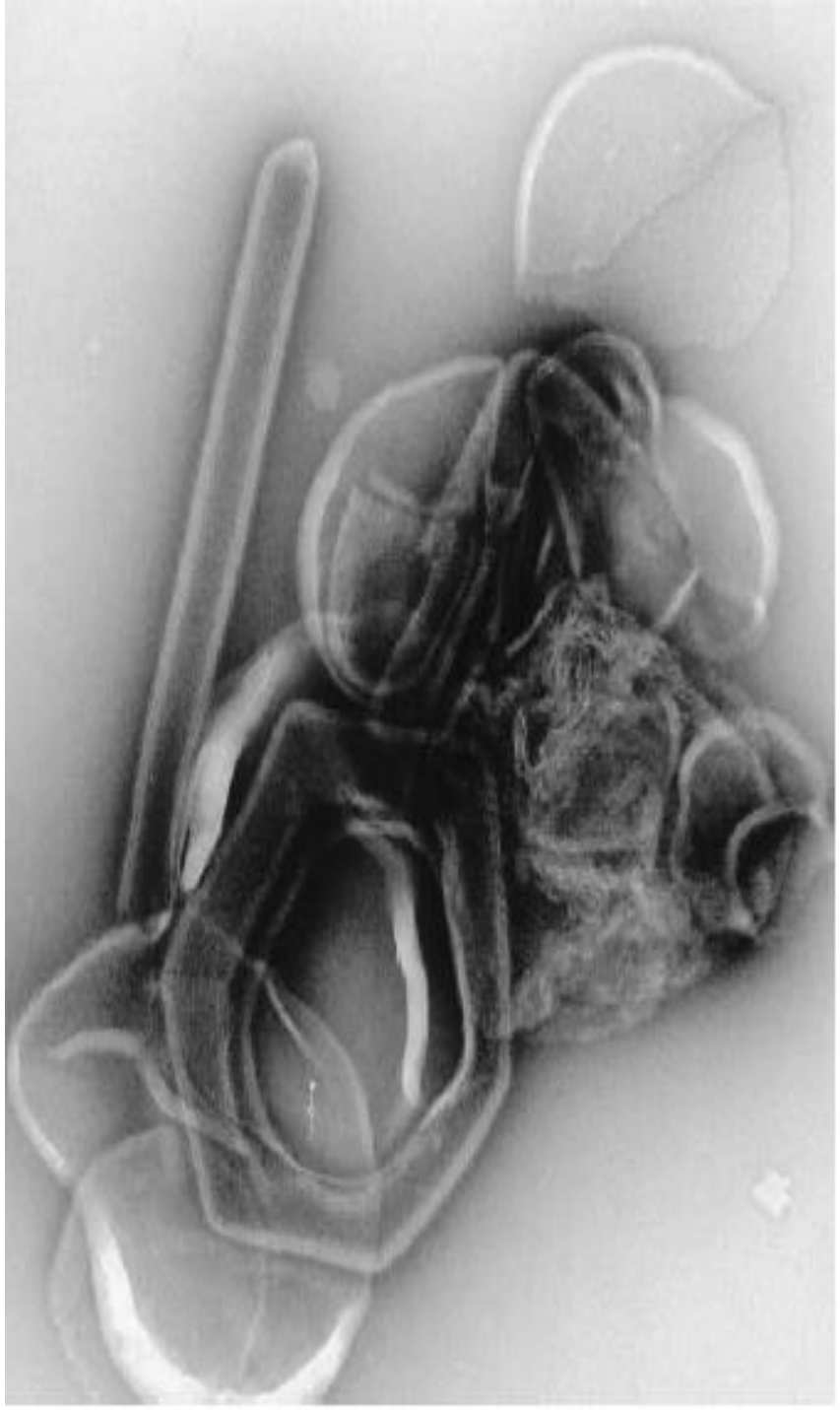
-isolated from the African ouabio tree (*Acokanthera ouabio*) in 1888 by Arnaud

-cardiac glycoside elicits effect by binding to myocardial Na^+, K^+-ATPase (responsible for regulating intracellular Na^+ transport

-used in Africa to make poison arrows

-unique steroid structure:

- -sugar at the 3β position
- -β butenolide ring at C17



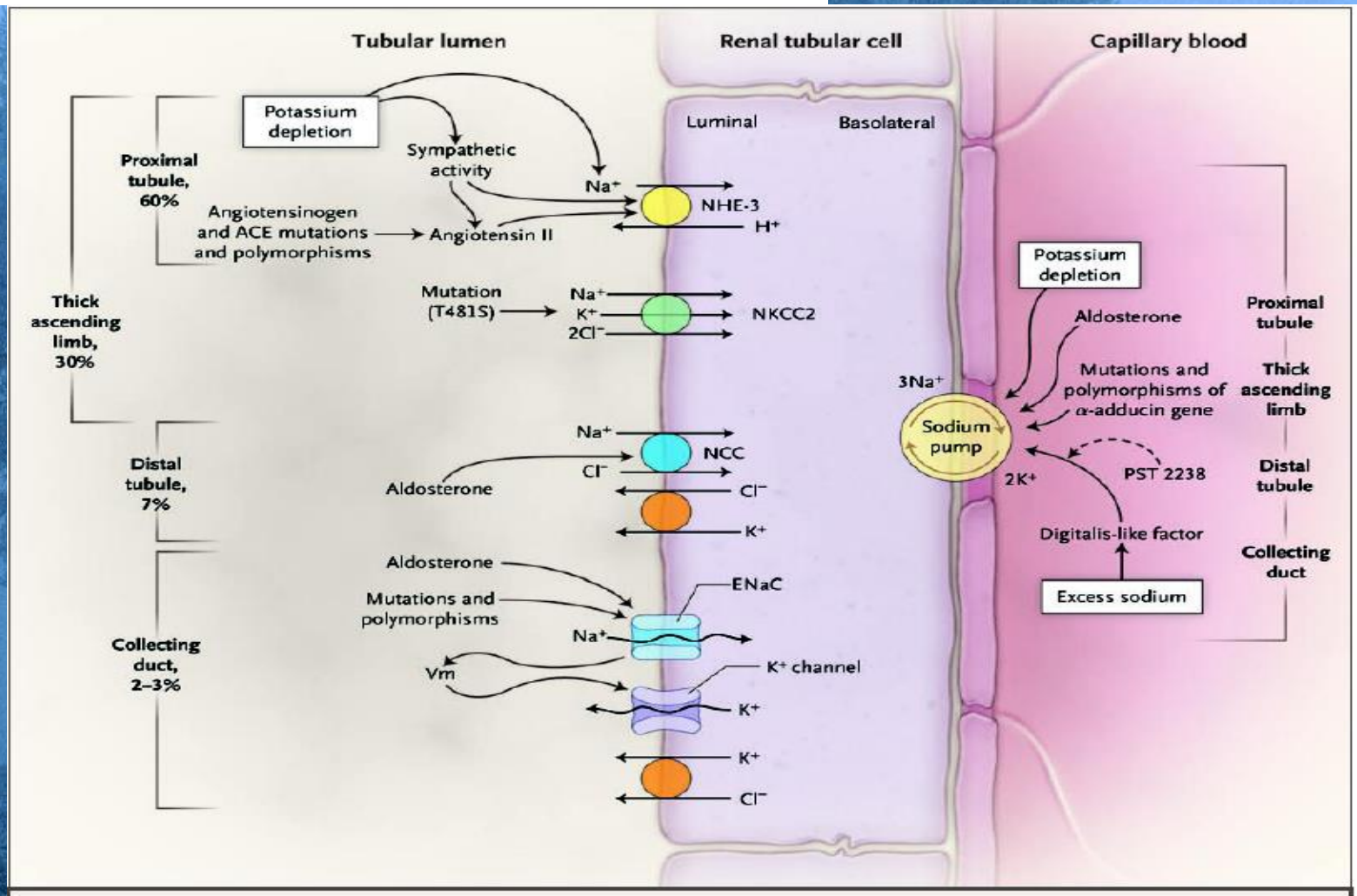
The NEW ENGLAND JOURNAL of MEDICINE

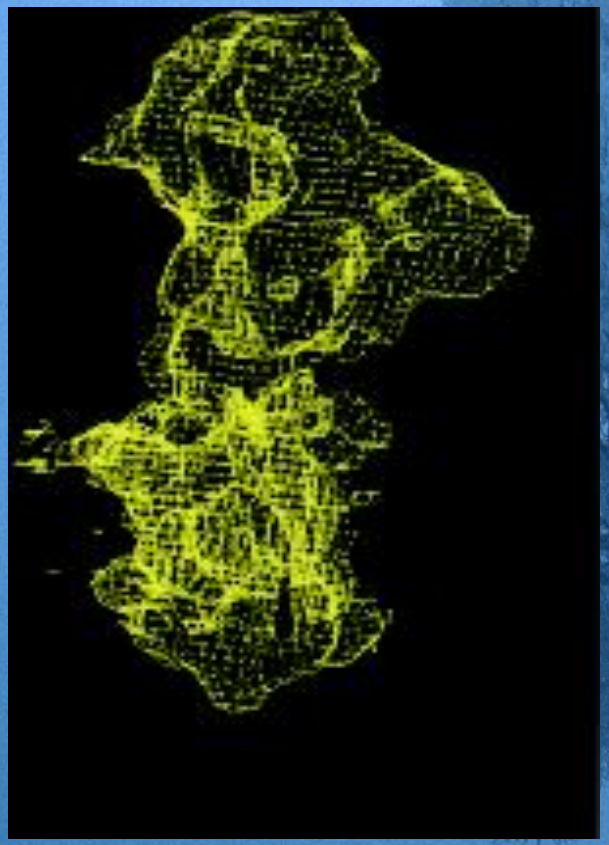
REVIEW ARTICLE

MECHANISMS OF DISEASE

Sodium and Potassium in the Pathogenesis of Hypertension

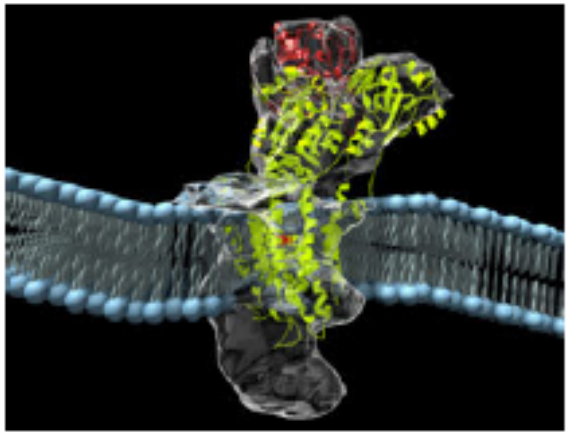
Horacio J. Adrogué, M.D., and Nicolaos E. Madias, M.D.

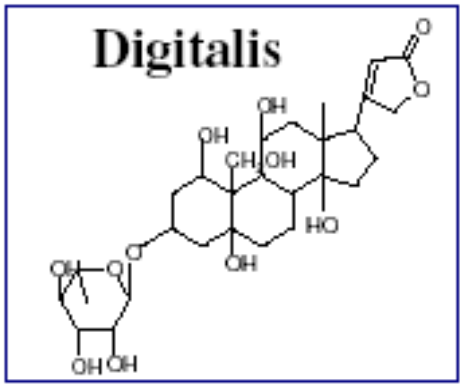


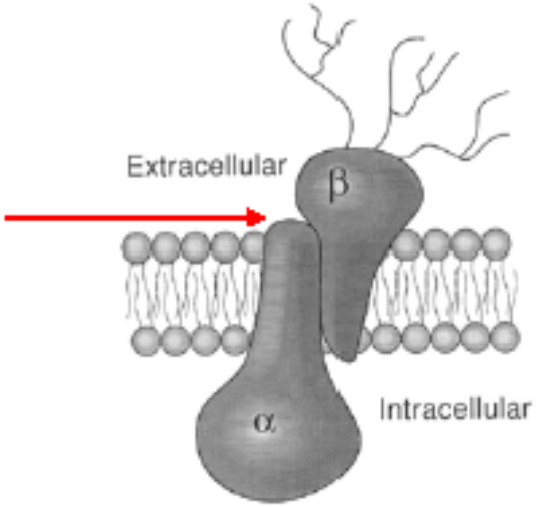


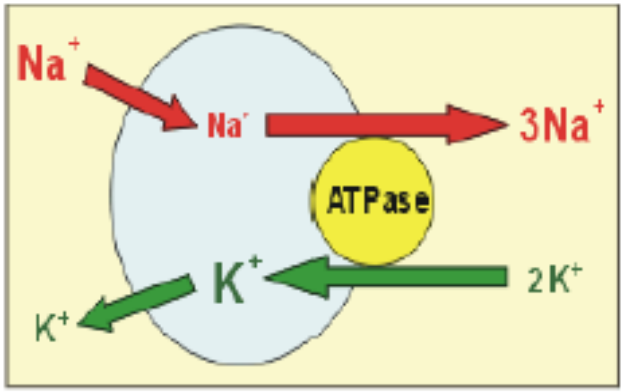
CELL
BIOLOGY

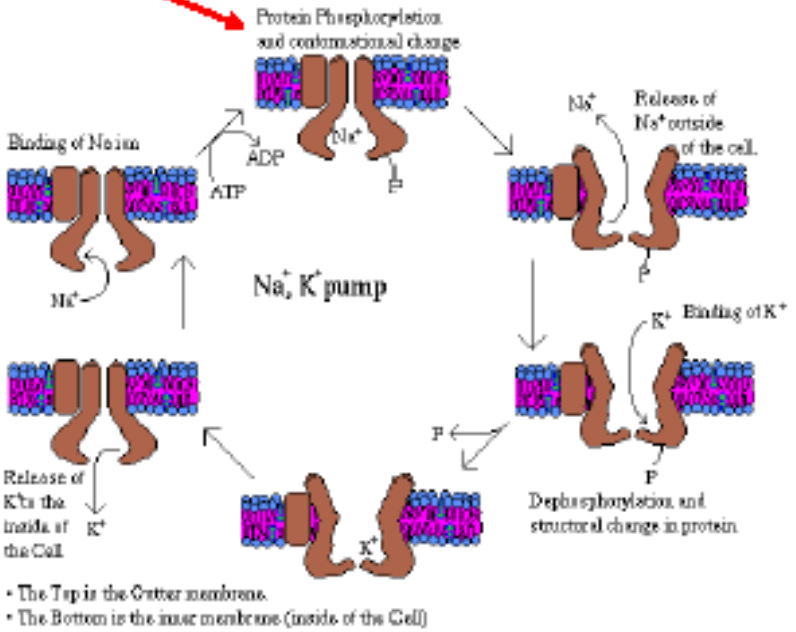
Na/K ATPase




Digitalis

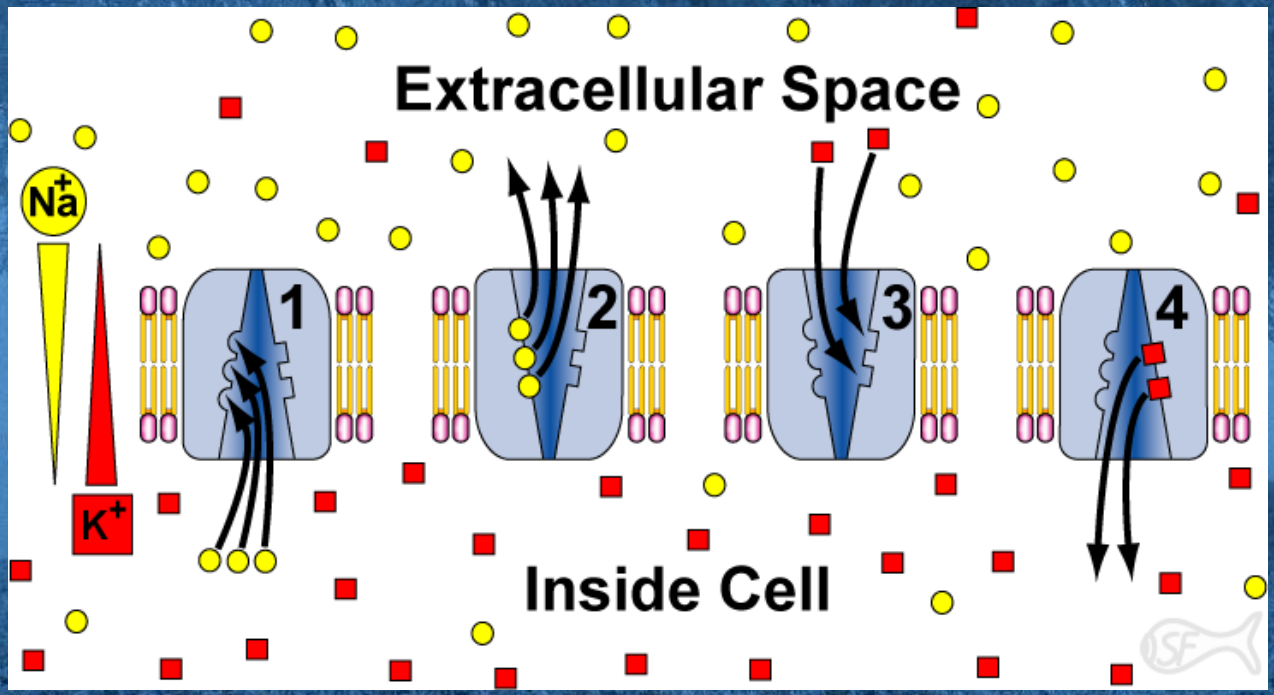

Extracellular
β
α
Intracellular


Na+
Na+
3Na+
ATPase
K+
2K+
K+


Protein Phosphorylation and conformational change
Binding of Na ion
ADP
ATP
Na+
P
Na+
Release of Na+ outside of the cell.
Na+, K+ pump
K+ Binding of K+
P
P
Dephosphorylation and structural change in protein
Release of K+ to the inside of the Cell
K+
K+
• The Top is the Outter membrane.
• The Bottom is the inner membrane (inside of the Cell)

Primary active transport example: The Na^+- K^+ antiporter pump

- Pumps 3 Na^+ ions out of cell & 2 K^+ ions in
- Maintains Na^+ & K^+ cell membrane gradients
- Each cycle uses one ATP, 100 cycles/sec
- Uses ¼ energy of most cells, ¾ for neurons

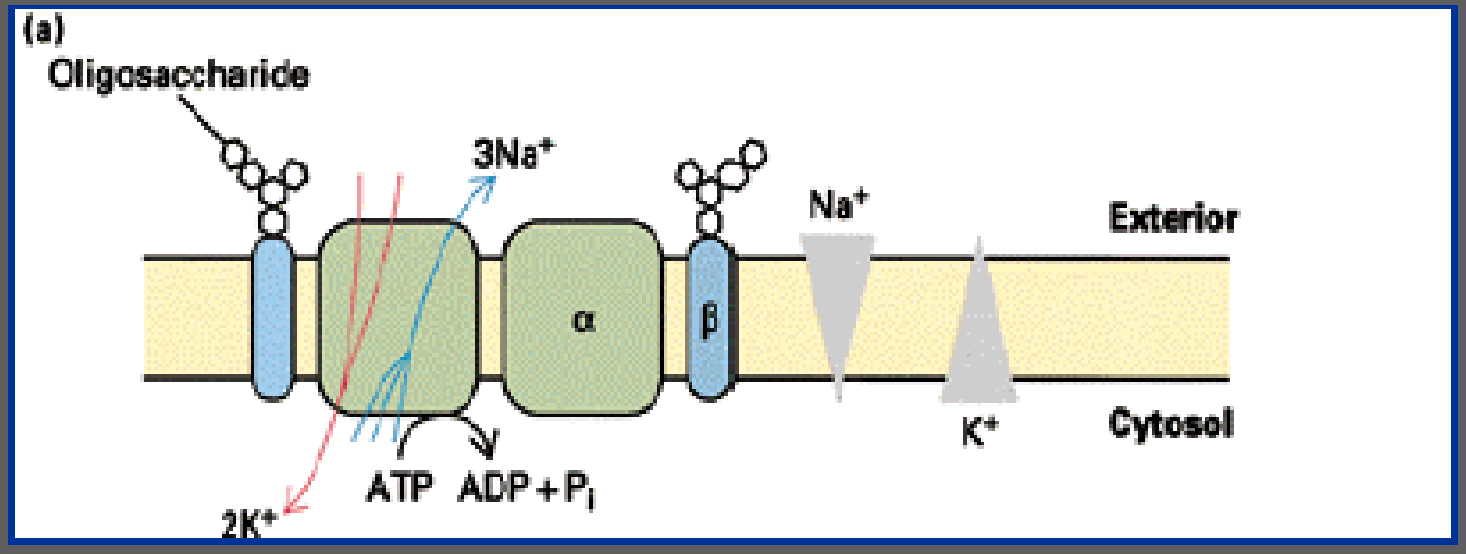


Na^+/ K^+ ATPase

Greatest consumer cellular energy

Sets up concentration & electrical gradients

Hydrolysis of 1 ATP moves $2K^+$ in and $3Na^+$ out against their concentration gradients

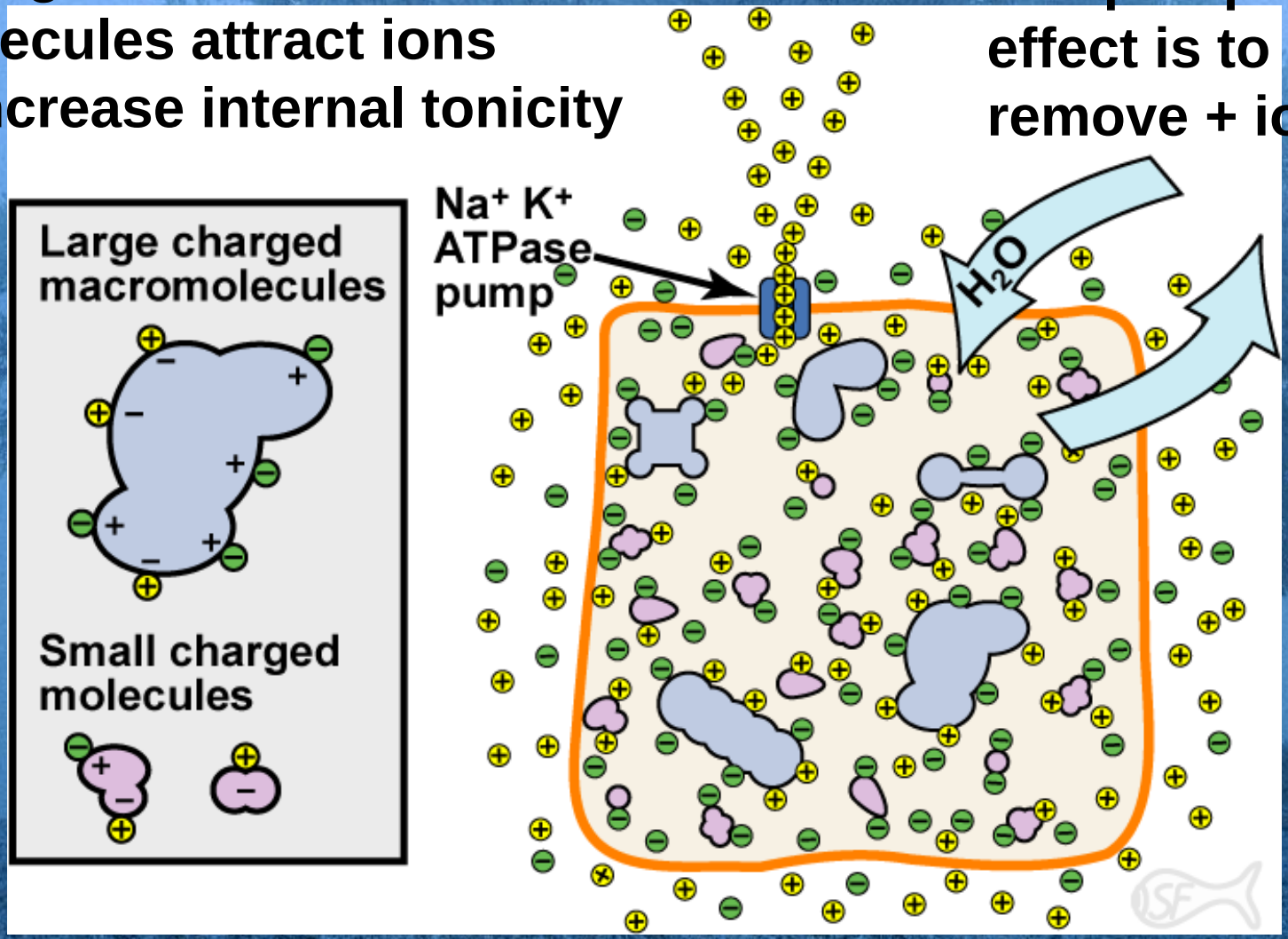


Na,K-ATPase is a receptor of digitalis and related cardiac glycosides used to strengthen the heartbeat

