

Low-dose Cardiotonic Steroids Increase Sodium-potassium ATPase Activity that Protects Hippocampal Slice Cultures from Experimental Ischemia

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Rationale for this study

- Ischemic preconditioning in hippocampal slice cultures
- Brief hypoxia-hypoglycemia protects from a lethal episode of hypoxia-hypoglycemia (Hassen, et al., 2004)
- Ischemic preconditioning induces Na/K ATPase and blocking Na/K ATPase abolishes neuroprotection (Tian, et al., 2008).
- Ischemic preconditioning has minimal clinical use
- Small molecule that induces Na/K ATPase activity

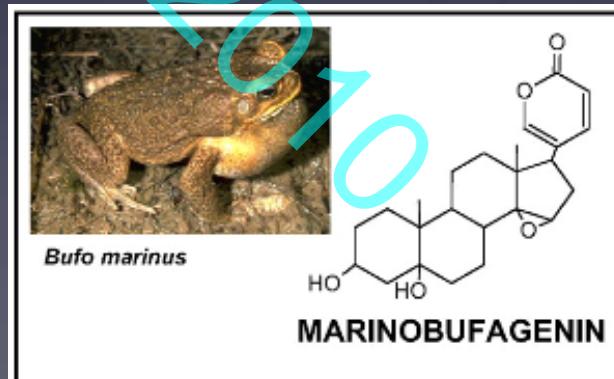
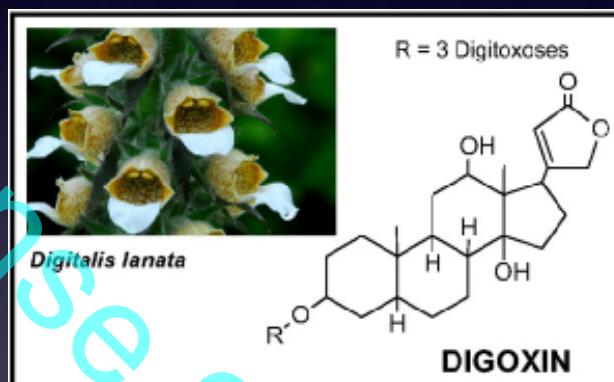
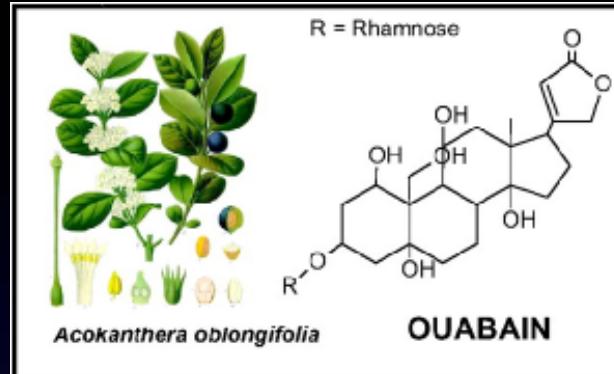
Stimulation of Na/K ATPase by cardiotonic steroids

- Cardiotonic steroids are well-known inhibitors of Na/K ATPase
- Multiple studies starting in the 1940's showed stimulation of Na/K ATPase at low doses
- Most recent Gao, et al., 2002.
- Studies were ignored

Cardiotonic steroids

- Cardenolides
- Ouabain
- Digoxin
- Bufadienolides
- Marinobufagenin

Dose-Response 2010



Exogenous and endogenous cardiotonic steroids

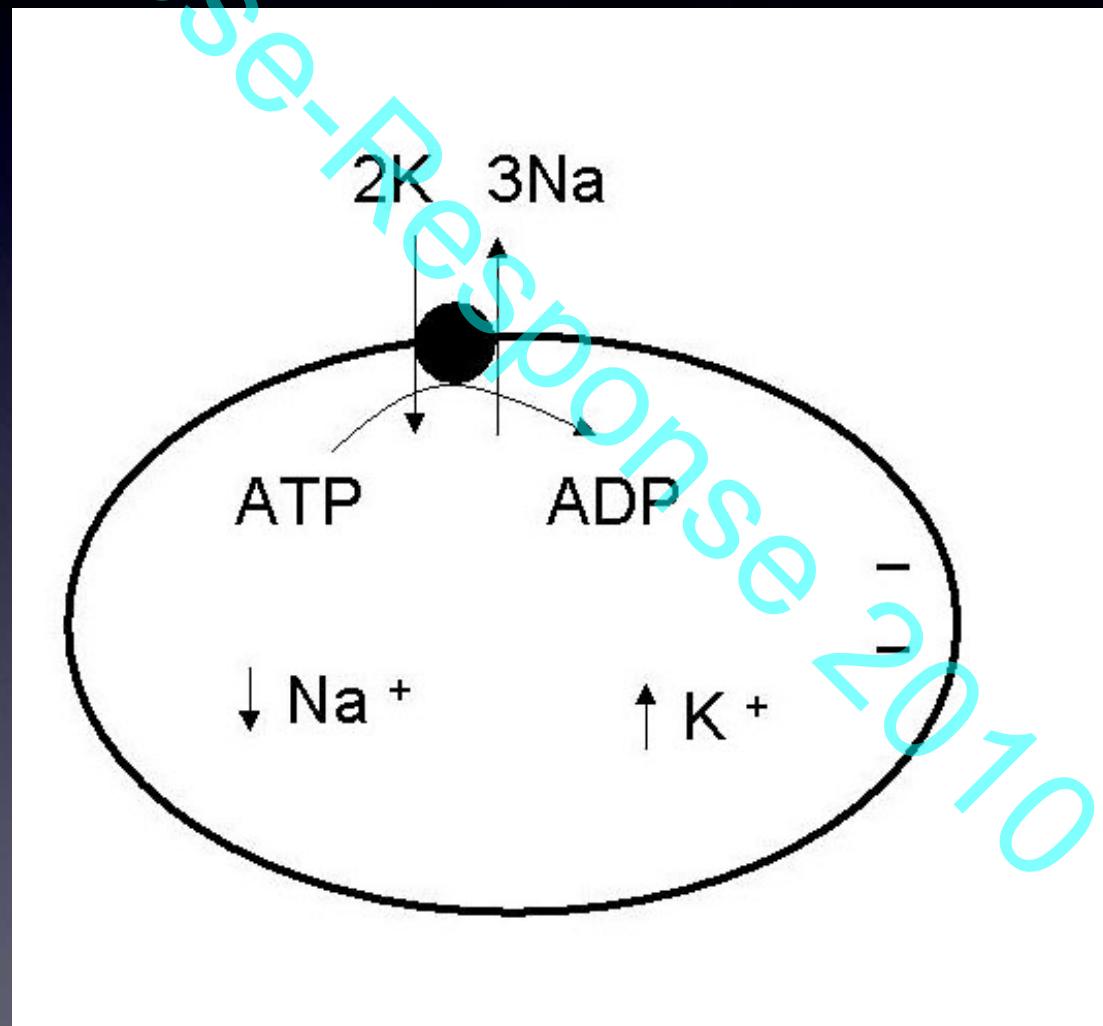
- Exogenous
 - Used for centuries to treat congestive heart disease
 - Poison arrows for hunting
 - Digoxin still used clinically
 - Narrow therapeutic index
- Endogenous
 - Idea is slowly being accepted in last the 10 years
 - Effective at many logs lower than pharmacological dose

