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
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
Sodium and Potassium transport by Na/K ATPase
Rubidium assay of Na/K ATPase

- Incubate explant cultures with $^{86}$RbCl
- Wash
- Count
Na/K ATPase Isoforms

• $\alpha$ and $\beta$ heterodimer
• Steroid binding site is on the $\alpha$ subunit
• Three major isoforms ($\alpha_1$, $\alpha_2$, $\alpha_3$)
  • 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 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 \textit{in vitro}

Oselkin, et al., 2010
Propidium Iodide assay of cell loss in hippocampal slice cultures

Loss of membrane integrity → Nuclear PI binding

UV → hv
Experimental Ischemia

- 20% O₂, 75% N₂, 5% CO₂
- or
- 95% N₂, 5% CO₂

- Syringe Filter
- #10 Two-Holed Rubber Stopper
- 20 Gauge Needle
- 100 ml beaker containing 50 ml EBSS
- Slice cultures on Millipore-CM filter
Ouabain pretreatment protects hippocampal neurons

MBG (2nM) inhibits $\alpha_1$

DHO (20µM) inhibits $\alpha_2, \alpha_3$

Oselkin, et al., 2010
Ouabain post-treatment protects hippocampal neurons

Oselkin, et al., 2010

Dose-Response 2010
Cardiotonic Steroids
Low dose- Endogenous Action

Because of low levels of endogenous ligand, very little Na,K-ATPase would be inhibited

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
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