The Na^+- K^+ ATPase pump is responsible for maintaining cellular osmotic balance

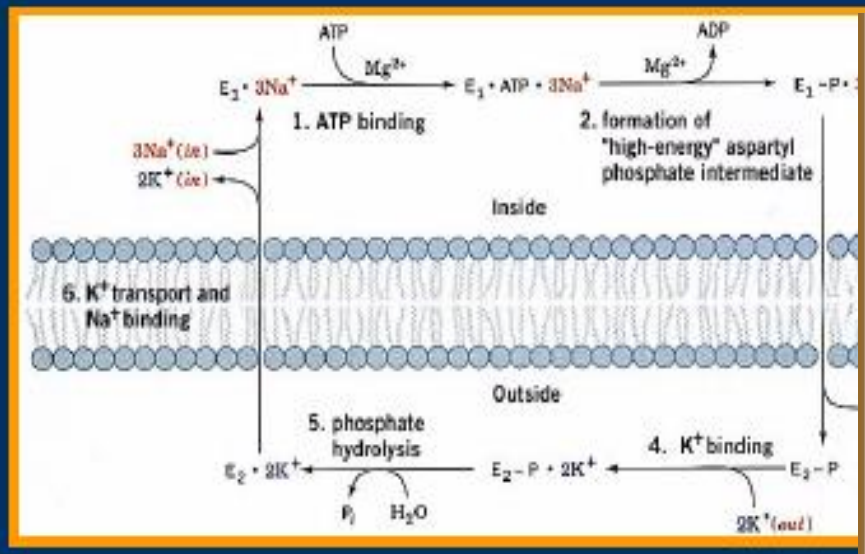
Charged intracellular molecules attract ions & increase internal tonicity

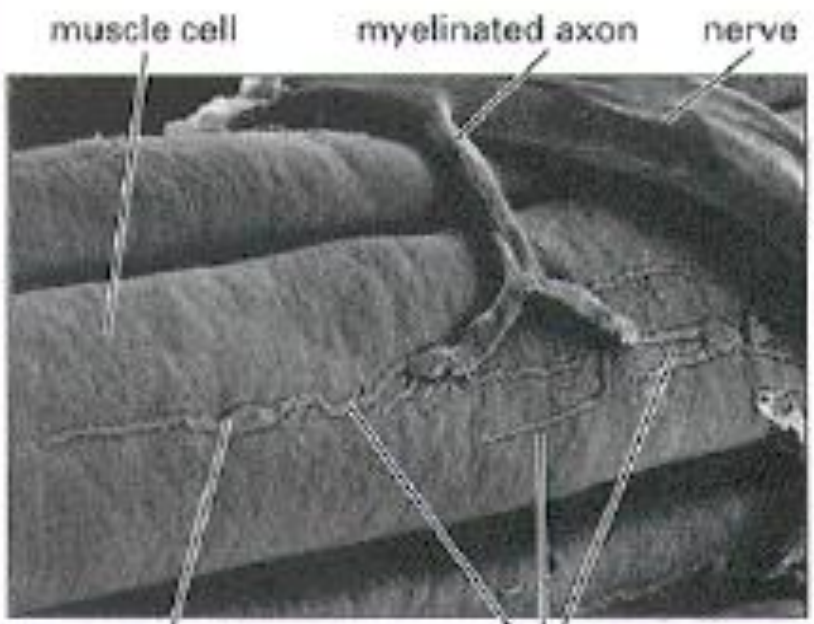
The pump's net effect is to remove + ions



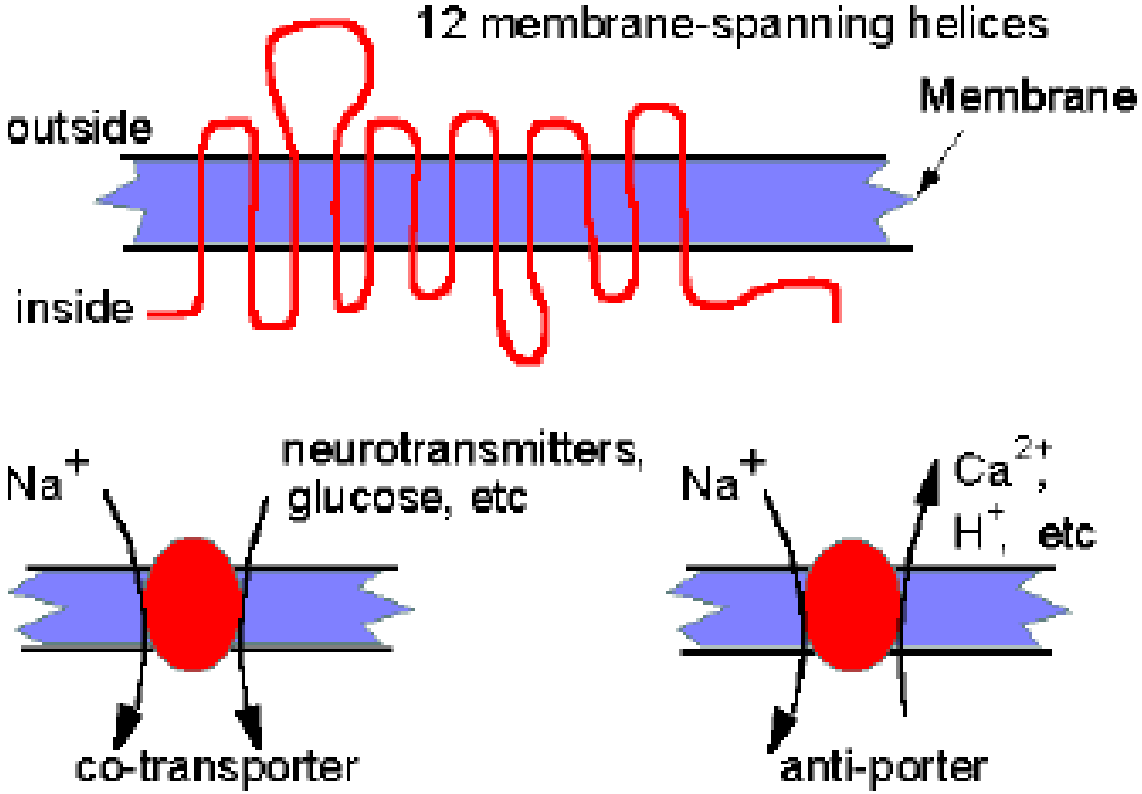
Na^+,K^+ -ATPase

- maintains uneven distribution of Na^+ and K^+ ions across cell membrane by transporting $3Na^+$ and $2K^+$ per each ATP hydrolyzed
- during the transport cycle the ions become "occluded"
- cycles between two major conformational states E_1 which has high affinity for Na^+ and ATP and E_2, which has high affinity for K^+-ions
- can be specifically inactivated by ouabain

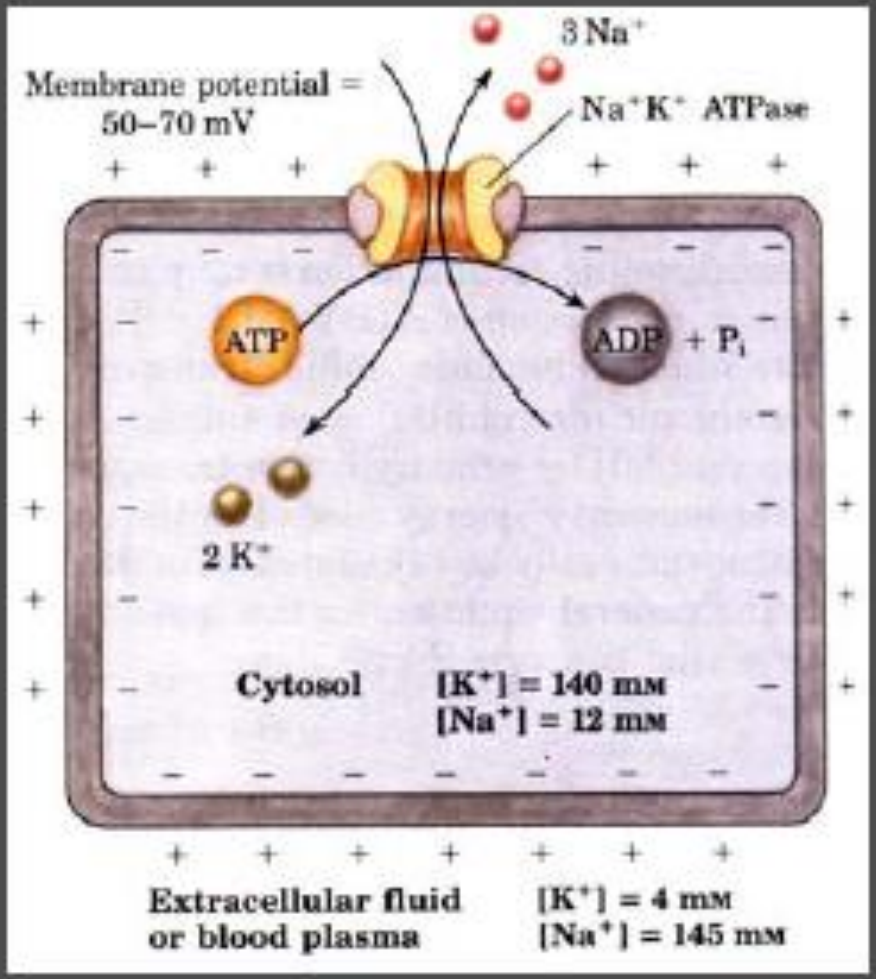


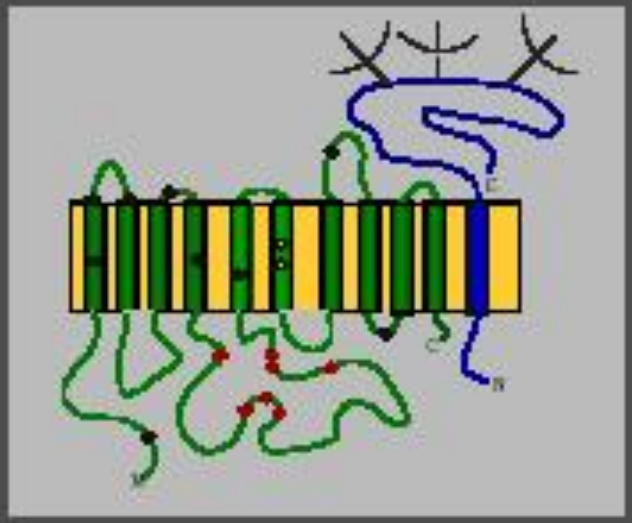


Sodium-coupled Transporters



In a cell Na^+,K^+-ATPase generates unequal distribution of Na^+ and K^+ across the membrane





- Composed of two subunits: the catalytic α-subunit and β-subunit which is required for proper folding of the α-subunit and for modulation of K binding and occlusion
- α-subunit contains two major "domains": the ATP-binding domain and the membrane portion that forms the cation-translocation pathway
- the ATP-binding domain is directly connected to transmembrane segments providing direct structural link between two functional domains and "coupling" transport and ATP-hydrolysis

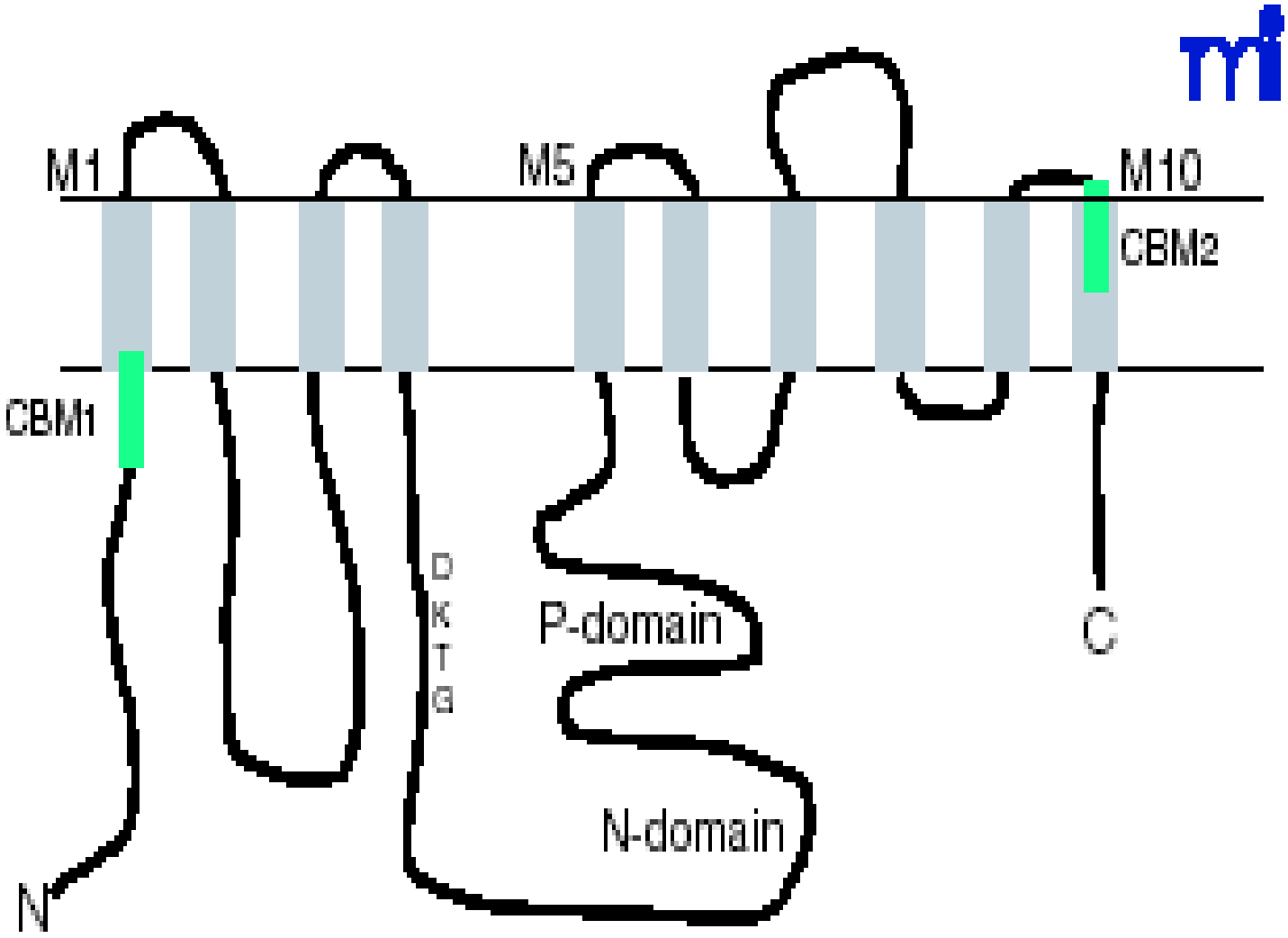
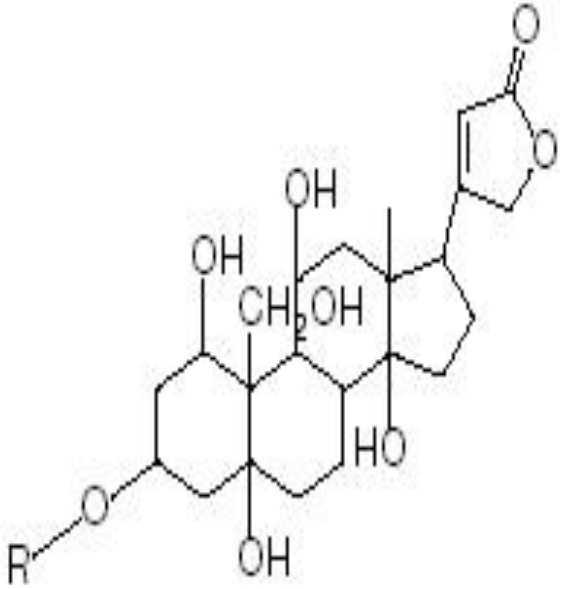


Figure 1. Schematic presentation of the α_1 subunit of Na^+-K^+–ATPase. The ten transmembrane domains are marked as M1 to M10. The third intracellular loop, connecting the M4 and M5, contains both P (phosphorylation) domain and N (nucleotide-binding) domain. The green bars indicate caveolin-binding motifs (CBMs).

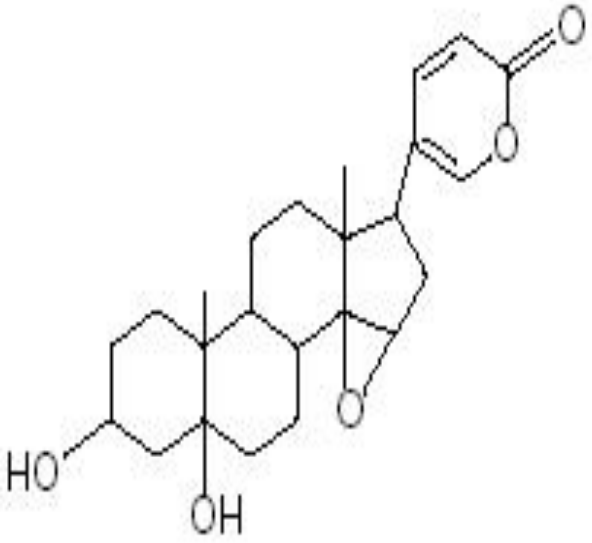
Digitalis purpurea

Bufo marinus

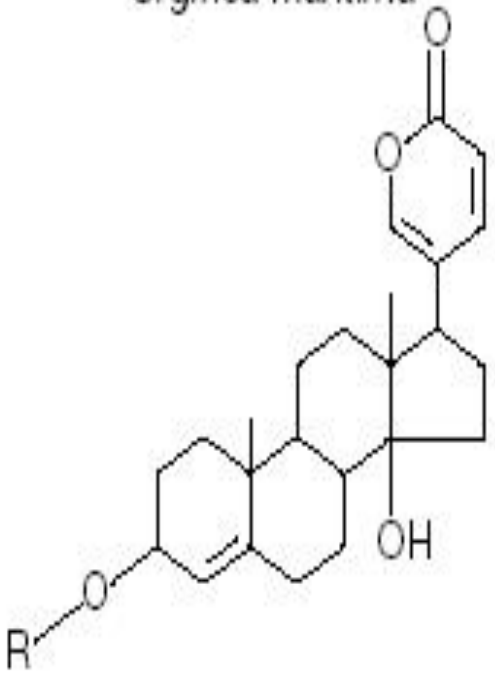
Urginea maritima



Ouabain



Marinobufagenin



Proscillaridin A

ENDOGENOUS DIGITALIS:

Ringer – 1902
Szent-Gyorgyi – 1953
De Wardener - 1980

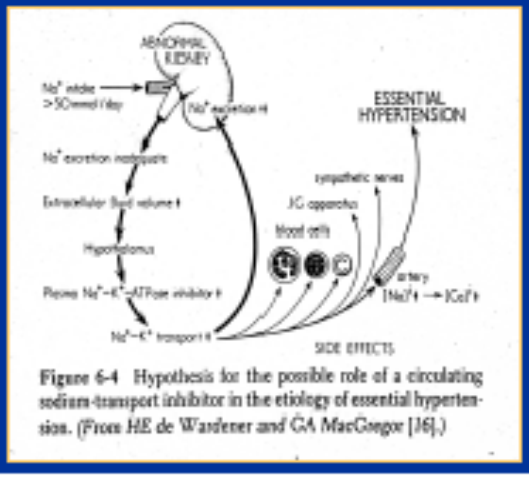
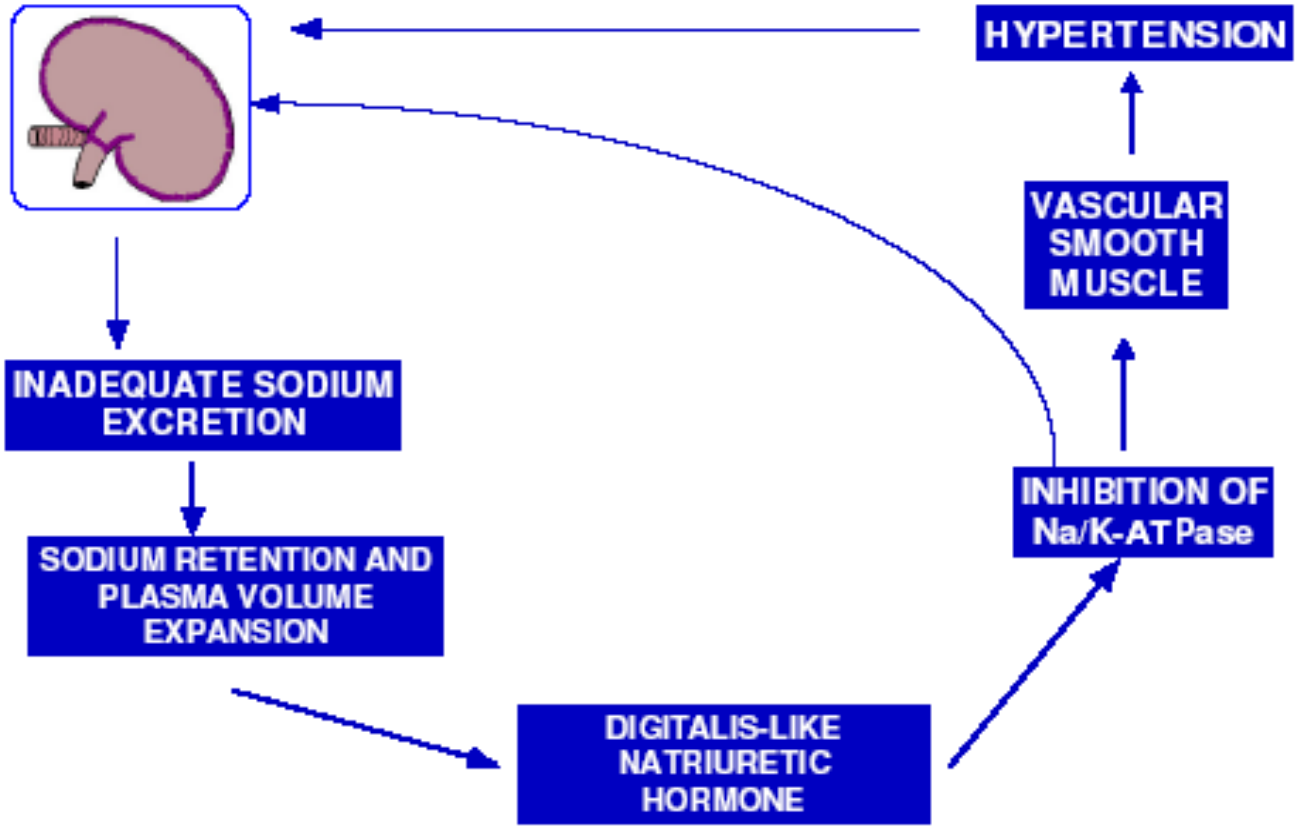
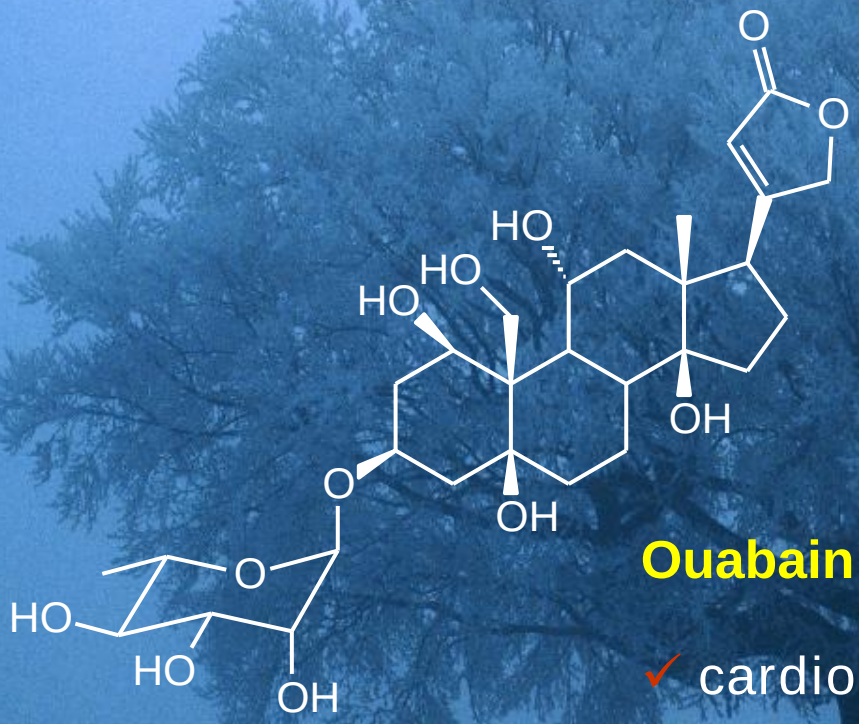


Figure 6-4 Hypothesis for the possible role of a circulating sodium-transport inhibitor in the etiology of essential hypertension. (From HE de Wardener and GA MacGregor [16].)