Endogenous ouabain

- Produced by adrenal gland
- Human plasma levels range from 30 - 380 pM

Dose-Response 2010

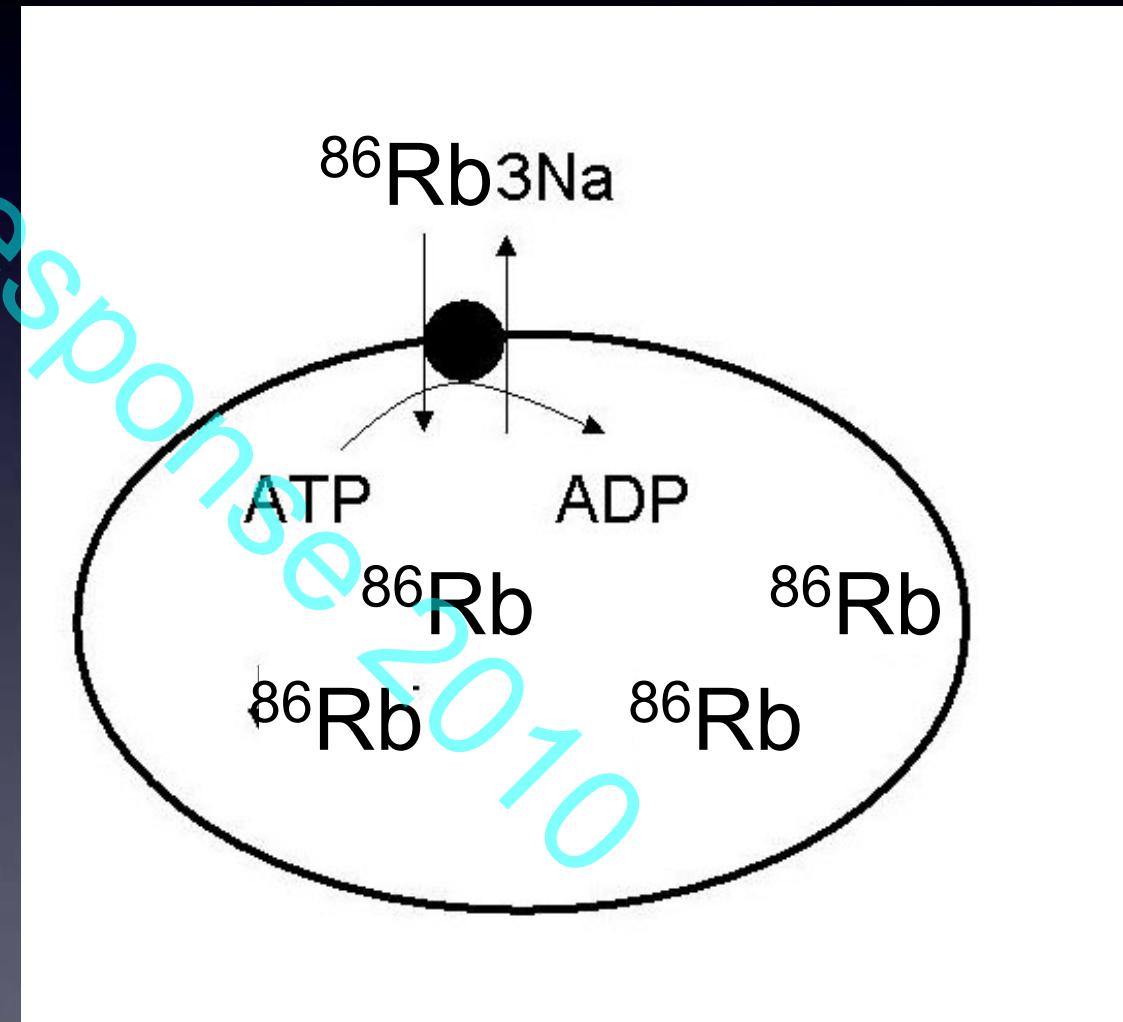
Sodium and Potassium transport by Na/K ATPase