Ouabain

Structural formula of ouabain



Ouabain:

- ✓ cardiotonic steroid (analogue of digitalis)
- ✓ cardenolides

Nephrol Dial Transplant (2008) 1 of 7
doi: 10.1093/ndt/gfn325

NDT
Nephrology Dialysis Transplantation

Editorial Comment

Role of endogenous cardiotonic steroids in sodium homeostasis

Wilhelm Schoner and Georgios Scheiner-Bobis

Institute of Biochemistry and Endocrinology, Justus-Liebig-University Giessen, Frankfurter Str. 100, D-35392 Giessen, Germany

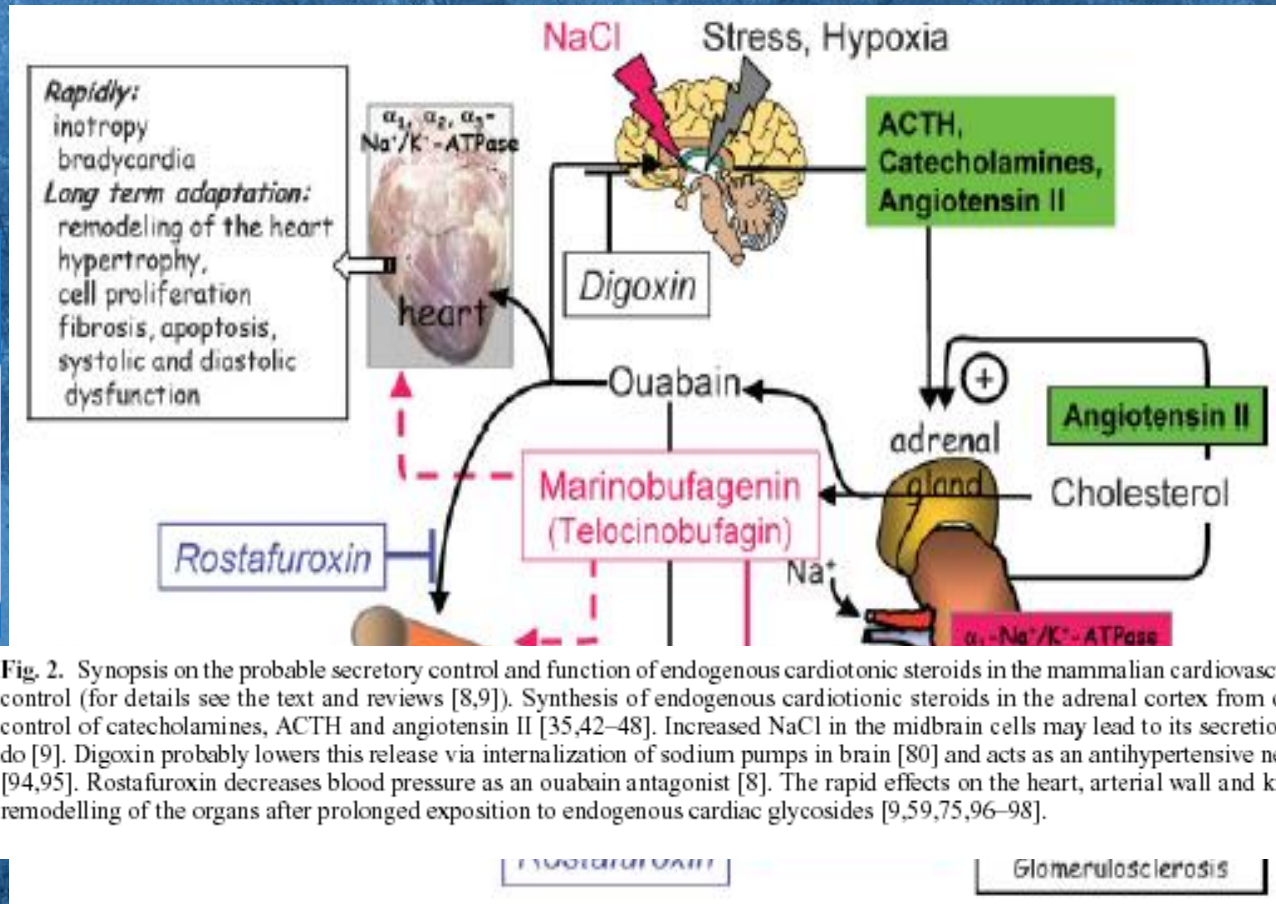
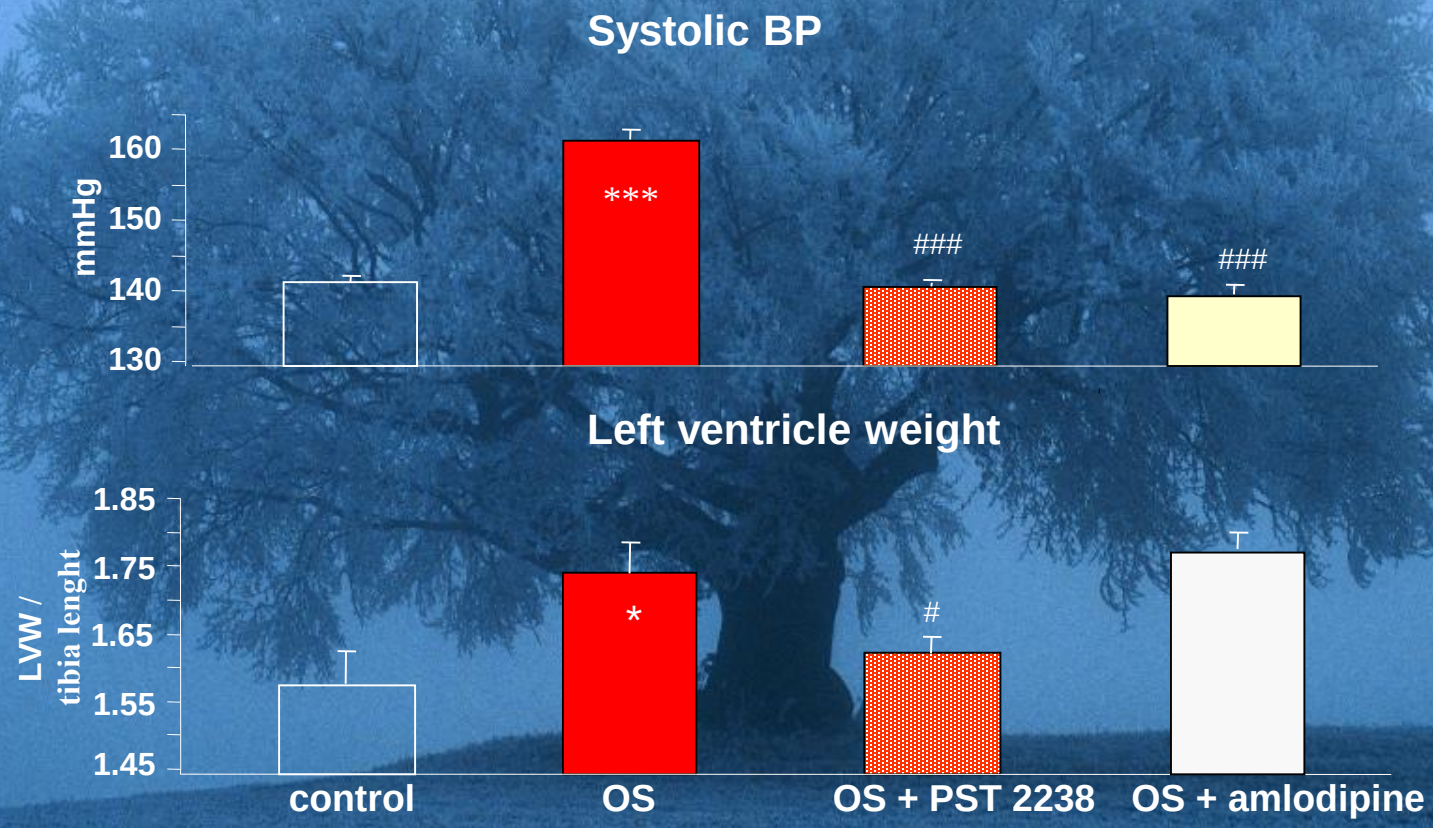


Fig. 2. Synopsis on the probable secretory control and function of endogenous cardiotonic steroids in the mammalian cardiovascular system and volume control (for details see the text and reviews [8,9]). Synthesis of endogenous cardiotonic steroids in the adrenal cortex from cholesterol is under the control of catecholamines, ACTH and angiotensin II [35,42–48]. Increased NaCl in the midbrain cells may lead to its secretion as ouabain and stress do [9]. Digoxin probably lowers this release via internalization of sodium pumps in brain [80] and acts as an antihypertensive neurohormonal regulator [94,95]. Rostafuroxin decreases blood pressure as an ouabain antagonist [8]. The rapid effects on the heart, arterial wall and kidney are followed by a remodelling of the organs after prolonged exposition to endogenous cardiac glycosides [9,59,75,96–98].

Ouabain Infusion in rats (15 mg/kg/day)

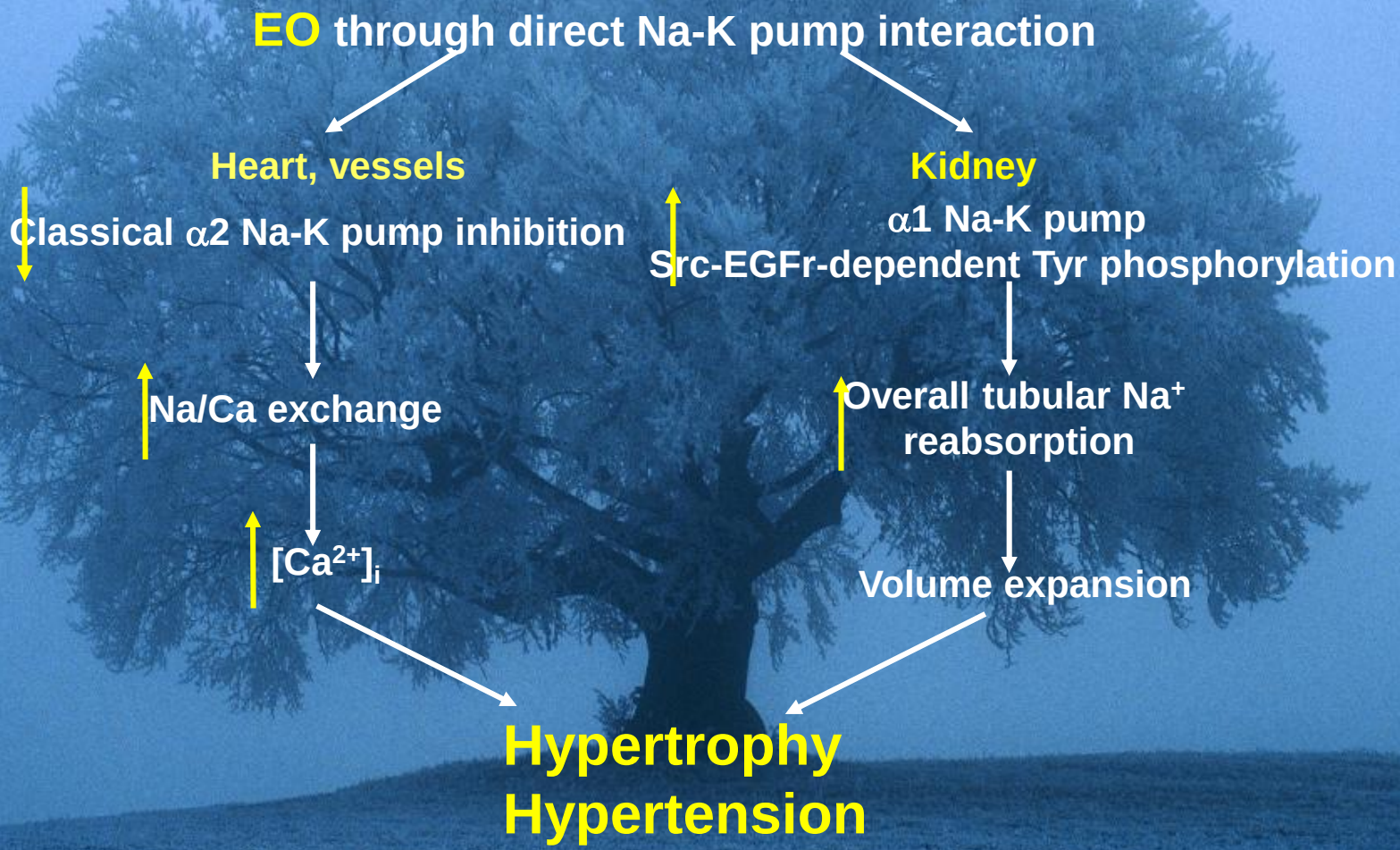
Plus oral treatment with PST 2238 (10 mg/kg) or amlodipine (5 mg/kg)



*p<0.05, **p<0.01 vs control; #p<0.05, ###p<0.001 vs ouabain

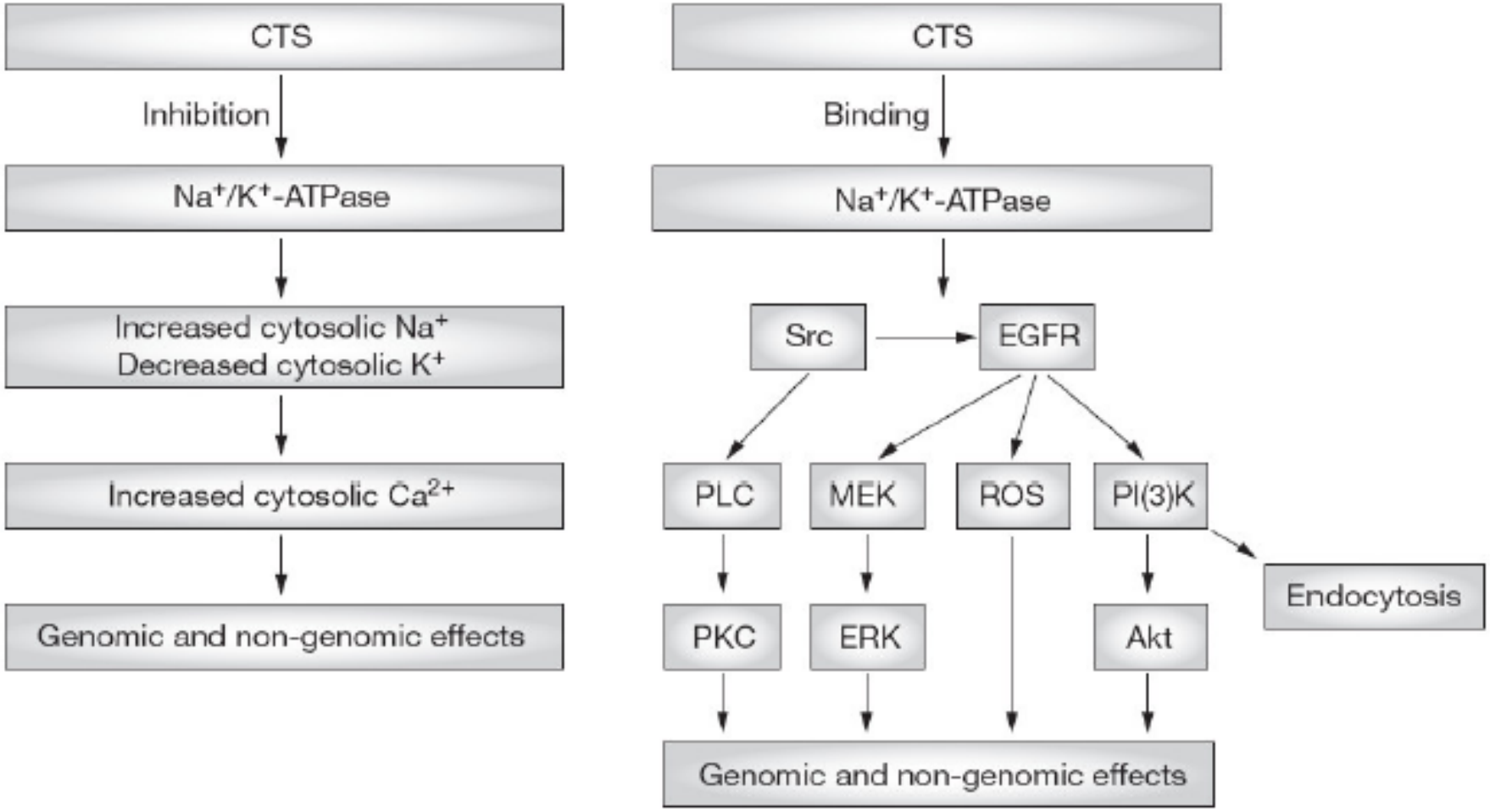
Ouabain

Modulator of the Na^+- K^+ pump

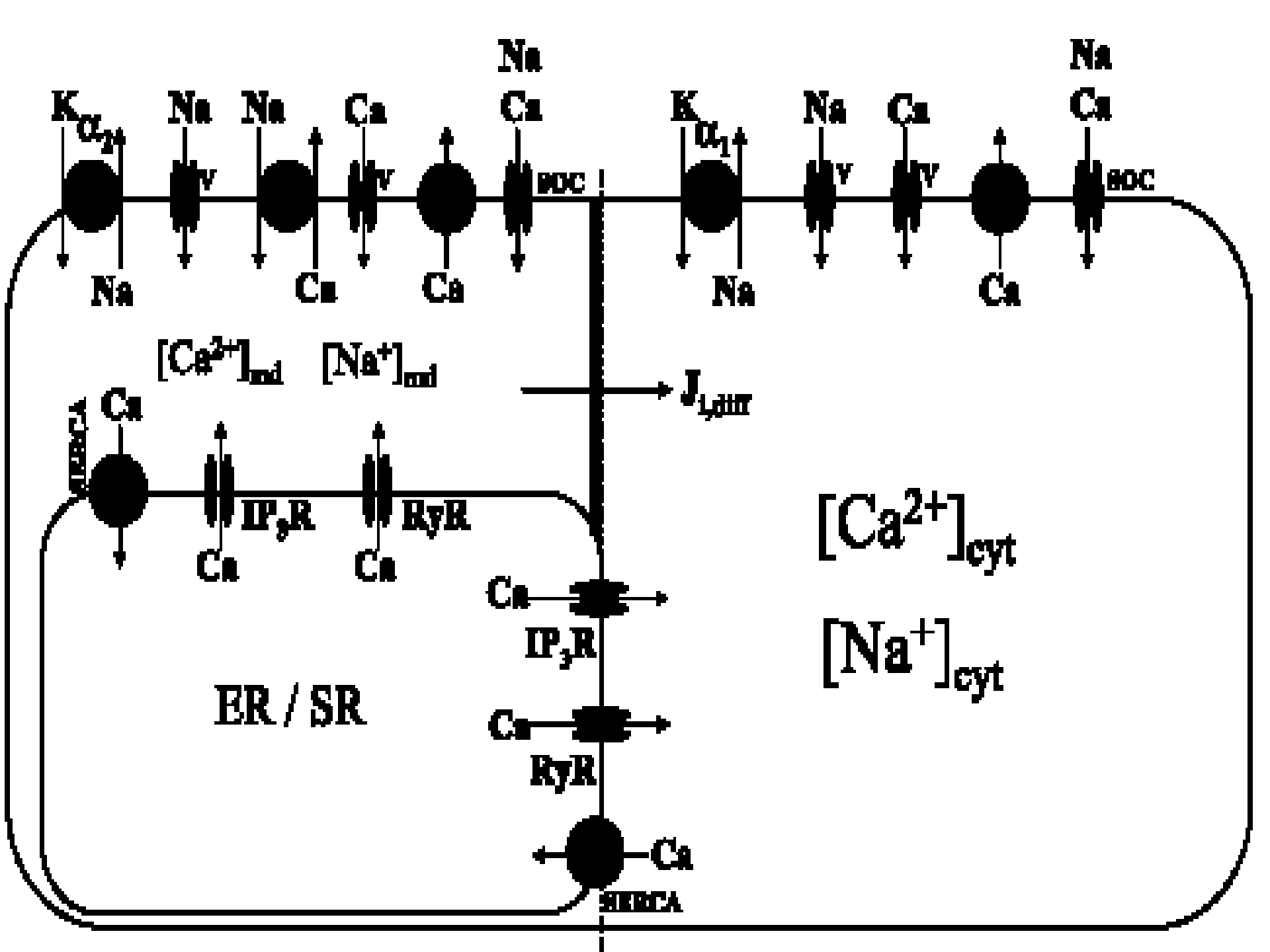


Ferrari P *et al*., *Am J Physiology* 2006;290:R529-R535

Classical vs. novel functions of Na/K-ATPase and of endogenous cardiotonic steroids



Bagrov, Shapiro, Nat Clin Pract Nephrol, 2008


Na
Na
Na
K
Na
Ca
Na
Ca
Na
Ca
Na
Ca
Na
Ca
Ca
Ca
Na
Ca
SOC
SOC
$[Ca^{2+}]_{mid}$
$[Na^+]_{mid}$
$J_{i,diff}$
SERCA
Ca
IP_3R
RyR
Ca
Ca
$[Ca^{2+}]_{cyt}$
Ca
IP_3R
$[Na^+]_{cyt}$
ER / SR
Ca
RyR
Ca
SERCA

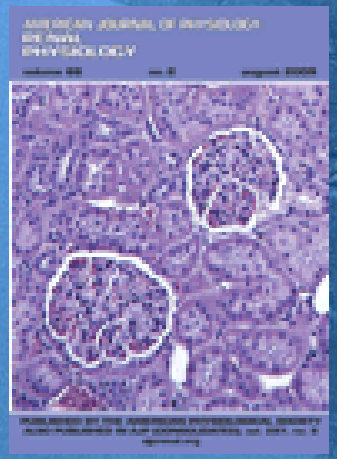
Am J Physiol Renal Physiol 293: F1518–F1532, 2007.
First published August 1, 2007; doi:10.1152/ajprenal.00251.2007.