^{86}Rb assay of Na/K ATPase

- Incubate explant cultures with $^{86}\text{RbCl}$
- Wash
- Count

Dose-Response



Na/K ATPase Isoforms

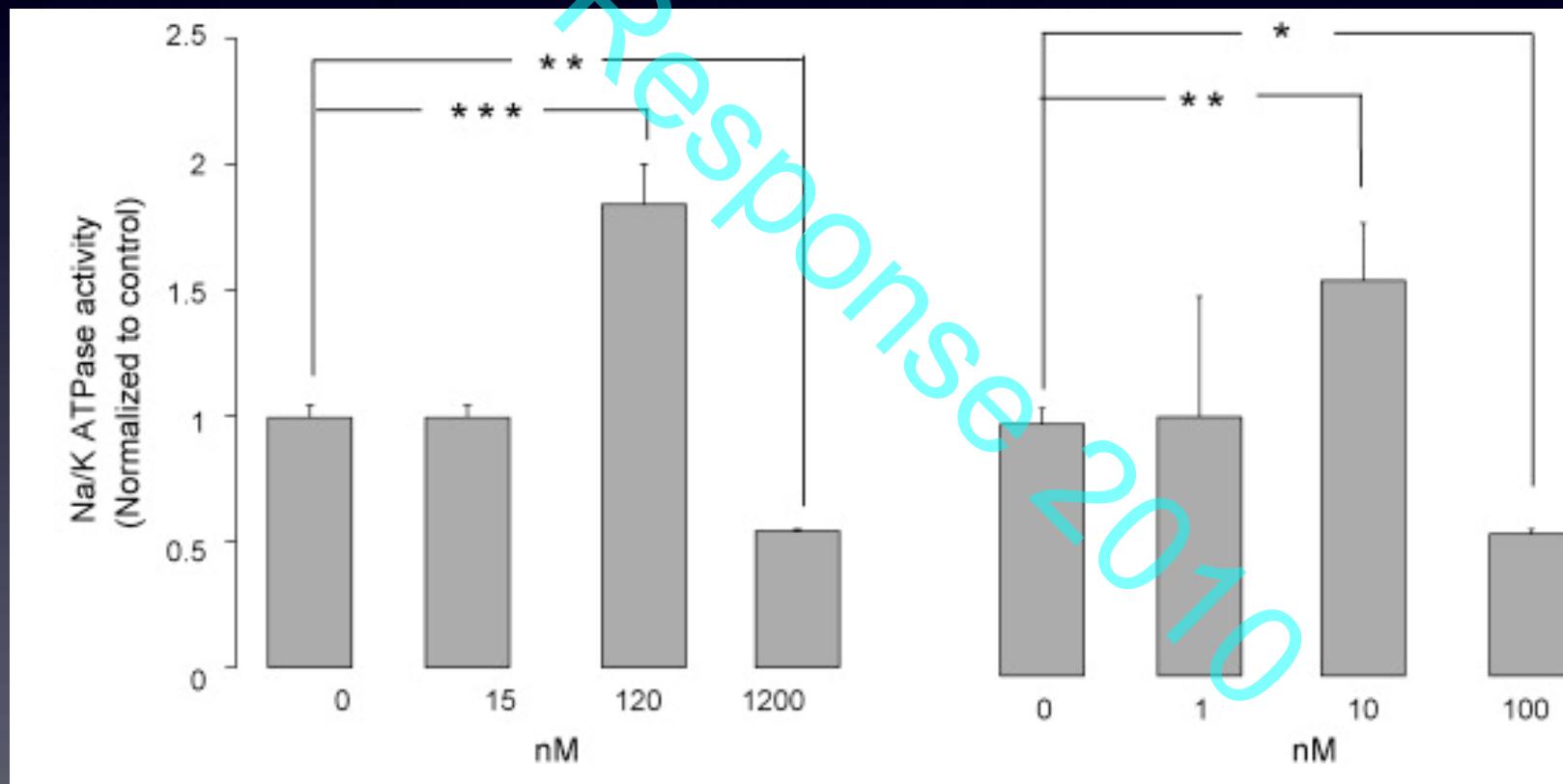
- α and β heterodimer
- steroid binding site is on the α subunit
- Three major isoforms (α_1 , α_2 , α_3)
 - Different steroid binding sites
 - $\alpha_1 \uparrow$ MBG, \downarrow Ouabain, Dig
 - $\alpha_{2/3} \downarrow$ MBG, \uparrow Ouabain, Dig

Cardenolides have an inverted U-shaped dose-response to Na/K

Dose-Response ATPase *in vitro*

Ouabain

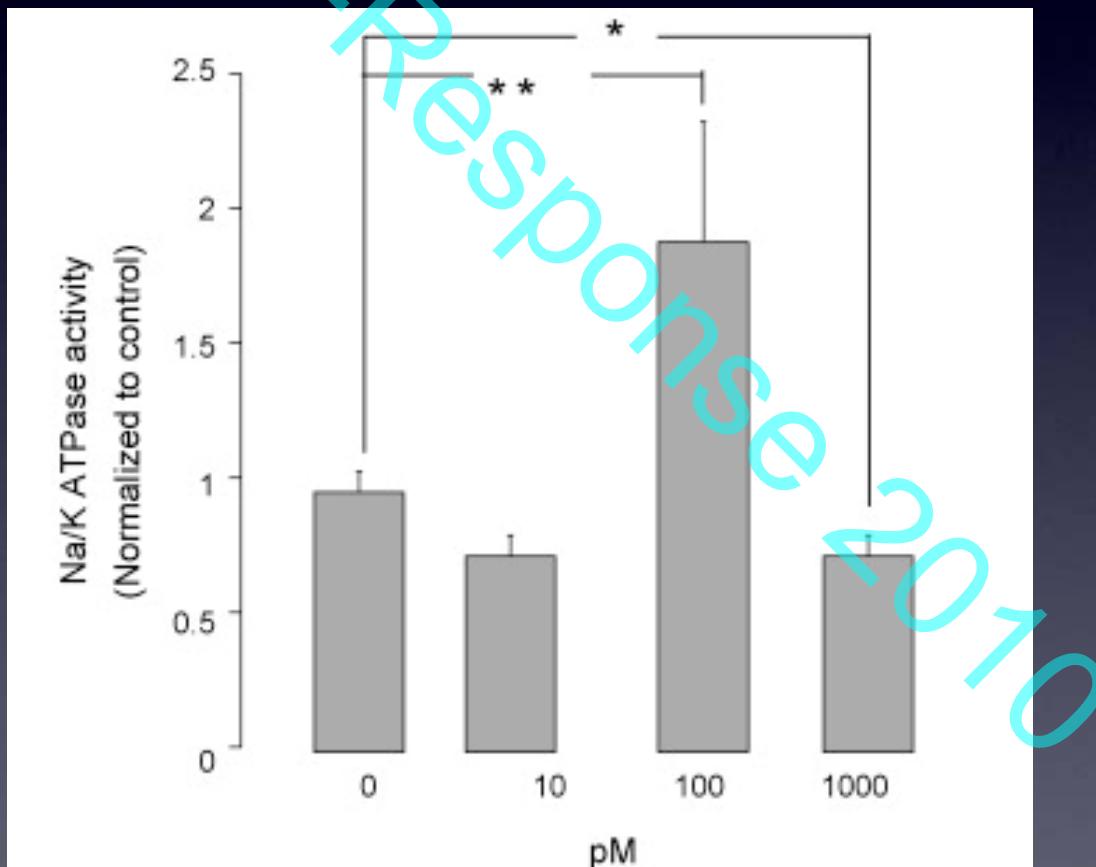
Digoxin



Oselkin, et al., 2010

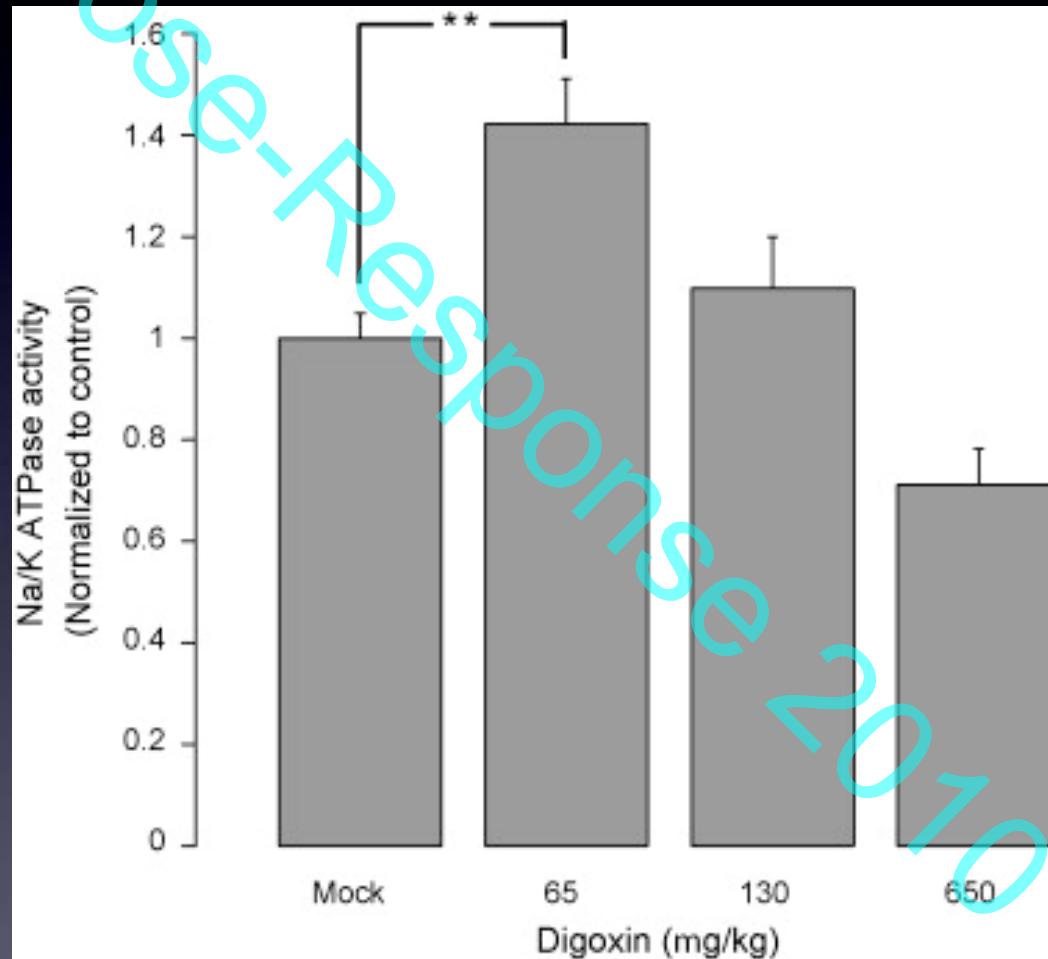
Bufadienolides have an inverted U-shaped dose-response to Na/K ATPase