Ouabain modulation of cellular calcium stores and signaling

Aurélie Edwards[1] and Thomas L. Pallone[2]

[1]*Department of Chemical and Biological Engineering, Tufts University, Medford, Massachusetts; and* [2]*Departments of Medicine and Physiology, University of Maryland School of Medicine, Baltimore, Maryland*

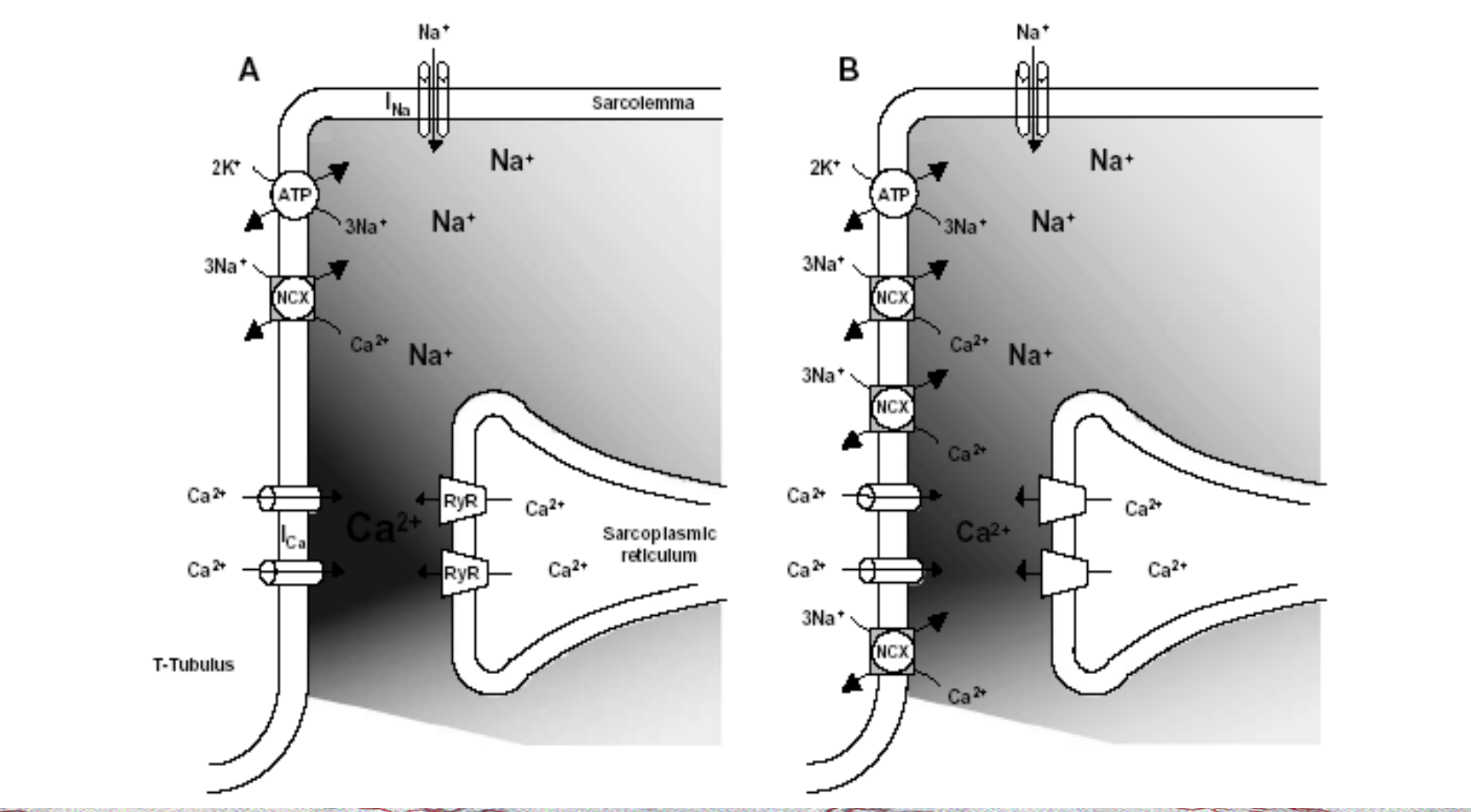
Submitted 30 May 2007; accepted in final form 27 July 2007

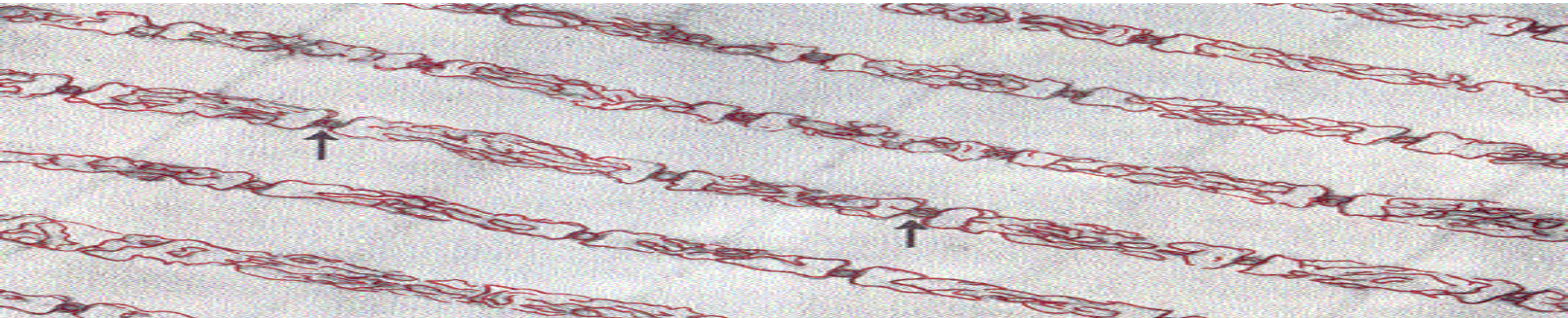


$$J_{i,\mathrm{diff}} = AP_{i,\mathrm{diff}}\xi_i\left(\frac{[i]_{\mathrm{md}} - [i]_{\mathrm{cyt}}\exp(-\xi_i)}{1-\exp(-\xi_i)}\right) \qquad i = \mathrm{K}^+,\mathrm{Na}^+,\mathrm{Ca}^{2+} \qquad (16)$$

$$\xi_i = \frac{z_i F}{RT}\left(V_{\mathrm{m}}^{\mathrm{md}} - V_{\mathrm{m}}^{\mathrm{cyt}}\right) \qquad (17)$$

$$P_{i,\mathrm{diff}} = \frac{hD_i}{L} \qquad (18)$$


A
B
Na+
I_{Na}
Sarcolemma
2K+
ATP
3Na+
NCX
Ca2+
I_{Ca}
RyR
Sarcoplasmic reticulum
T-Tubulus



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Ouabain modulation of cellular calcium stores and signaling

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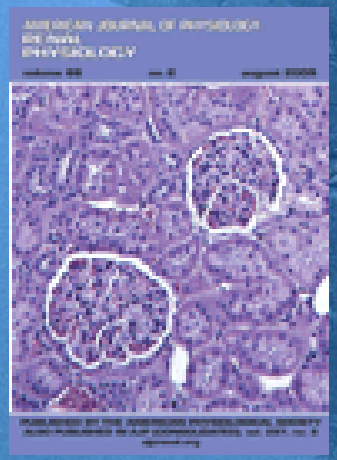

Motivated by the importance of ouabain and OLF in modulation of myocyte contractility and generation of hypertension, we simulated the pathways through which ouabain affects Ca^{2+} signaling in cells: *1*) inhibition of active transport by Na^+-K^+-ATPase α_1- and α_2-isoforms; *2*) activation of PLC-γ_1, leading to IP_3 production; and *3*) stimulation of tyrosine phosphorylation of IP_3R, leading to an increase in its conductance.

REVIEWS

PHYSIOLOGY 23: 205–211, 2008; doi:10.1152/physiol.00008.2008

The Na-K-ATPase and Calcium-Signaling Microdomains

Jiang Tian and Zi-jian Xie

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zi-jian.xie@utoledo.edu

The Na-K-ATPase is an energy-transducing ion pump that converts the free energy of ATP into transmembrane ion gradients. It also serves as a functional receptor for cardiotonic steroids such as ouabain and digoxin. Binding of ouabain to the Na-K-ATPase can activate calcium signaling in a cell-specific manner. The exquisite calcium modulation via the Na-K-ATPase is achieved by the ability of the pump to integrate signals from numerous protein and non-protein molecules, including ion transporters, channels, protein kinases/phosphatases, as well as cellular Na^+. This review focuses on the unique properties of the Na-K-ATPase and its role in the formation of different calcium-signaling microdomains.

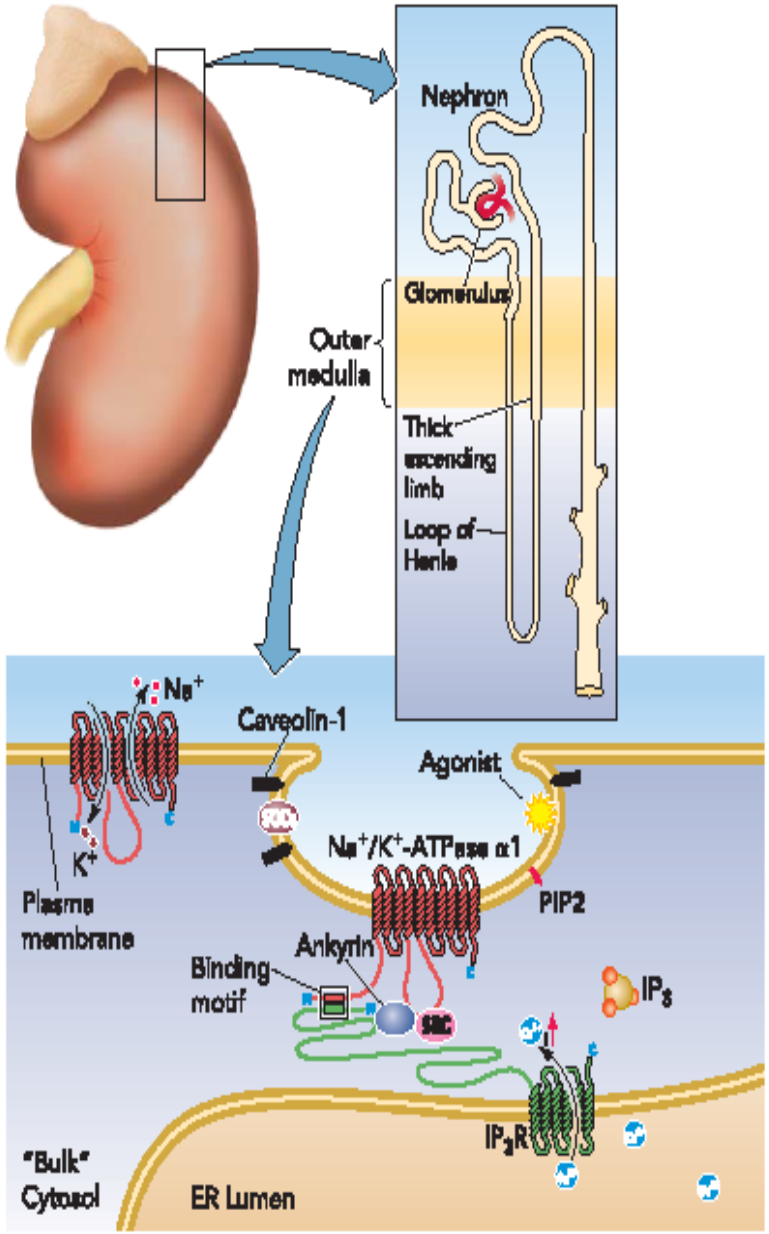

FIGURE 1. The potential role of Na-K-ATPase in the formation of a junctional calcium-signaling microdomain in renal epithelial cells

PHYSIOLOGY 23: 205–211, 2008; doi:10.1152/physiol.00008.2008

The Na-K-ATPase and Calcium-Signaling Microdomains

Jiang Tian and Zi-jian Xie
Department of Physiology and Pharmacology, University of Toledo Health Science Campus, Toledo, Ohio
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"In addition to its effect on calcium entry through NCX, early studies suggested that ouabain and other cardiotonic steroids might activate L-type Ca^{2+} channels and stimulate Ca^{2+} release from SR and/or ER."

Invited Review

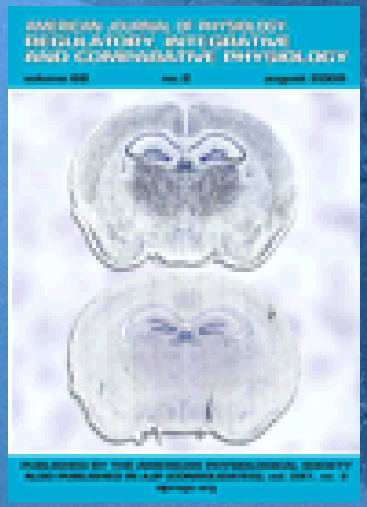
Am J Physiol Regul Integr Comp Physiol 290: R514–R523, 2006;
doi:10.1152/ajpregu.00819.2005.

CALL FOR PAPERS | *Molecular Mechanisms Linking Salt to Hypertension*

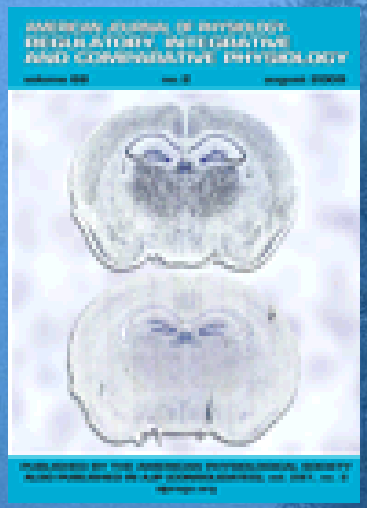
How does salt retention raise blood pressure?

Mordecai P. Blaustein,[1,2] Jin Zhang,[1] Ling Chen,[3] and Bruce P. Hamilton[3,4]

Departments of [1]Physiology and [2]Medicine and [3]Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Maryland School of Medicine; and [4]Division of Endocrinology, Department of Medicine, Baltimore Veterans Affairs Medical Center and University of Maryland School of Medicine, Baltimore, Maryland



proposal was that, by reducing Na^+ pumping, the inhibitor might depolarize vascular smooth muscle myocytes directly because Na^+ pumps are electrogenic and make a small contribution to the membrane potential (39). The depolarization should activate Ca^{2+} entry (presumably via voltage-gated Ca^{2+} channels), which would be expected to augment vasoconstriction. Such an effect can be true only transiently, how-



Etiology:

↑ NaCl Intake
or ↓ Renal Na^+ Excretion or ↑ Na^+ Re-absorption

↓

Na(Cl) and Water Retention

↓

↑Plasma Volume

Interventions:

ACTH → ↑Plasma Endogenous Ouabain (EO)

Exogenous Ouabain →

Ouabain Antagonists - - - →

"Digibind" - - - →

Na^+ Pump $\alpha2^{R/R}$ - - - →

Na^+ Pump $\alpha2^{+/-}$ → ↓Na^+ Pump $\alpha2$ Activity

↓

↑$[Na^+]_{CYT}$

NCX Inhibitors - - - →

$mNCX1.3^{Ts/Ts}$ - - - →

$NCX1^{+/-}$ - - - →

$NCX1.3^{Ts/Ts}$ → Na/Ca Exchange (↑Ca^{2+} Entry)

↓

↑$[Ca^{2+}]_{CYT}$

↓

↑ Ca^{2+} Signaling

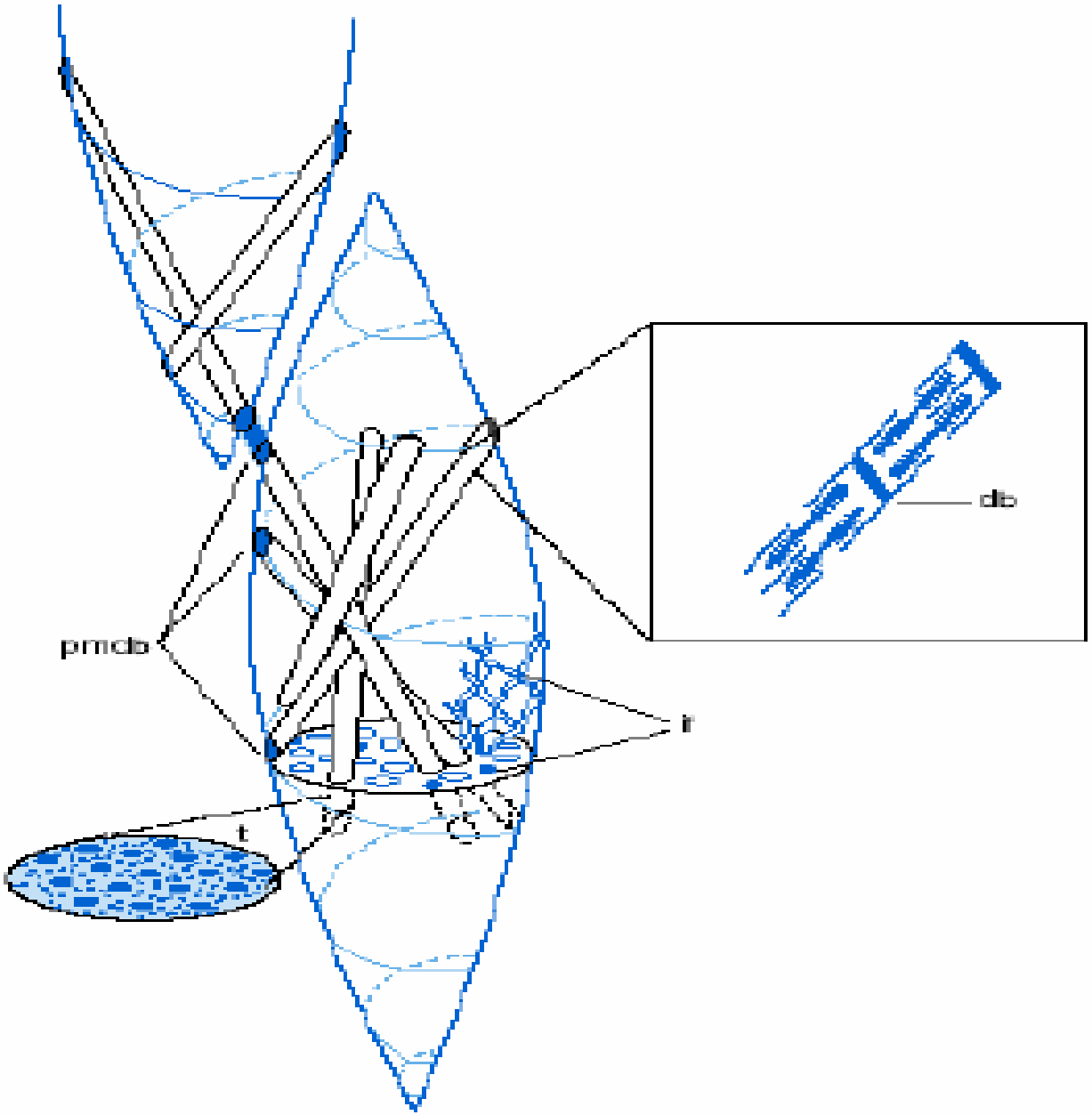
↓

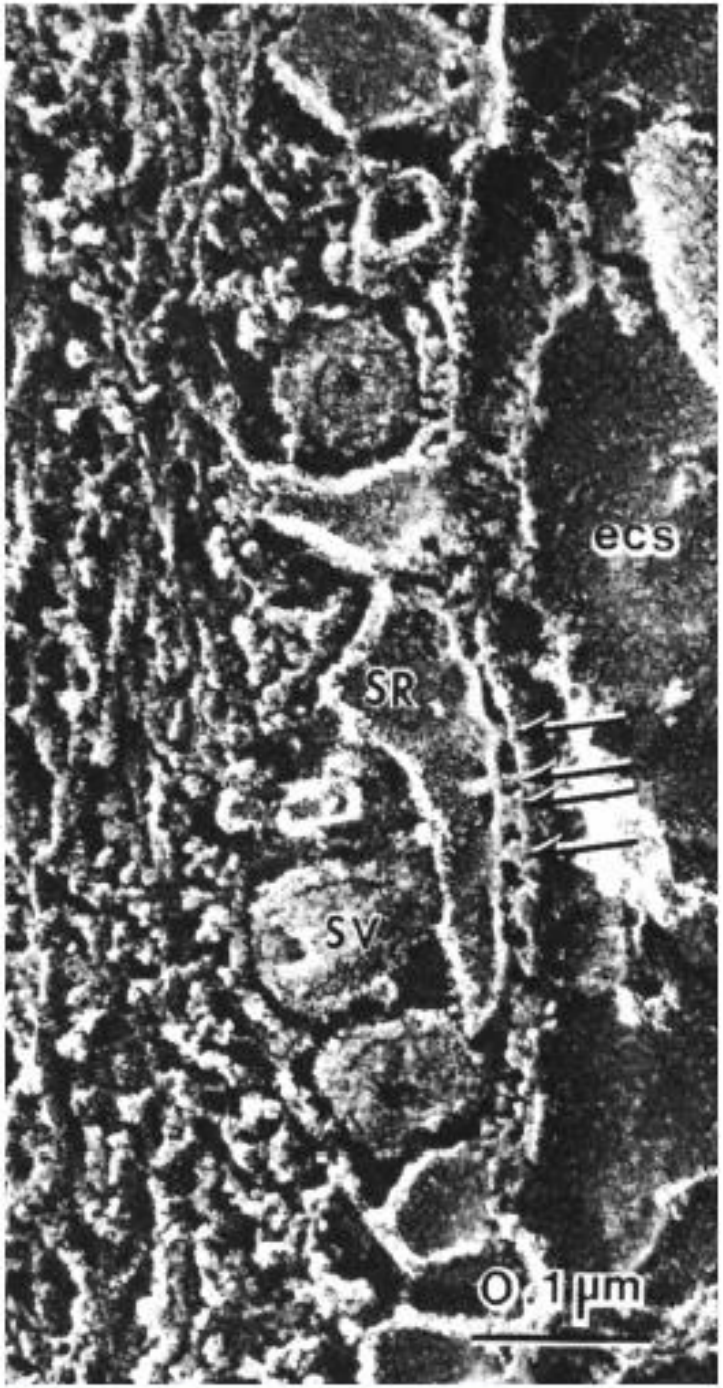
↑Vascular Tone and Contractility

↓

↑ Blood Pressure

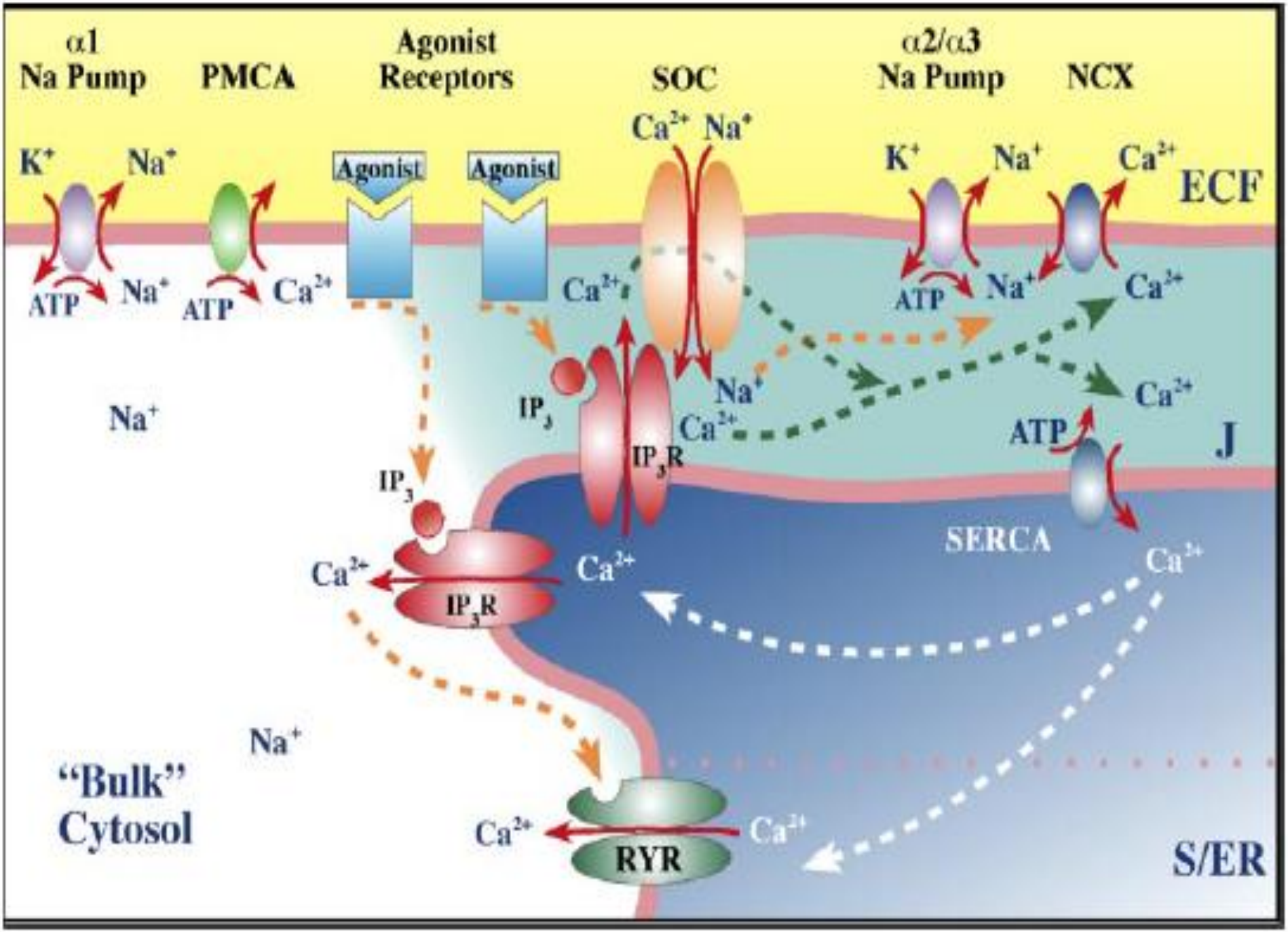
Structure of Smooth Muscle

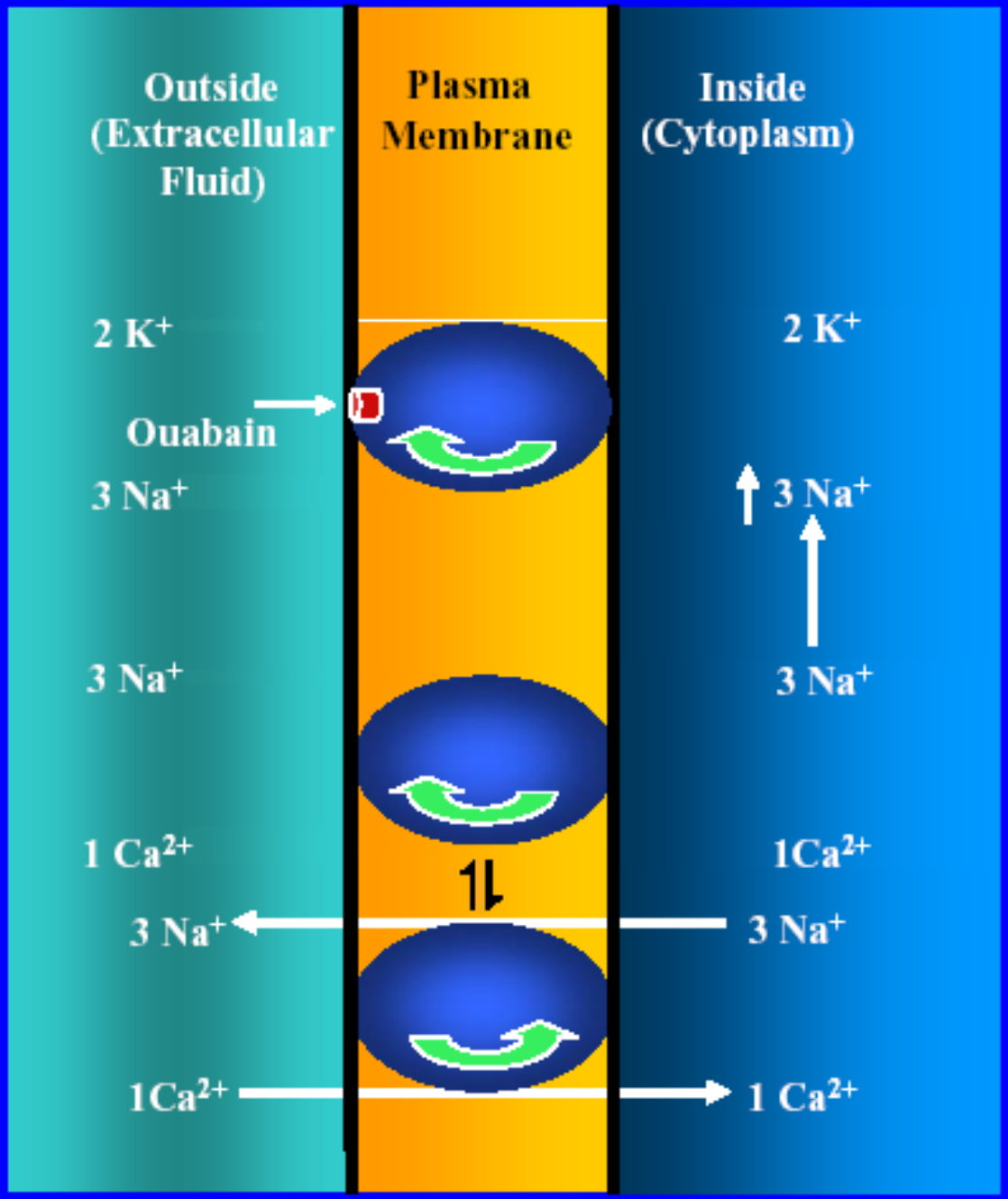




Electron micrograph of frozen-etched rabbit venous smooth muscle cell.
ecs = extracellular space; arrows point to plasma membrane; SR = sub-plasma membrane ("junctional") sarcoplasmic reticulum (jSR).

A.V. Somlyo & C. Franzini-Armstrong, *Experientia* (1985)


α1
Na Pump
PMCA
Agonist
Receptors
SOC
α2/α3
Na Pump
NCX
K+
Na+
ATP
Na+
ATP
Ca2+
Agonist
Agonist
Ca2+
Na+
K+
Na+
Ca2+
ECF
ATP
Na+
Ca2+
Na+
IP3
Ca2+
IP3R
Ca2+
ATP
J
IP3
SERCA
Ca2+
Ca2+
IP3R
Ca2+
Na+
"Bulk"
Cytosol
Ca2+
Ca2+
RYR
S/ER

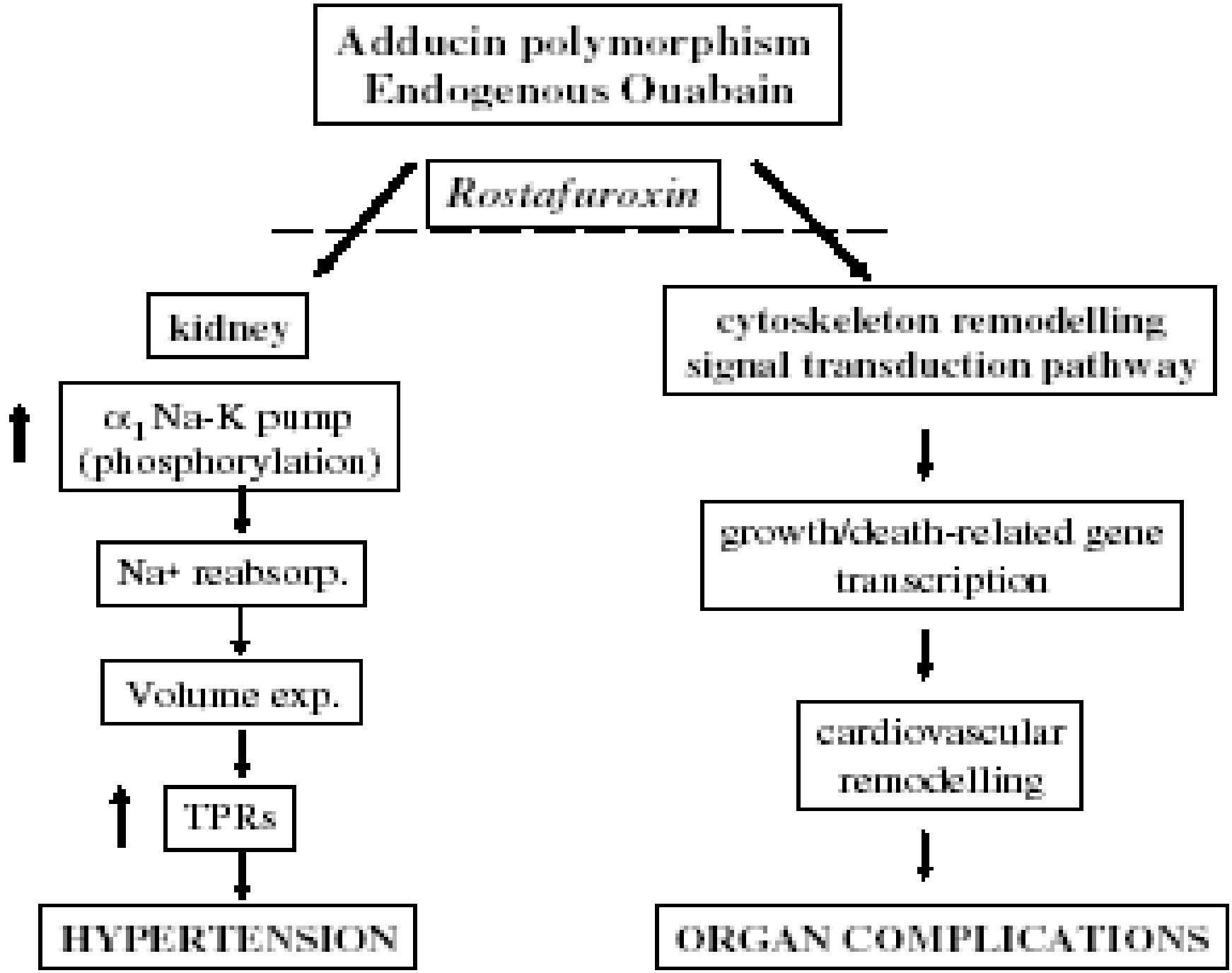


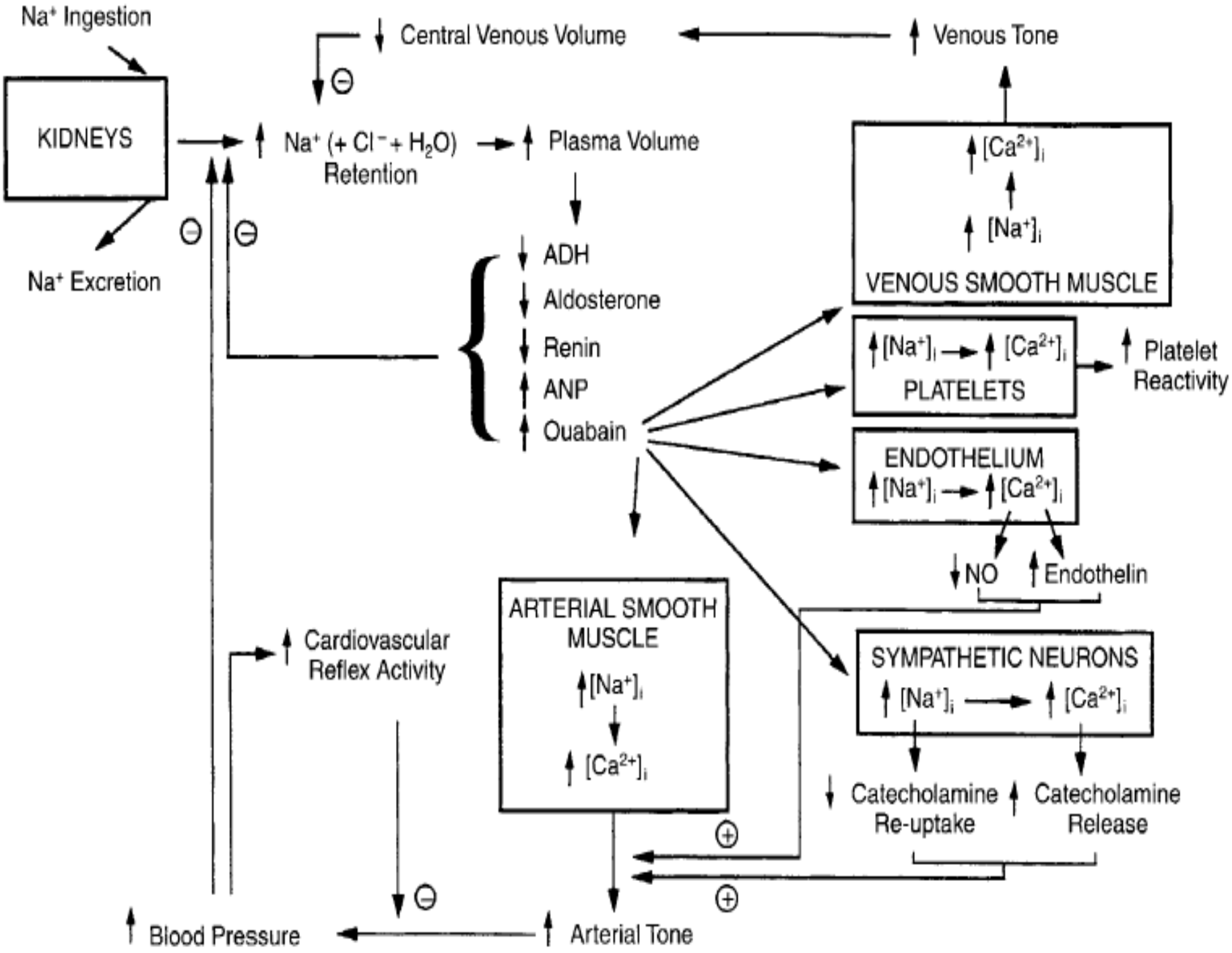
Sodium Pump

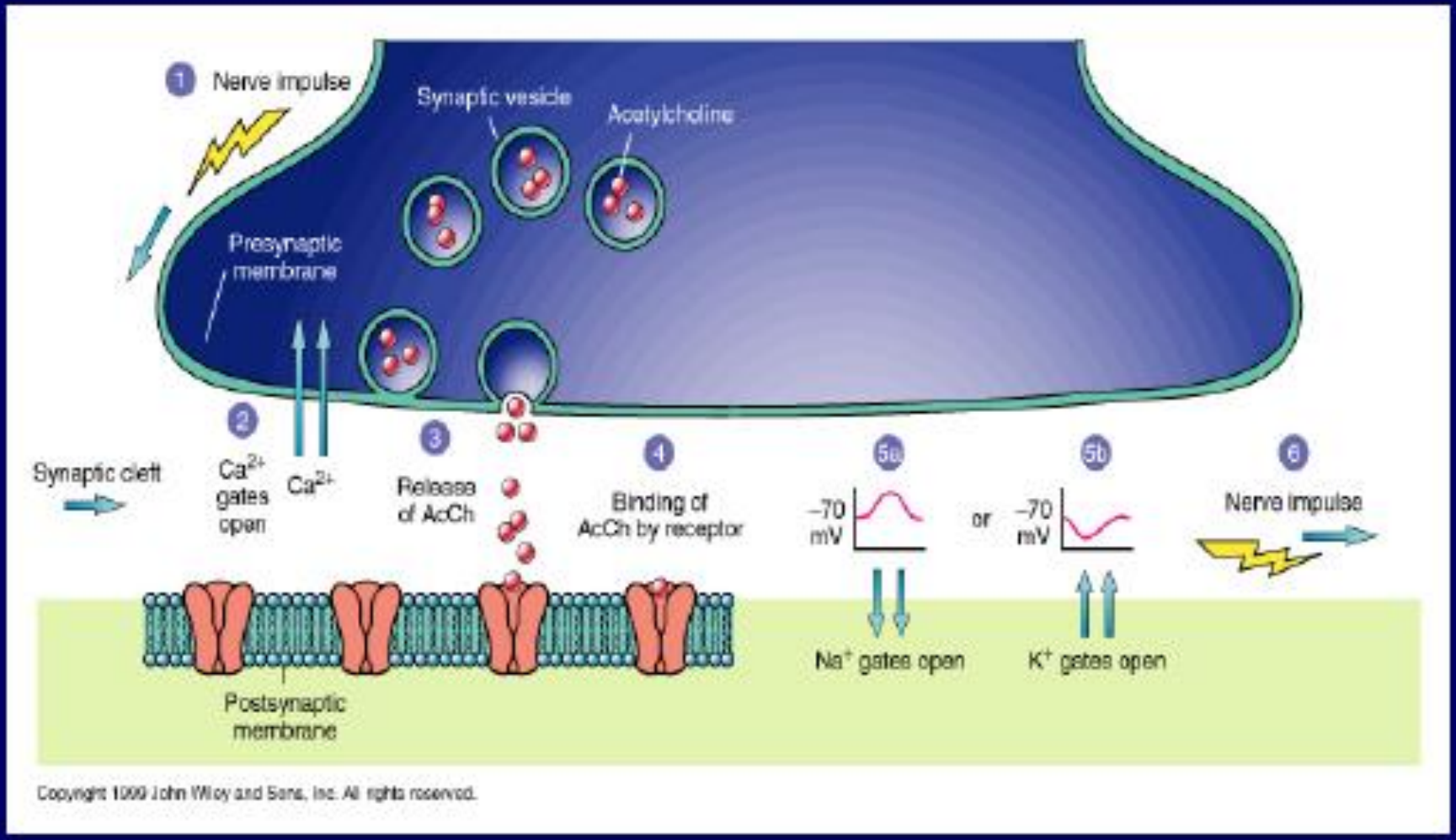
Sodium/Calcium Exchanger

Ca^{2+} efflux mode

Ca^{2+} influx mode


Adducin polymorphism
Endogenous Ouabain
Rostafuroxin
kidney
α_1 Na-K pump (phosphorylation)
Na+ reabsorp.
Volume exp.
TPRs
HYPERTENSION
cytoskeleton remodelling signal transduction pathway
growth/death-related gene transcription
cardiovascular remodelling
ORGAN COMPLICATIONS


Na+ Ingestion
KIDNEYS
Na+ Excretion
Central Venous Volume
Venous Tone
Na+ (+ Cl− + H2O) Retention
Plasma Volume
ADH
Aldosterone
Renin
ANP
Ouabain
[Ca2+]i
[Na+]i
VENOUS SMOOTH MUSCLE
[Na+]i → [Ca2+]i
PLATELETS
Platelet Reactivity
ENDOTHELIUM
[Na+]i → [Ca2+]i
NO
Endothelin
ARTERIAL SMOOTH MUSCLE
[Na+]i
[Ca2+]i
SYMPATHETIC NEURONS
[Na+]i → [Ca2+]i
Catecholamine Re-uptake
Catecholamine Release
Cardiovascular Reflex Activity
Blood Pressure
Arterial Tone