Marinobufagenin



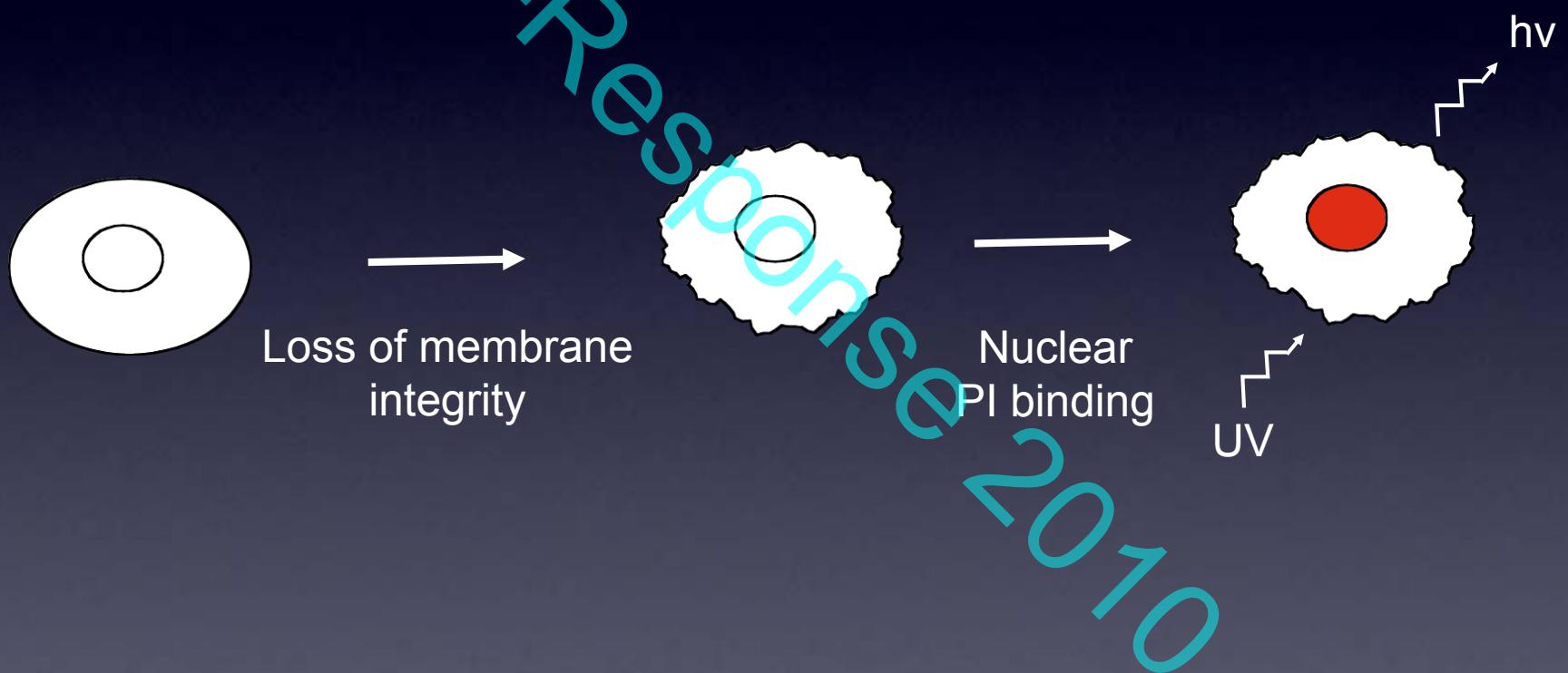
Oselkin, et al., 2010

Digoxin induces Na/K ATPase *in vitro*

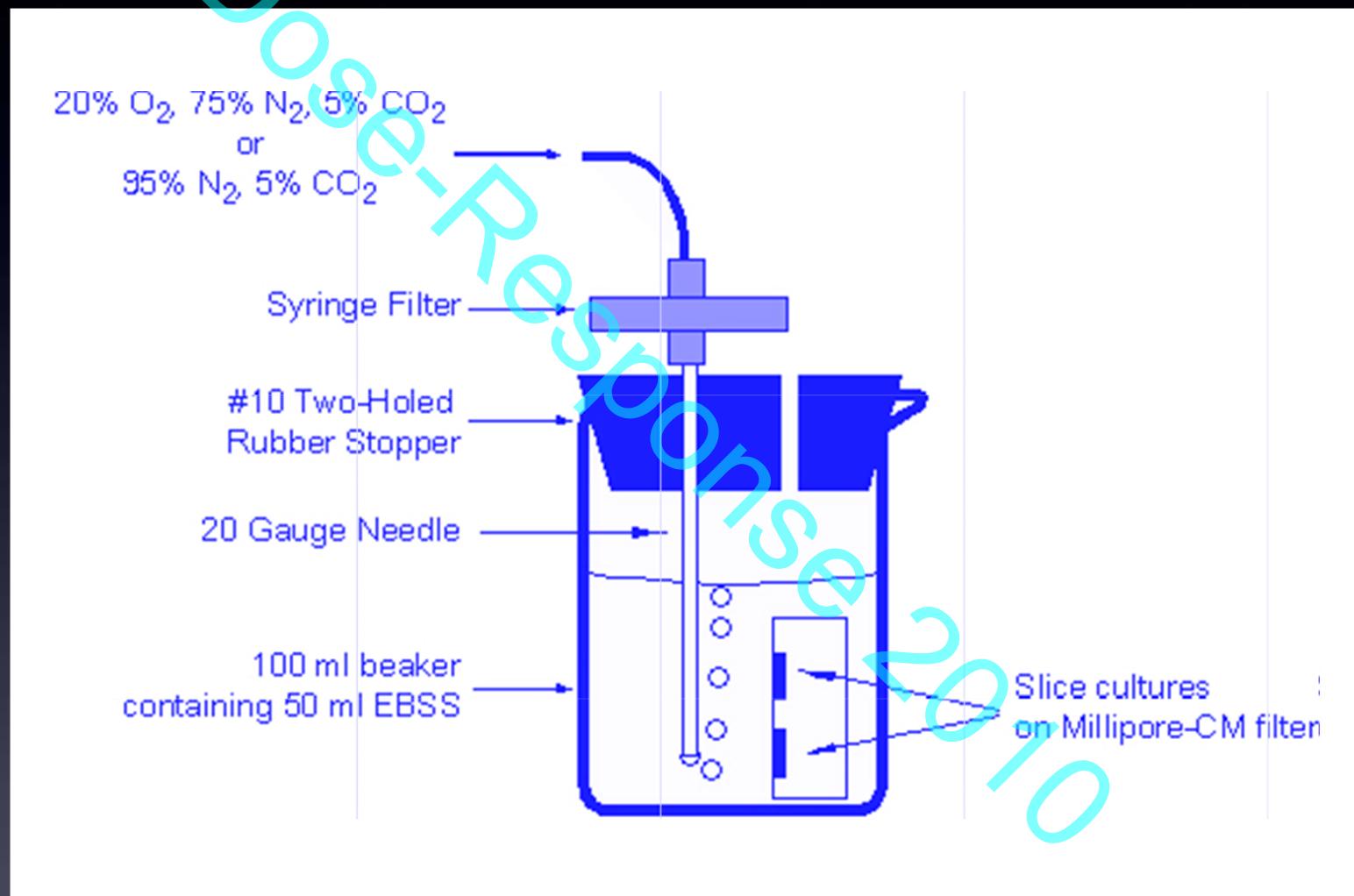


Oselkin, et al., 2010

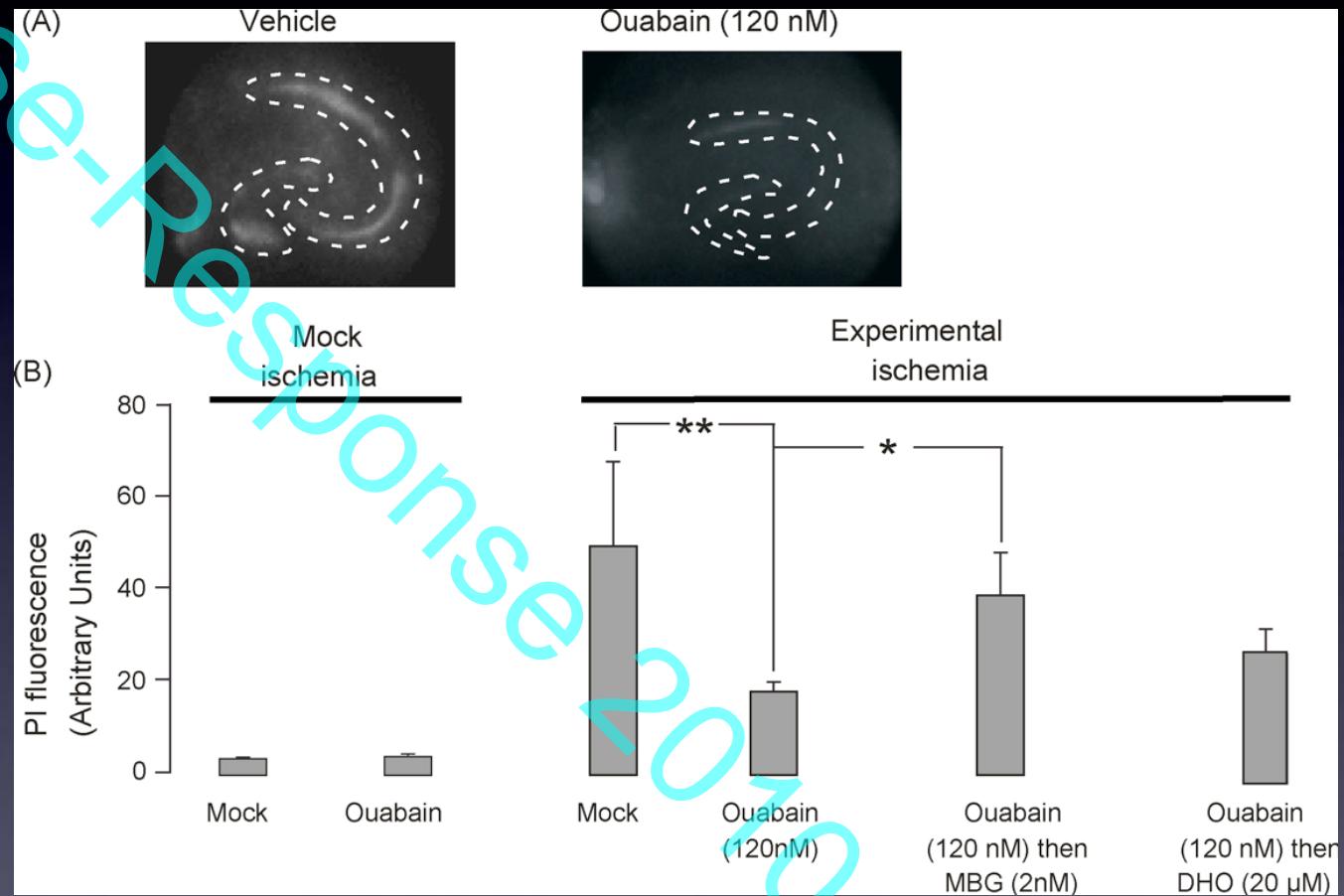
Propidium iodide assay of cell loss in hippocampal slice cultures