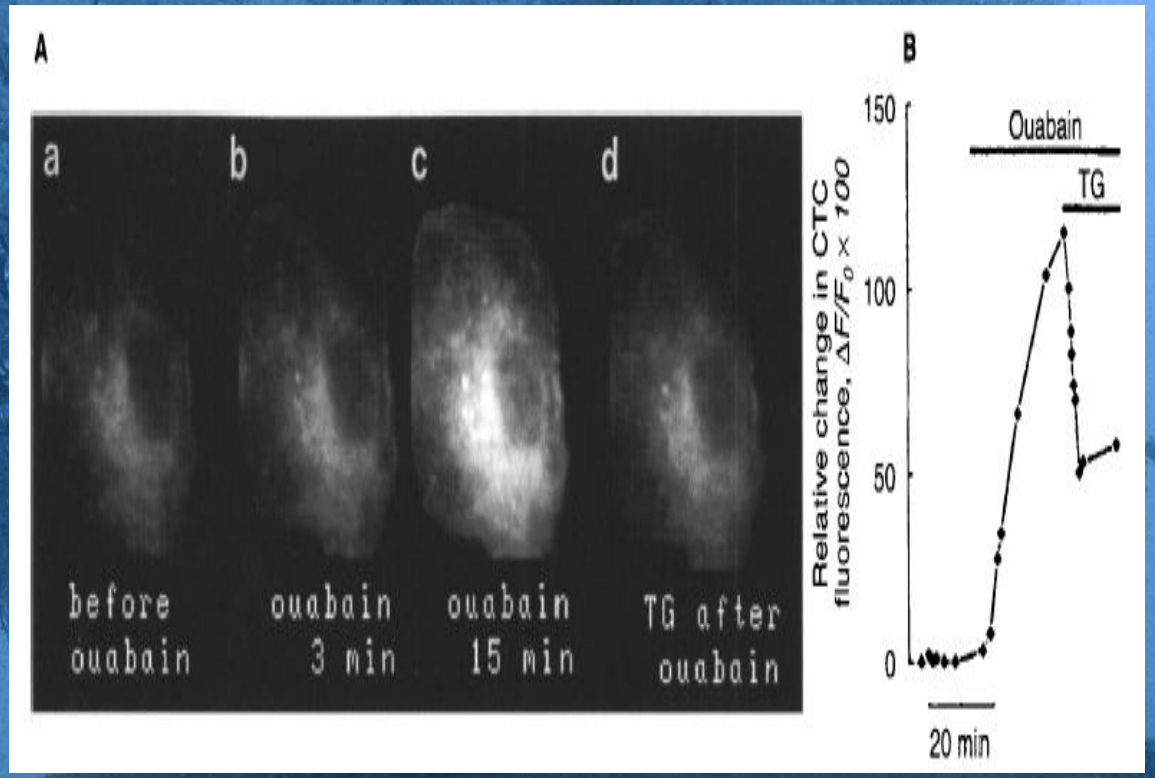


Kidney International, Vol. 49 (1996), pp. 1748–1753

Endogenous ouabain: Role in the pathogenesis of hypertension

MORDECAI P. BLAUSTEIN

Department of Physiology and the Center for Vascular Biology and Hypertension, University of Maryland School of Medicine, Baltimore, Maryland, USA

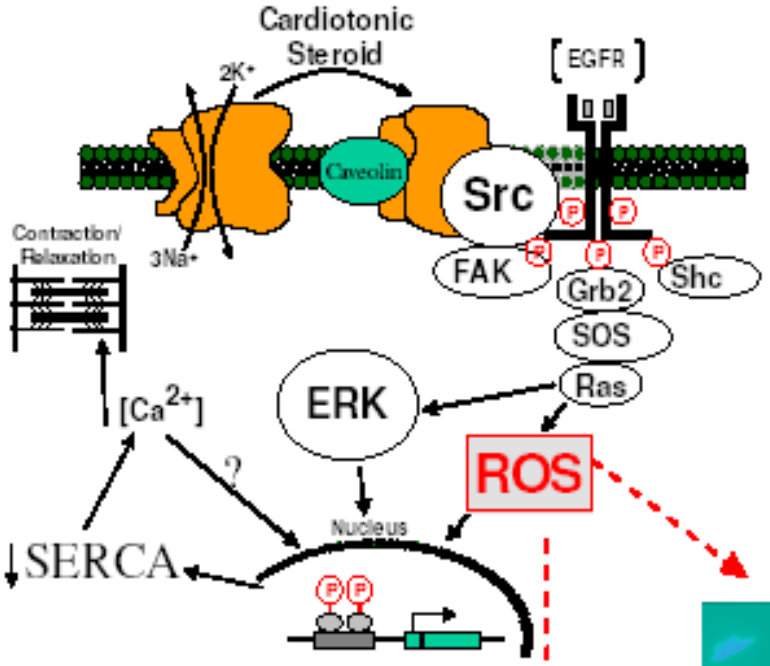


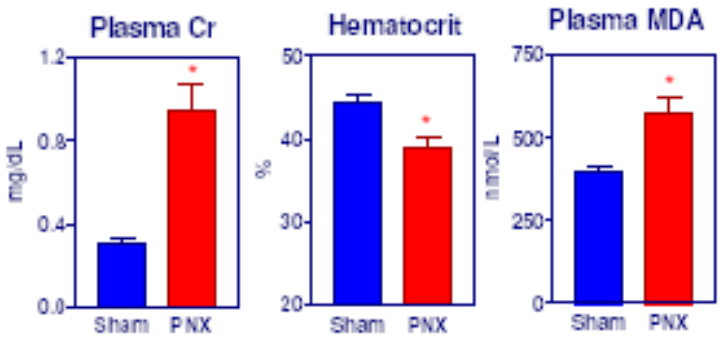
Uremic Cardiomyopathy—An Endogenous Digitalis Intoxication?

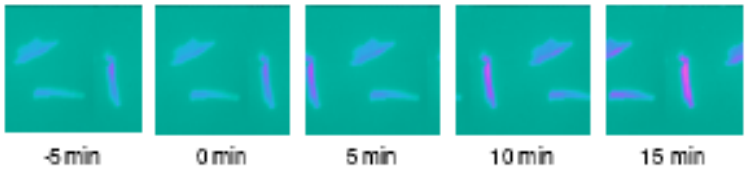
Central Role for the Cardiotonic Steroid Marinobufagenin in the Pathogenesis of Experimental Uremic Cardiomyopathy. ***Hypertension*** **47: 488–495, 2006**
Kennedy DJ, Vetteth S, Periyasamy SM, Kanj M, Fedorova L, Khouri S, Kahaleh MB, Xie Z, Malhotra D, Kolodkin NI, Lakatta EG, Fedorova OV, Bagrov AY, Shapiro JI

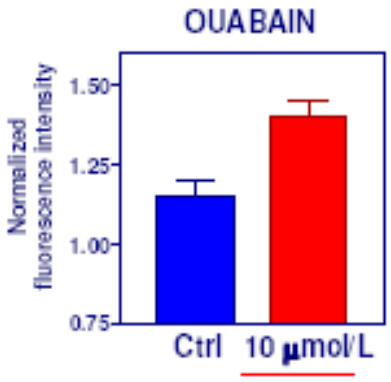
heart muscle, ouabain causes a Ca^{2+}-dependent early gene response (c-fos, c-jun), stimulates Ras and p42/44, and increases protein biosynthesis (24). In vascular smooth muscle cells, both ouabain and marinobufagenin also stimulate proliferation at concentrations lower than those normally required to alter intracellular ion concentrations (25).

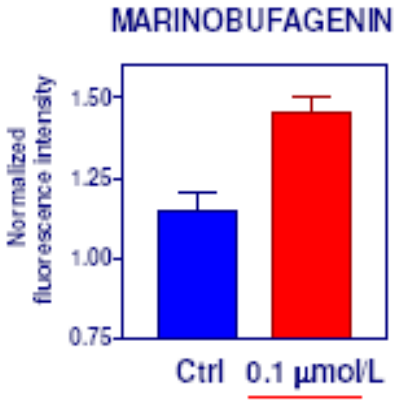
Uremic cardiomyopathy and oxidant stress

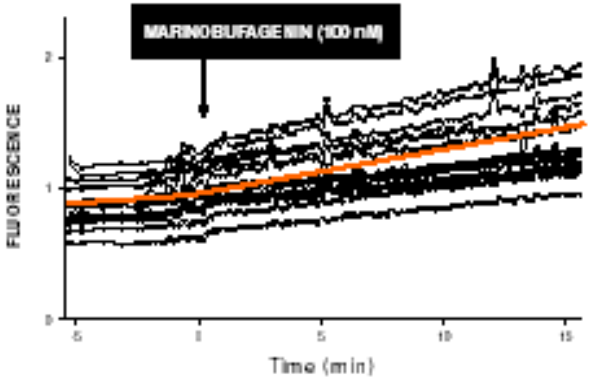




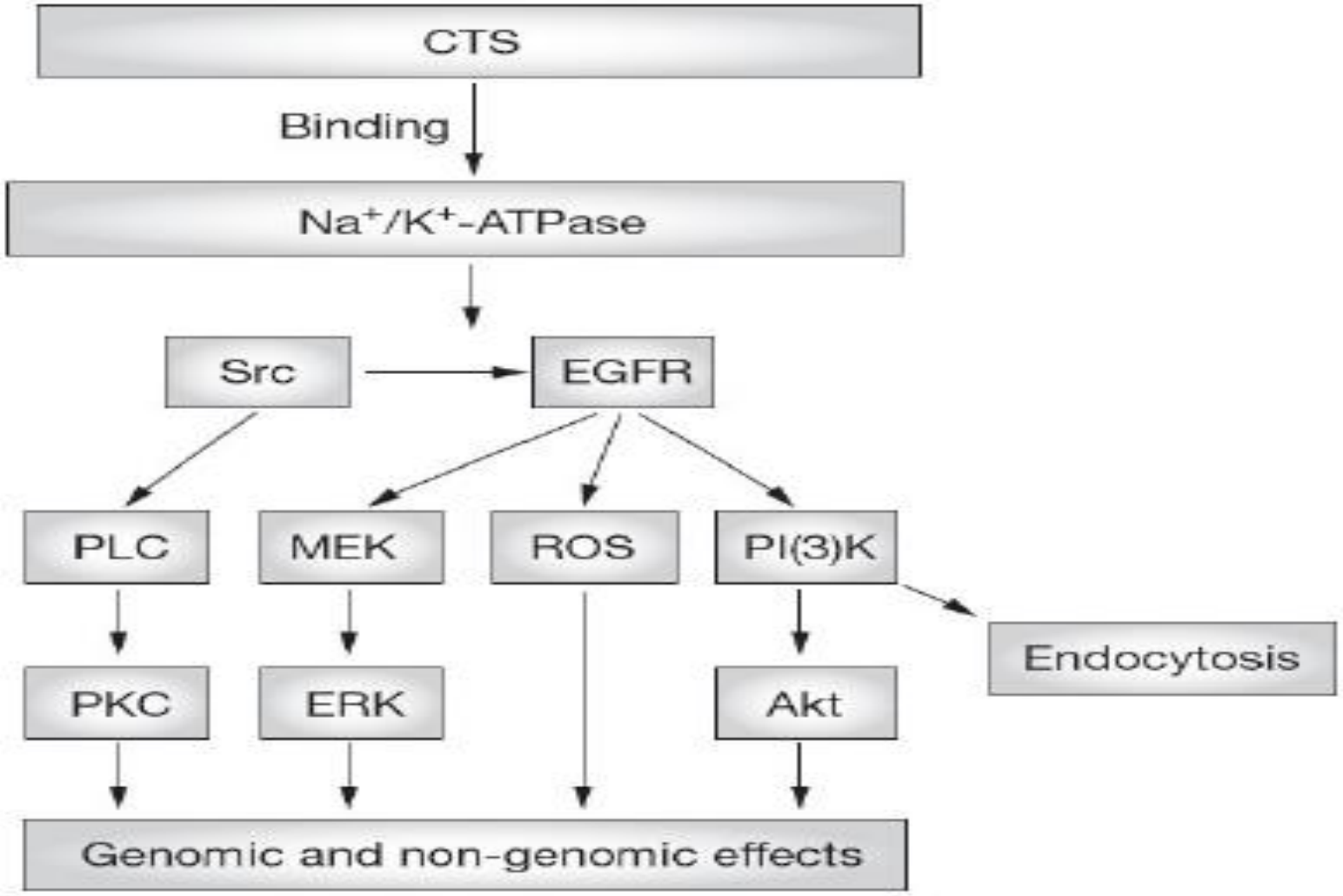








Novel functions of Na/K-ATPase and of endogenous cardiotonic steroids



MOL PHARM
MOLECULAR PHARMACOLOGY

New Molecular Determinants Controlling the Accessibility of Ouabain to Its Binding Site in Human Na,K-ATPase α Isoforms

Gilles Crambert, Daniele Schaer, Sophie Roy, and Käthi Geering

Institute of Pharmacology and Toxicology of the University, Lausanne, Switzerland

Received August 26, 2004; accepted October 22, 2003

This article is available online at http://molpharm.aspetjournals.org

In conclusion, we have identified new amino acids in the Na,K-ATPase that differentially control discrete steps in the ouabain binding to α1 and α2 isoforms. These findings, which explain the isoform-specific differences in ouabain binding kinetics, may be of importance for the development of new drugs that are able to discriminate between the 'inotropic' α2 and the 'toxic' α1 isoform of Na,K-ATPase.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 32, Issue of August 6, pp. 33306–33314, 2004
Printed in U.S.A.

Organ Hypertrophic Signaling within Caveolae Membrane Subdomains Triggered by Ouabain and Antagonized by PST 2238*

Received for publication, February 27, 2004, and in revised form, May 20, 2004
Published, JBC Papers in Press, May 25, 2004, DOI 10.1074/jbc.M402187200

Mara Ferrandi‡§, Isabella Molinari‡, Paolo Barassi‡, Elena Minotti‡, Giuseppe Bianchi¶, and Patrizia Ferrari‡

From the ‡Prassis sigma-tau Research Institute, Settimo Milanese, 20019 Milan, Italy and the ¶Division of Nephrology, Dialysis and Hypertension, Vita e Salute University, San Raffaele Hospital, 20132 Milan, Italy

ouabain activates: (*a*) tyrosine phosphorylation of the epidermal growth factor receptor (EGFr),[1] Src, and p42/44 mitogen-activated protein kinase (MAPKs) in both neonatal rat cardiac myocytes and A7r5 cells (4, 6); (*b*) the same signaling pathway within the cellular membrane microdomain of caveolae in isolated perfused rat heart (7); and (*c*) slow intracellular Ca^{2+} oscillations in rat tubular cells that favor the association of Na-K ATPase with the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) in a signaling microdomain (8). Recently, it has been

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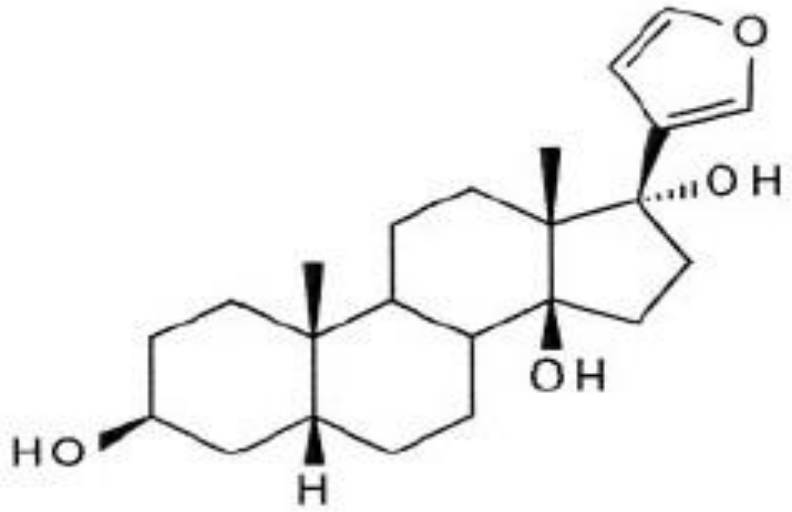
PST 2238



17β-(3-furyl)-5β-androstan-3β, 14β, 17α-triol

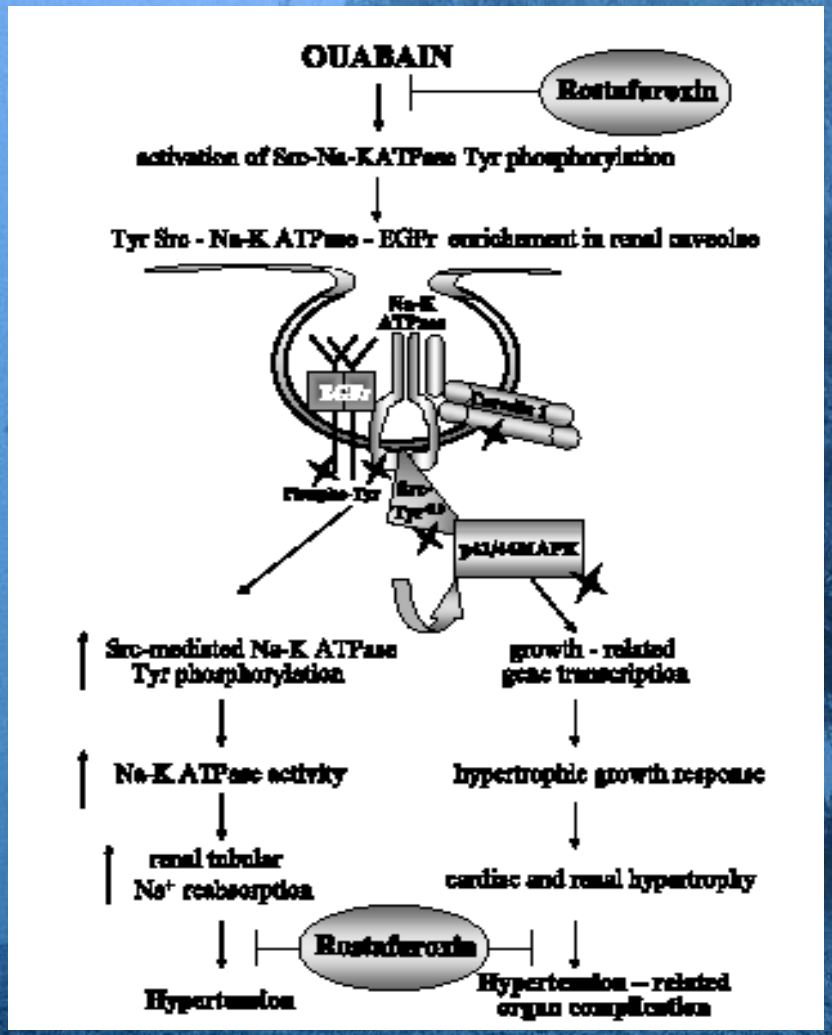
Am J Physiol Regul Integr Comp Physiol 290: R529–R535, 2006;
doi:10.1152/ajpregu.00518.2005.

Invited Review

CALL FOR PAPERS | *Molecular Mechanisms Linking Salt to Hypertension*

Rostafuroxin: an ouabain antagonist that corrects renal and vascular Na^+-K^+-ATPase alterations in ouabain and adducin-dependent hypertension

Patrizia Ferrari,[1] Mara Ferrandi,[1] Giovanni Valentini,[1] and Giuseppe Bianchi[2]
[1]Prassis Research Institute Sigma-Tau, Settimo Milanese (Milan), Medical Department Research & Development Division, Sigma-Tau, Pomezia (Rome); and [2]Vita-Salute University, San Raffaele Hospital, Milan, Italy



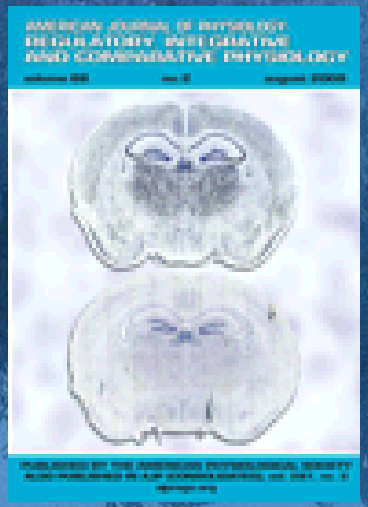
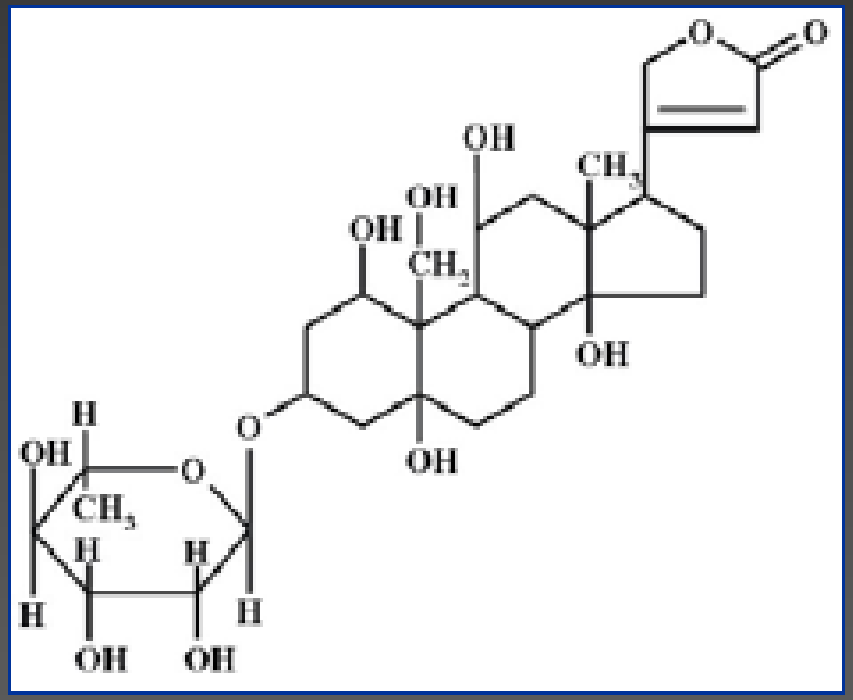


Fig. 4. Molecular mechanism of action of the ouabain antagonist rostafuroxin in renal caveolae: subnanomolar ouabain/EO concentrations activate an Src-dependent signaling pathway that induces enrichment of the tyrosine-phosphorylated forms of Src, epidermal growth factor receptor (EGFr), and Na^+-K^+-ATPase in renal caveolae, increases Na^+-K^+-ATPase activity on the cell membrane and activates the p42/44 MAPK in the cytosol, thus leading to hypertension and organ hypertrophy. Rostafuroxin, at nanomolar concentrations, antagonizes the ouabain/EO-Src-Na^+-K^+-ATPase interaction, normalizes the Na^+-K^+-ATPase and p42/44 MAPK activities, thus reducing blood pressure and preventing organ hypertrophy.

Therapeutic action of cardiotonic steroids like digitalis (ouabain derivatives)



ouabain

- Inhibition of Na,K-ATPase by ouabain-like cardiotonic steroids leads to decrease in Na^+ -gradient and decrease in the activity of Na^+/Ca^{2+} exchanger
- This in turn leads to increases in intracellular Ca^{2+} concentration and better cardiac muscle contraction

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Conclusions—The present study provides evidence that sub-nanomolar concentrations of ouabain, close to those of the circulating EO in humans, activate a Src kinase-dependent signaling pathway correlated with organ hypertrophy *in vivo*.

REVIEWS
PHYSIOLOGY 23: 205–211, 2008; doi:10.1152/physiol.00008.2008

The Na-K-ATPase and Calcium-Signaling Microdomains

Jiang Tian and Zi-jian Xie
Department of Physiology and Pharmacology, University of Toledo Health Science Campus, Toledo, Ohio
zi-jian.xie@utoledo.edu

The Na-K-ATPase is an energy-transducing ion pump that converts the free energy of ATP into transmembrane ion gradients. It also serves as a functional receptor for cardiotonic steroids such as ouabain and digoxin. Binding of ouabain to the Na-K-ATPase can activate calcium signaling in a cell-specific manner. The exquisite calcium modulation via the Na-K-ATPase is achieved by the ability of the pump to integrate signals from numerous protein and non-protein molecules, including ion transporters, channels, protein kinases/phosphatases, as well as cellular Na^+. This review focuses on the unique properties of the Na-K-ATPase and its role in the formation of different calcium-signaling microdomains.