Experimental Ischemia



Ouabain pretreatment protects hippocampal neurons

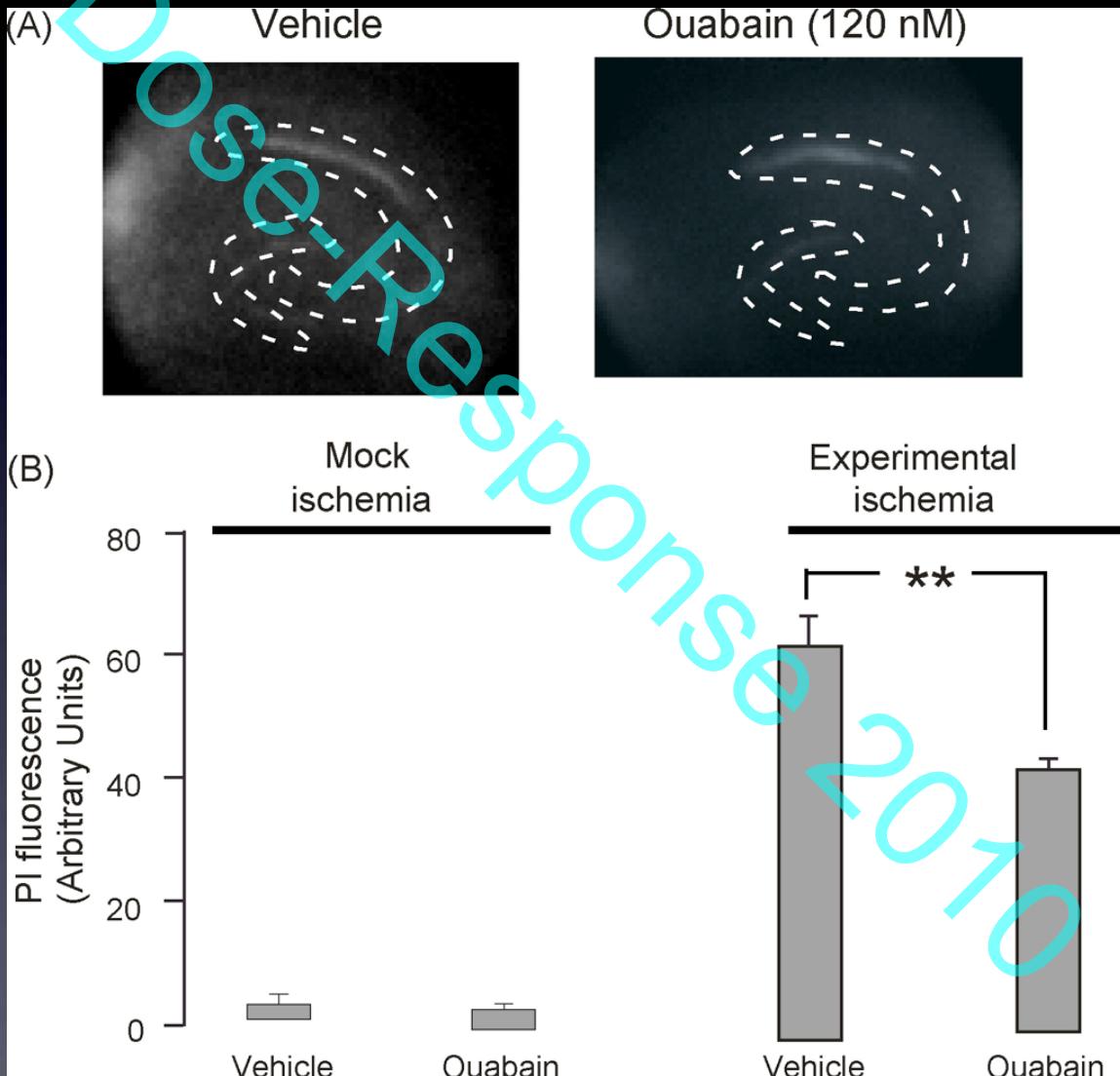


MBG (2nM) inhibits α_1

DHO (20 μ M) inhibits α_2 , α_3

Oselkin, et al., 2010

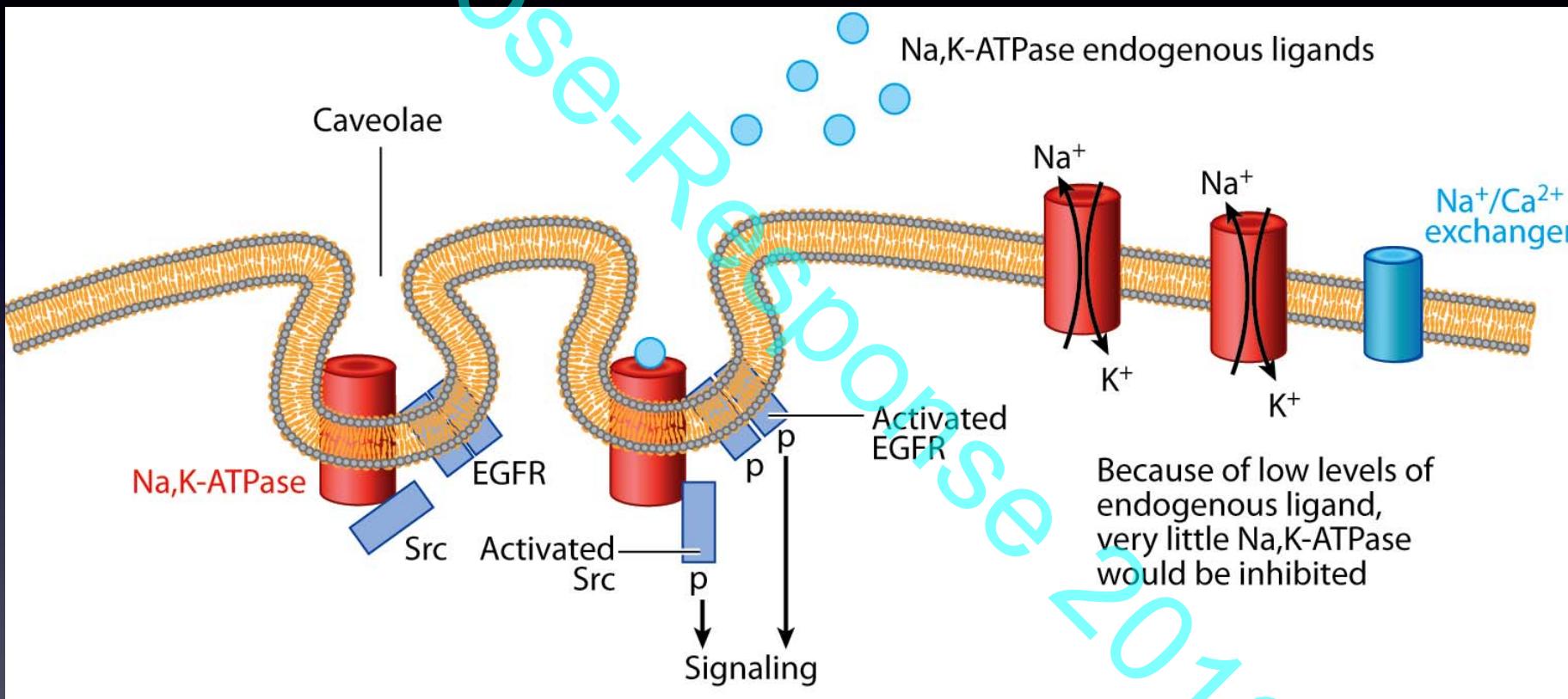
Ouabain post-treatment protects hippocampal neurons



Oselkin, et al., 2010

Cardiotonic Steroids

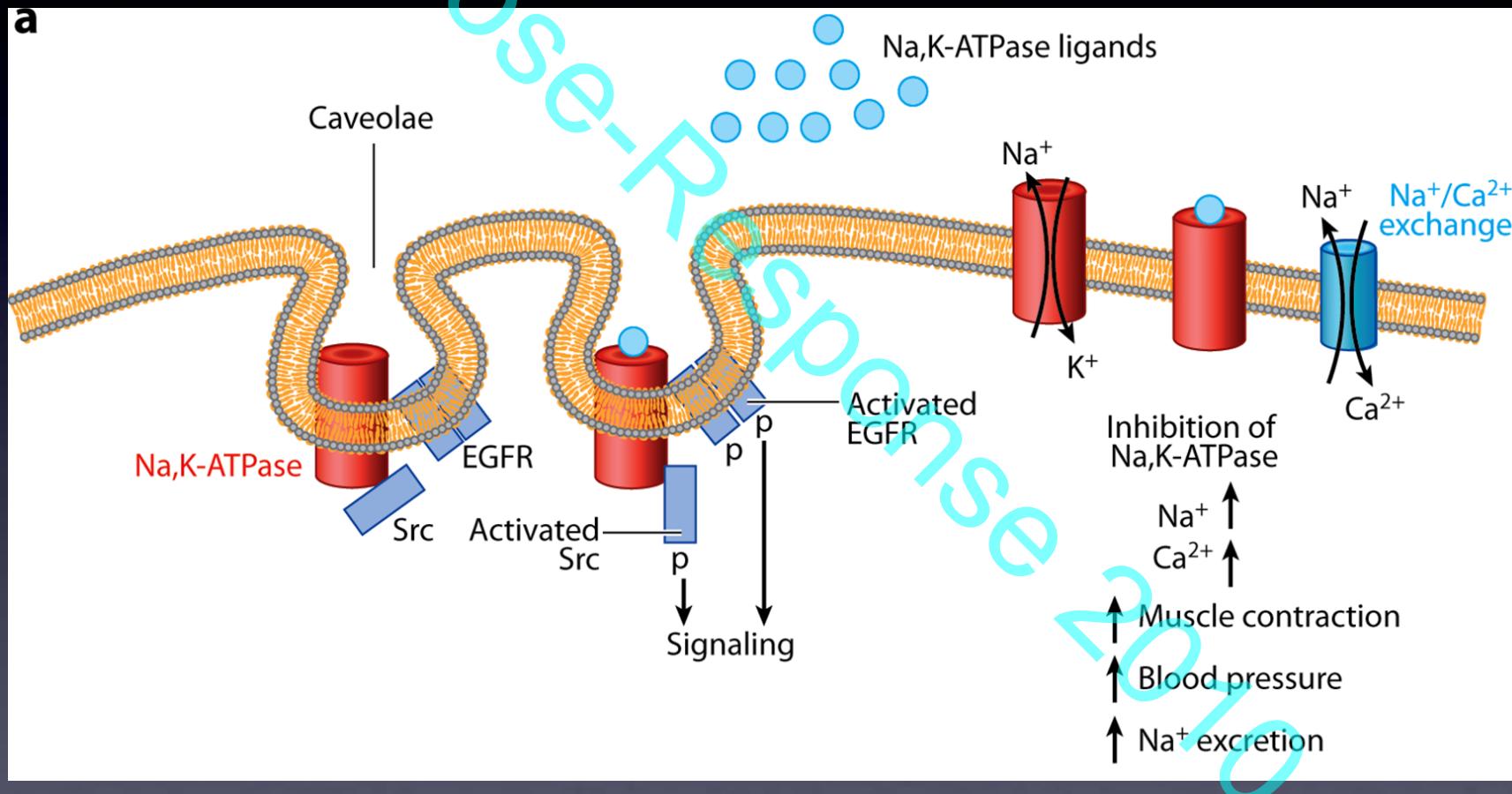
Low dose- Endogenous Action



Lingrel JB. 2010.
Annu. Rev. Physiol. 72:395–412

Cardiotonic Steroids

High dose- Pharmacological Action



Lingrel JB. 2010.
Annu. Rev. Physiol. 72:395–412

Conclusions

- Cardiotonic steroids, either inhibit or induce Na/K ATPase activity in a dose dependent manner
- Occurs both in vitro and in vivo
- Doses that induce Na/K ATPase activity also protect slice cultures from experimental ischemia
- Low-dose
 - Induce intracellular signalling
 - Mimic action of endogenous cardiotonic steroids