Studies of the past 10 years have identified many important protein interactions of the Na-K-ATPase. The interactions among the Na-K-ATPase, protein kinase, membrane transporters/channels, and structural proteins ensure formation of dynamic and cell-specific calcium-signaling microdomains. It is

and protein phosphatases (31, 62, 89), studies have to be conducted to understand the dynamics, regulation, and isoform- and cell-specific aspects of these interactions among the Na-K-ATPase and its partners. Further efforts of many laboratories are clearly required. It is

these cell functions. However, the continual efforts will eventually provide insights into the newly appreciated functions of the Na-K-ATPase and their roles in cell biology and animal physiology.

Nephrol Dial Transplant (2008) 1 of 7
doi: 10.1093/ndt/gfn325

NDT
Nephrology Dialysis Transplantation

Editorial Comment

Role of endogenous cardiotonic steroids in sodium homeostasis

Wilhelm Schoner and Georgios Scheiner-Bobis

Institute of Biochemistry and Endocrinology, Justus-Liebig-University Giessen, Frankfurter Str. 100, D-35392 Giessen, Germany

terial hypertension [67,68]. Consistent with the functioning of a hormone, low (nanomolar) concentrations of cardiac glycosides that do not inhibit the sodium pump may activate intracellular signalling cascades. This will lead to vasoconstriction, hypertension, natriuresis and, when such signals are of longer duration, also to tissue remodelling of the heart, arteries and kidneys [9,69] (Figure 2). Since

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It is evident now that long-term excessive sodium consumption stimulates, in addition to other known mechanisms, the generation of arterial hypertension via the release of various endogenous cardiac glycosides. A long-lasting rise of

ous endogenous cardiac glycosides. A long-lasting rise of this new type of steroid hormone in blood plasma, and especially that of ouabain and marinobufagenin, leads to arterial hypertension, natriuresis and finally, via altered gene expression patterns, to remodelling of the heart, arterial wall and kidneys. In particular, a prolonged increased secretion

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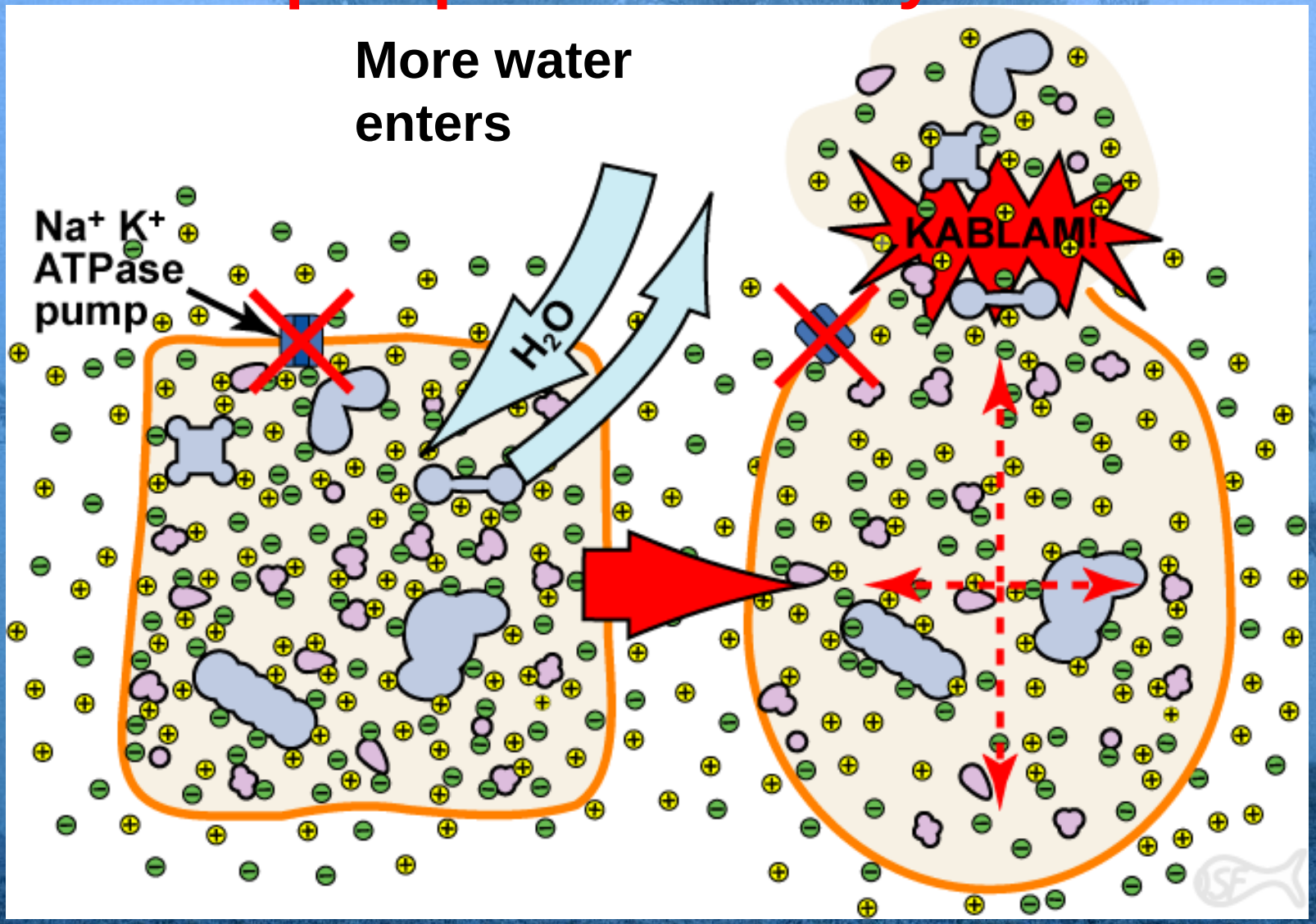
cardiomyopathy. Since 45% of hypertension cases are due to increased secretion of cardiac glycosides, it might be of diagnostic value to measure the plasma concentrations of endogenous cardiac glycosides to decide whether rostafuroxin, an ouabain antagonist and member of a new group of antihypertensives, might be the therapeutic agent of choice. The availability of an immunoassay for endoge-

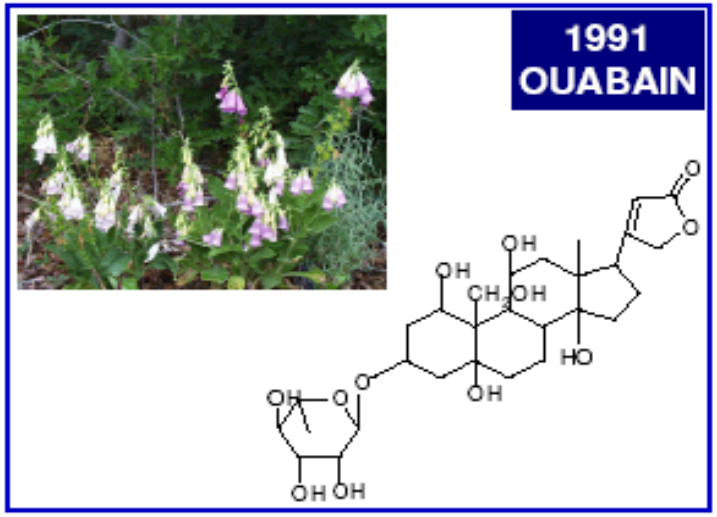
increase fibrosis. Therapy with rostafuroxin, accompanied by other means of therapy (diuretics, β blockers, Ca^{2+} antagonists), would presumably be a better choice to lower the likelihood of cardiac remodelling, fibrosis and heart failure.

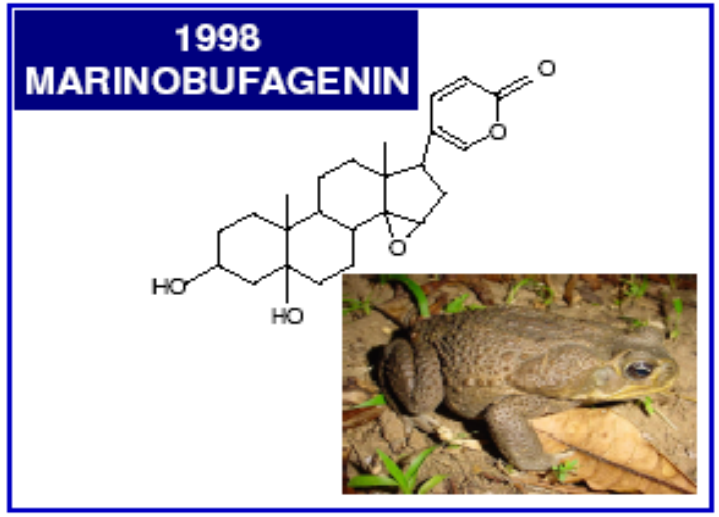
Conclusions

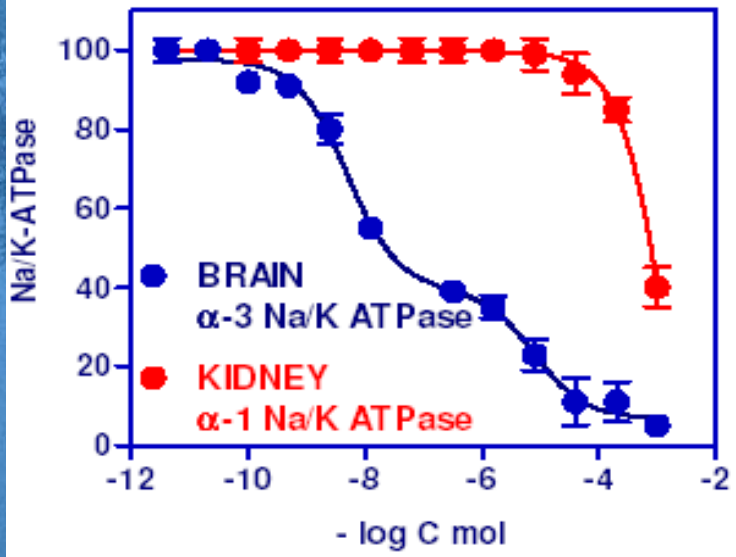
And beyond the confines of nephrology: if such Na^+-K^+-ATPase inhibitors have been conserved during evolution so long—from foxglove to *Homo sapiens*—it is difficult to believe that the only reason why nature preserved them was to make life difficult for nephrologists. The substance must obviously have more basic physiologic regulatory functions that so far escape us. The future will hopefully give the answer to the question of what role it plays in normal physiology.

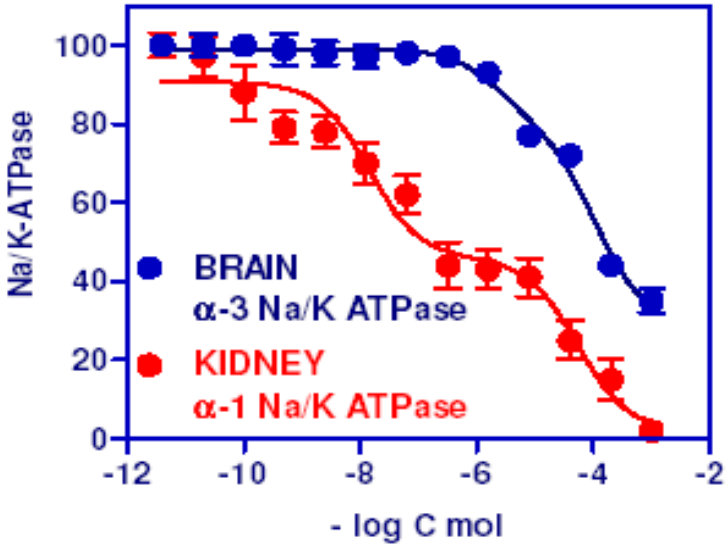
If the pump is blocked by ouabain











Fedorova et al, Circulation 2000: 102; 3009, Hypertension 2001: 37; 462.

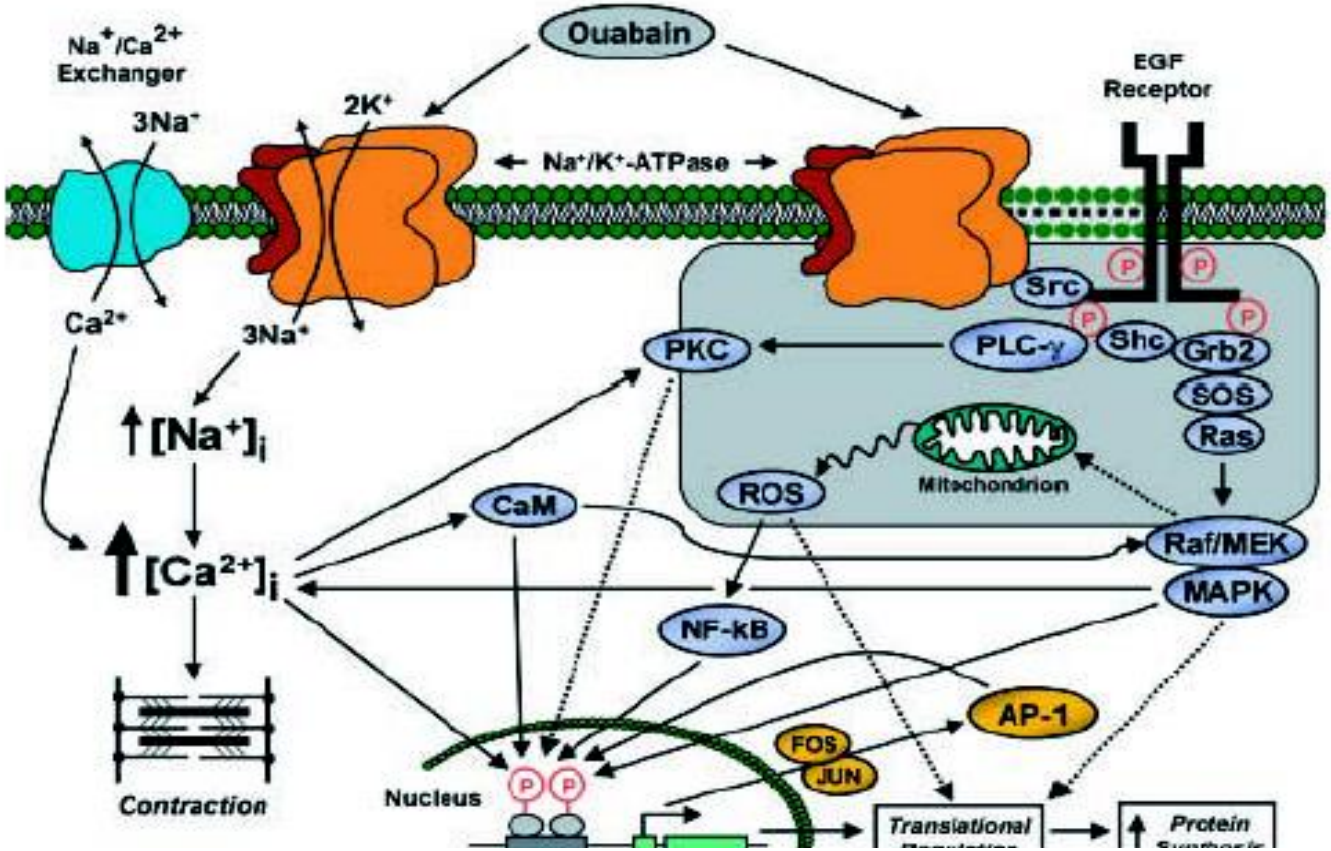
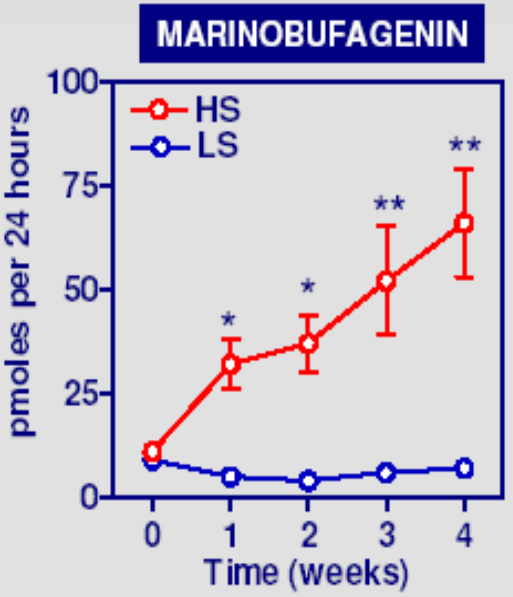
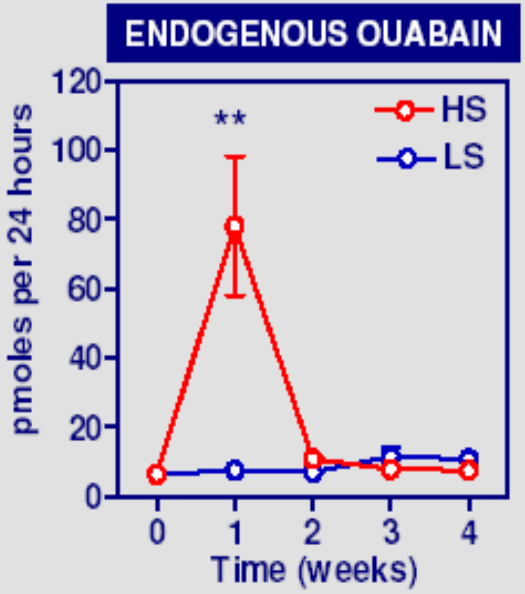
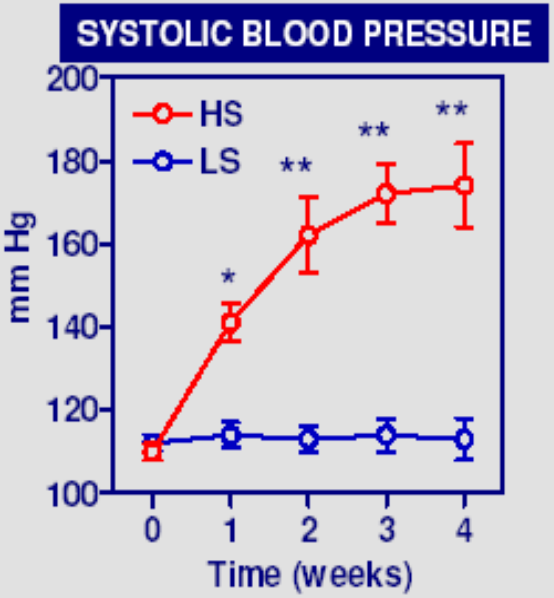


Fig. 1. The signal transducing function of Na^+/K^+-ATPase and its consequences in cardiac myocytes. Two pools of the enzyme, one pumping ions and the other interacting with neighboring proteins are suggested by the data. The partial inhibition of the pump by ouabain causes a modest change, if any, in $[Na^+]_i$ and $[K^+]_i$, but a significant change in $[Ca^{2+}]_i$ due to the presence of the Na^+/Ca^{2+}-exchanger. Ouabain interaction with the other pool alters protein–protein interactions to activate the indicated signaling pathways. The events placed in the grey box have been shown to be independent of changes in $[Na^+]_i$, $[K^+]_i$, and $[Ca^{2+}]_i$ that may occur. These activated pathways, the resulting increase in ROS, and the concomitant increase in $[Ca^{2+}]_i$ lead to activations of NF-κB and AP-1, transcriptional regulation of early response genes (c-fos, c-jun), and cardiac growth-related genes (those of atrial natriuretic factor, skeletal α-actin, and the α_3 subunit of Na^+/K^+-ATPase), stimulation of protein synthesis, and myocyte hypertrophy. The solid arrows indicate experimentally supported events induced by ouabain in myocytes, and the broken arrows indicate those with limited or indirect support. In several cell types other than cardiac myocytes, some of the same signaling events are induced by ouabain, but there are also significant cell-specific differences between the ouabain-induced pathways and the down-stream consequences (see text).

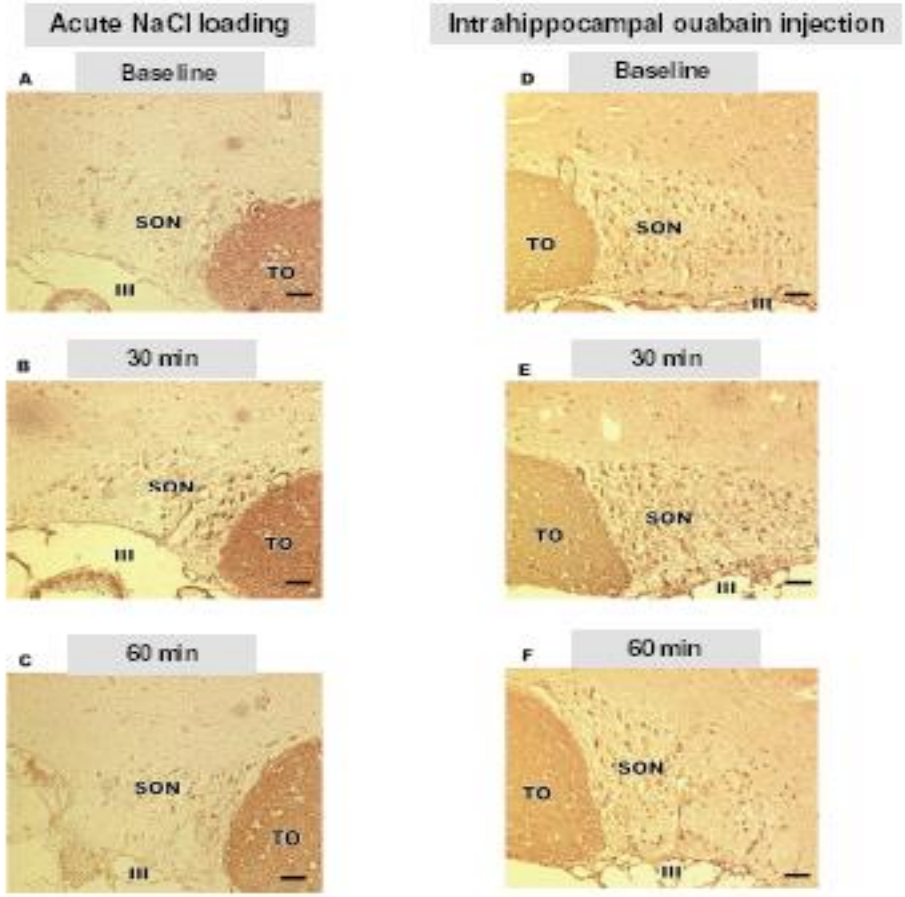
EXCRETION OF ENDOGENOUS OUABAIN AND MARINOBUFAGENIN BY NaCl LOADED DAHL RATS







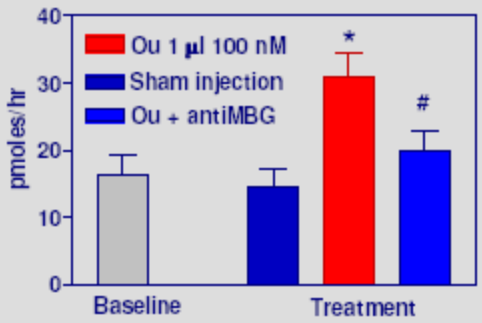
Angiotensin II in supraoptical nucleus of hypothalamus



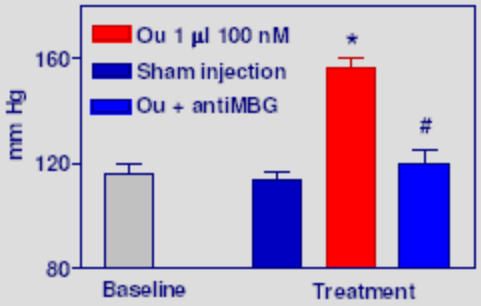
ATII staining in the supraoptical nucleus of hypothalamus following acute NaCl loading of DS, and intrahippocampal administration of 60 pg ouabain. Scale bar = 10 µm.

CENTRAL ADMINISTRATION OF OUABAIN MIMICS NaCl LOADING

Renal excretion of MBG



Systolic blood pressure



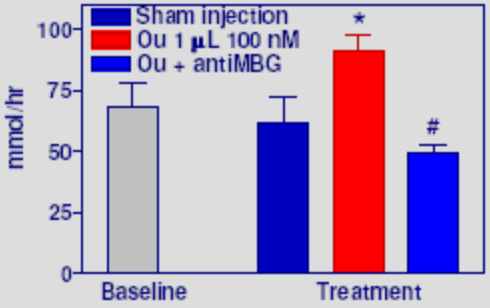
ADMINISTRATION OF OUABAIN TO DAHL RATS INDUCES:

- PRESSOR AND DIURETIC RESPONSE,

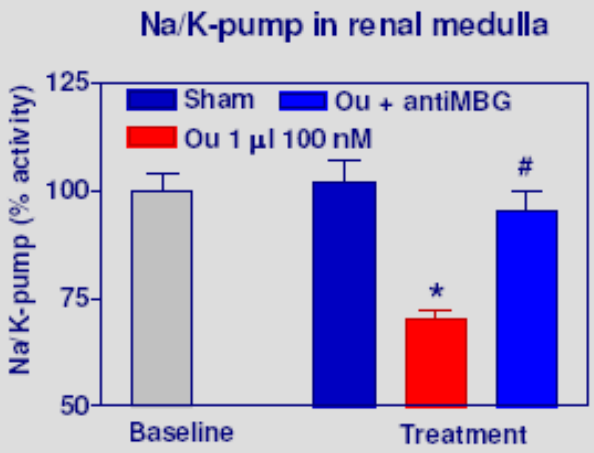
- DOUBLING OF MBG EXCRETION,

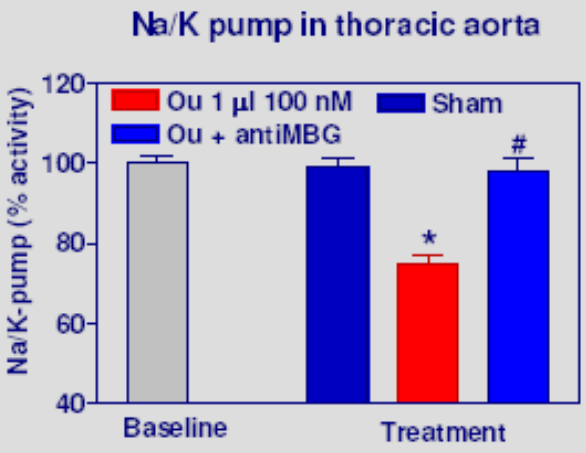
THESE EFFECTS WERE PREVENTED BY PRETREATMENT OF RATS WITH ANTI-MBG ANTIBODY

Renal sodium excretion

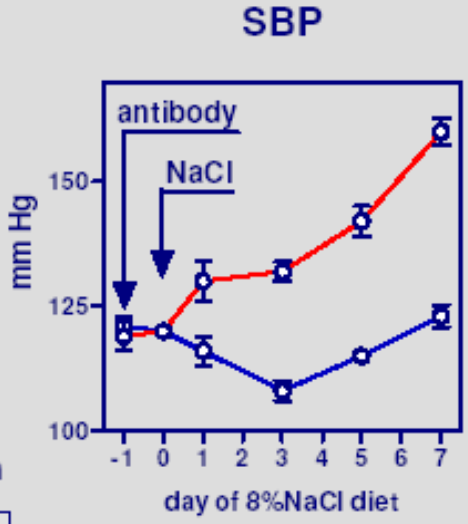


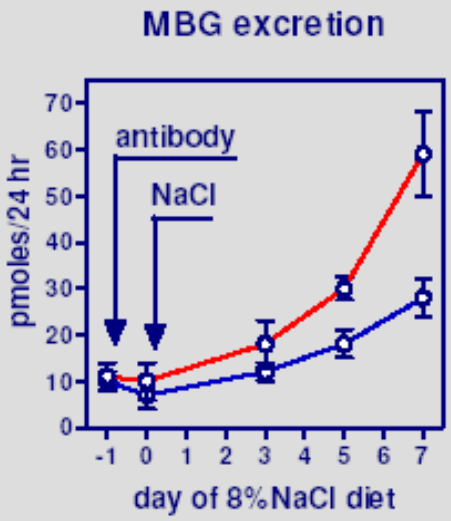
CENTRAL ADMINISTRATION OF OUABAIN MIMICS NaCl LOADING

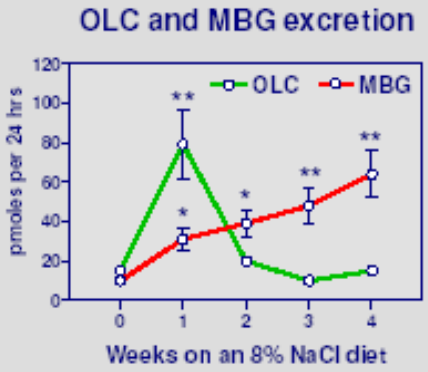




Administration of an anti-ouabain (aOU) antibody prior to NaCl loading of Dahl-S rats prevents elevation of systolic blood pressure (SBP) and decreases renal marinobufagenin (MBG) excretion

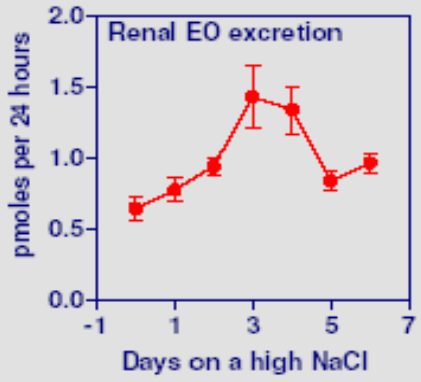


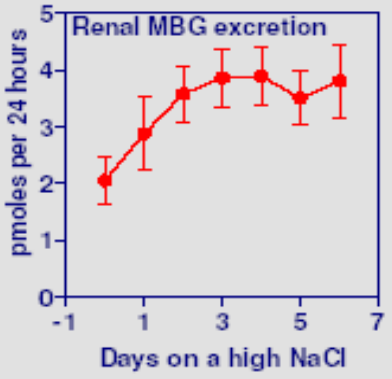


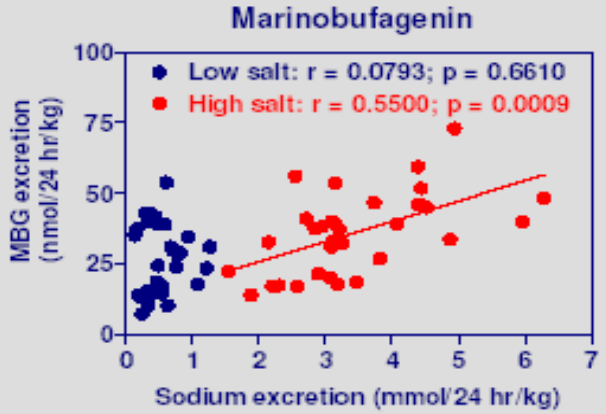


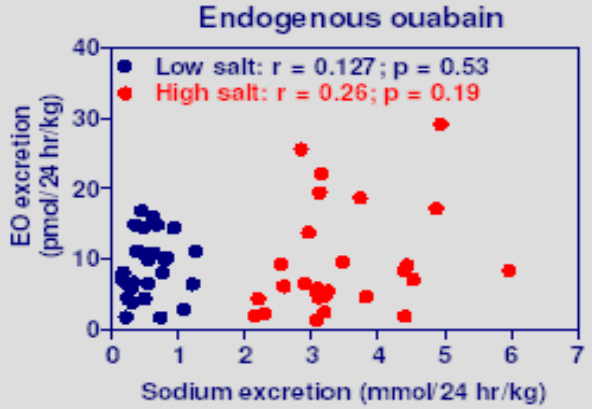
Control antibody
aOU antibody

Renal excretion of endogenous ouabain and marinobufagenin by healthy volunteers on a high (300 mmol/day) NaCl intake





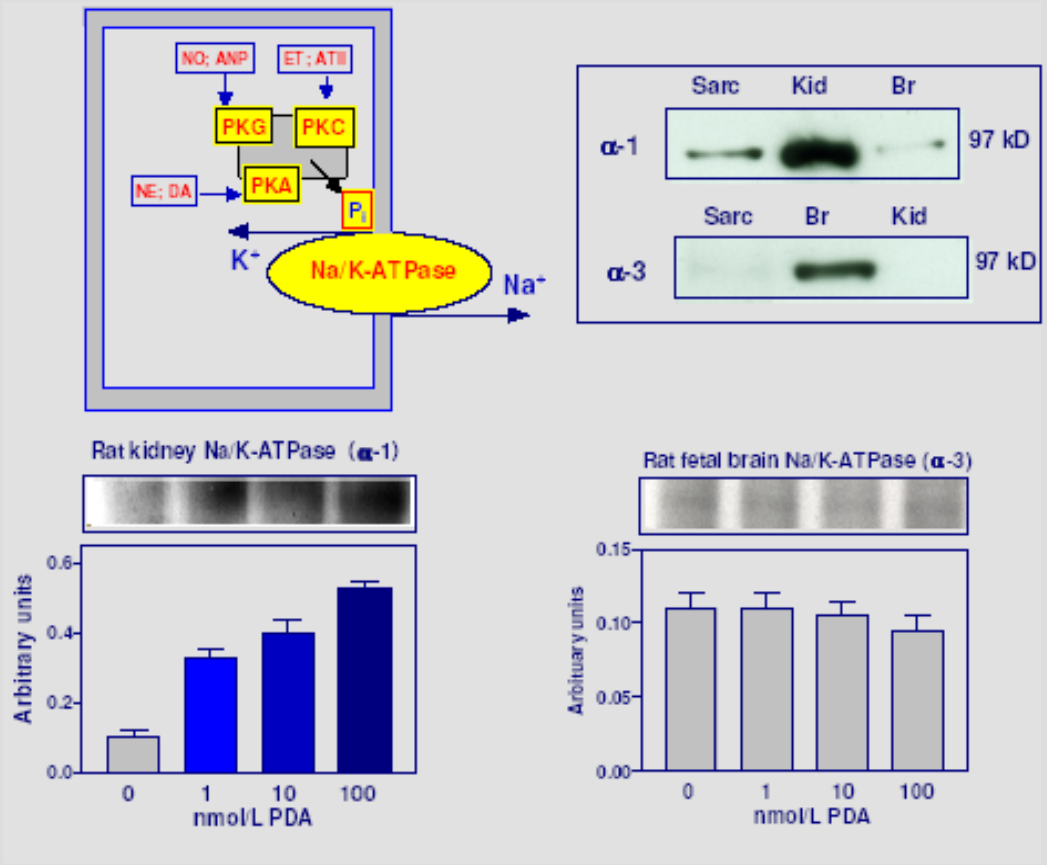




HOW DO LOW CONCENTRATIONS OF ENDOGENOUS DIGITALIS WORK?

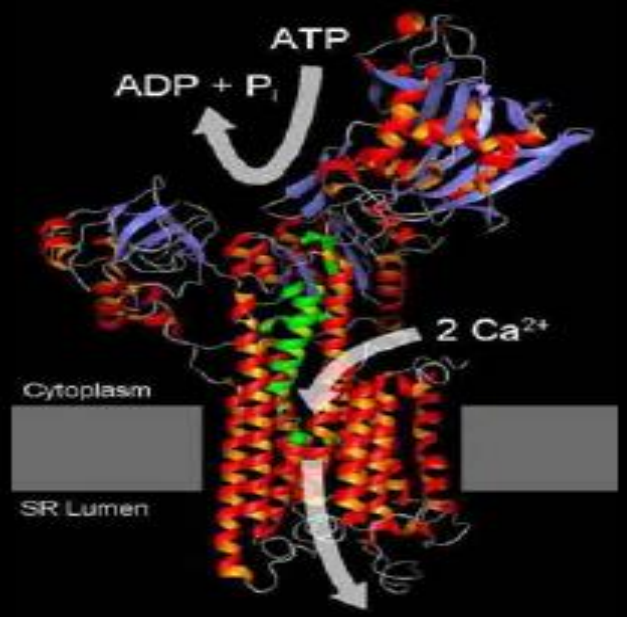
Isoform-sepcific phosphorylation of the Na/K-ATPase

ACTIVATION OF PKC INDUCES PHOSPHORYLATION OF ALPHA-1, BUT NOT ALPHA-3 ISOFORM OF THE Na/K-ATPase

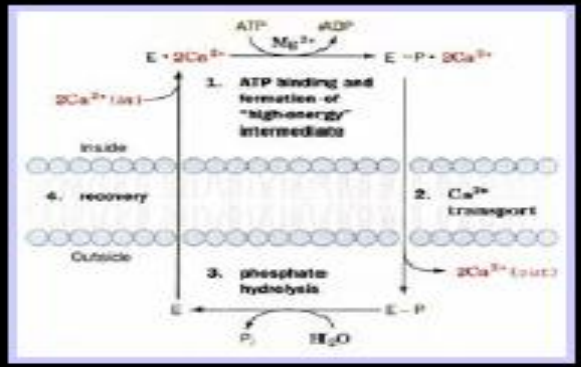


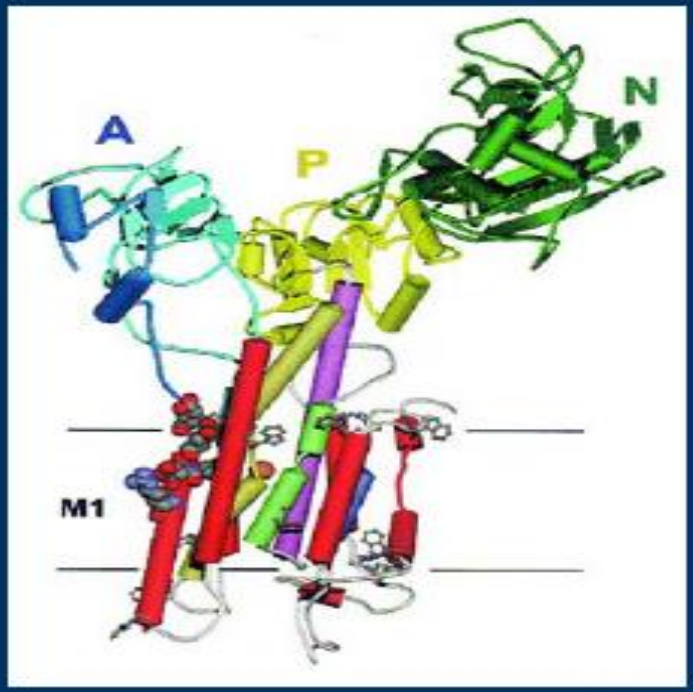
Hypertension 2002; 39: 298

Ca^{2+}-ATPase of Sarcoplasmic Reticulum



- Plays a major role in muscle relaxation by transporting released Ca back into SR
- A single subunit protein with 10 transmembrane fragments
- Is highly homologous to Na,K-ATPase





Four major domains:

M - Membrane-bound domain, which is composed of 10 transmembrane segments

N- Nucleotide-binding domain, where adenine moiety of ATP and ADP binds

P – Phosphatase domain, which contains invariant Asp residue, which became phosphorylated during the ATP hydrolysis

A domain – essential for conformational transitions between E1 and E2 states

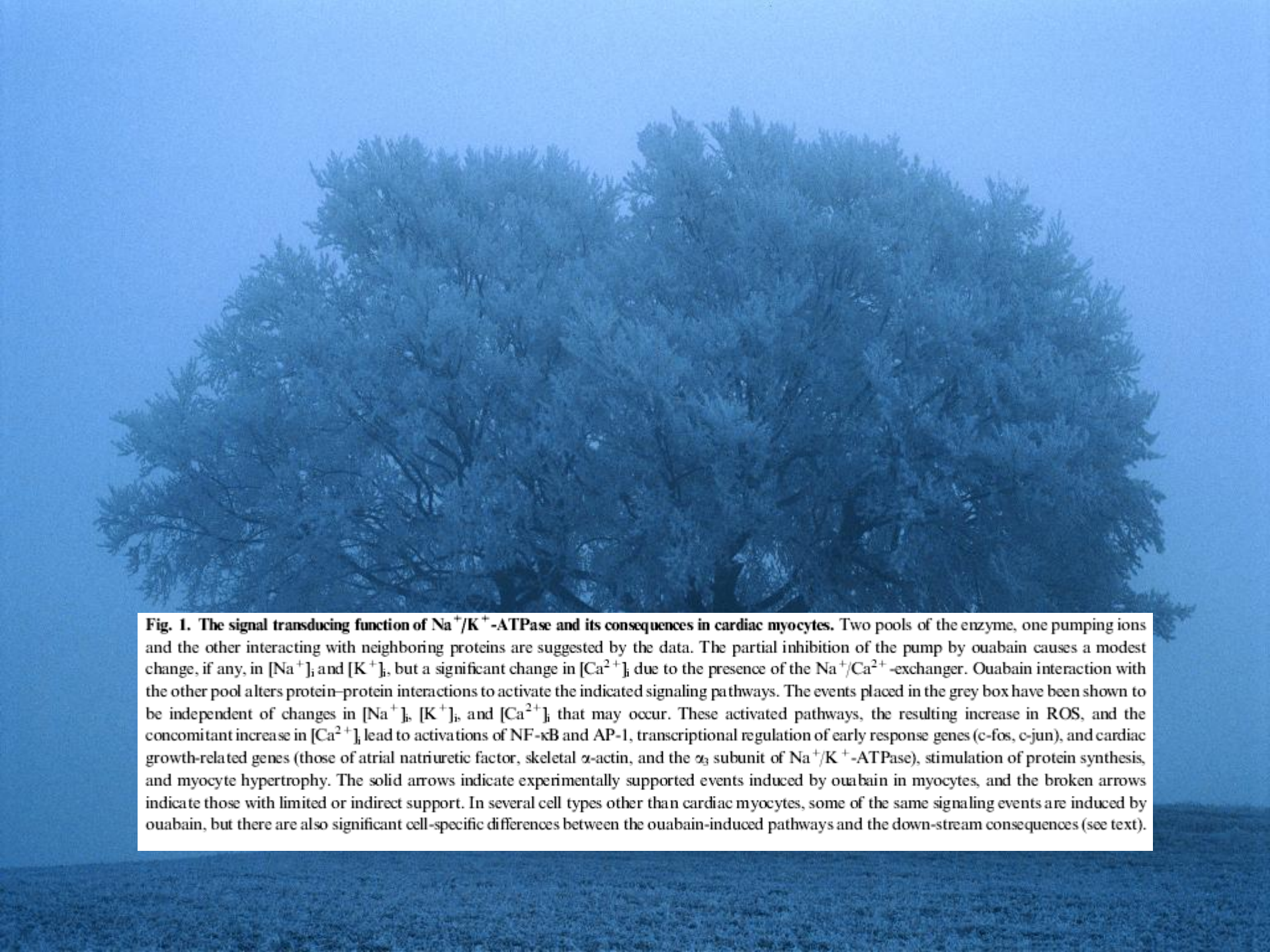
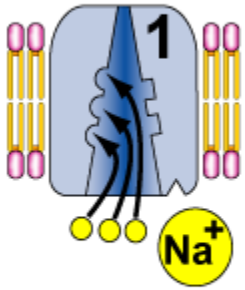


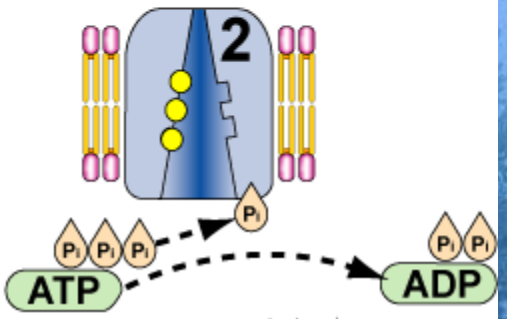
Fig. 1. The signal transducing function of Na^+/K^+-ATPase and its consequences in cardiac myocytes. Two pools of the enzyme, one pumping ions and the other interacting with neighboring proteins are suggested by the data. The partial inhibition of the pump by ouabain causes a modest change, if any, in $[Na^+]_i$ and $[K^+]_i$, but a significant change in $[Ca^{2+}]_i$ due to the presence of the Na^+/Ca^{2+}-exchanger. Ouabain interaction with the other pool alters protein–protein interactions to activate the indicated signaling pathways. The events placed in the grey box have been shown to be independent of changes in $[Na^+]_i$, $[K^+]_i$, and $[Ca^{2+}]_i$ that may occur. These activated pathways, the resulting increase in ROS, and the concomitant increase in $[Ca^{2+}]_i$ lead to activations of NF-κB and AP-1, transcriptional regulation of early response genes (c-fos, c-jun), and cardiac growth-related genes (those of atrial natriuretic factor, skeletal α-actin, and the α_3 subunit of Na^+/K^+-ATPase), stimulation of protein synthesis, and myocyte hypertrophy. The solid arrows indicate experimentally supported events induced by ouabain in myocytes, and the broken arrows indicate those with limited or indirect support. In several cell types other than cardiac myocytes, some of the same signaling events are induced by ouabain, but there are also significant cell-specific differences between the ouabain-induced pathways and the down-stream consequences (see text).

The Na^+- K^+ Pump Cycle

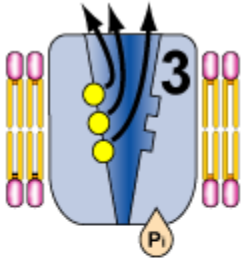
1. Cytosolic Na^+ binds the transporter.



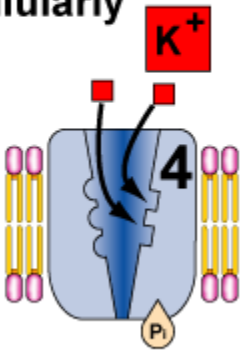
2. Binding initiates hydrolysis of ATP to ADP & phosphorylation of the transporter (binding of P_i).



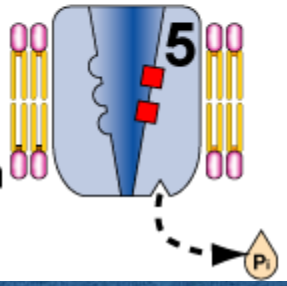
3. P_i binding induces a conformational change that releases Na^+ extracellularly



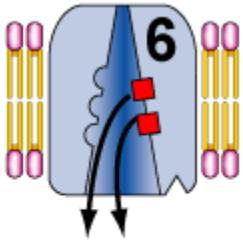
4. Exposed K^+ binding sites can now be filled from the cytosol.



5. Binding of K^+ induces release of P_i & allows the conformation to change back.



6. K^+ is released in the cytosol.



7. The pump is ready for another cycle.