Small-conductance Ca2+ activated K+ Channel Activation Reduces Hippocampal Damage after Cardiac Arrest

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ABSTRACT

Introduction: Small conductance Ca^{2+}-activated K^+ (SK) channels modulate negatively to NMDA-receptors in hippocampal CA1 neurons. We hypothesized that treatments of increased SK channel activity would protect ischemia-induced damage by decreasing NMDA receptor mediated excitotoxicity. Using a well-characterized animal model, we examined if activation of SK channel with either genetically (SK2-over expression; SK2 OE) or pharmacologically with SK channel agonist, 1-EBIO, reduce neuronal damage following cardiac arrest (CA). In addition, we examined SK2 knockout mice (SK2 KO).

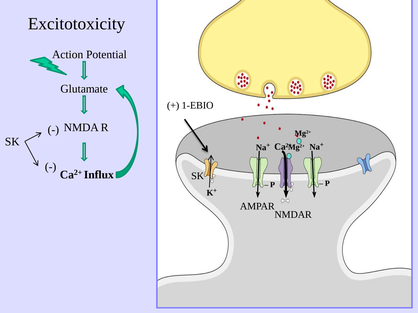
Methods: Isoflurane-anesthetized (2%) adult male WT, SK2 OE, and SK2 KO mice were subjected to CA induced by intravenous (IV) KCL. During CA, cranial temperature was raised to 38.8 ± 0.2˚C, and body temperature decreased from 37°C to 28˚C. After 8 min of CA, resuscitation was initiated with IV epinephrine (8µg in 0.5 ml 0.9% saline), ventilation with 100% oxygen and chest compressions (rate 300/min). WT male mice were injected 1-EBIO (16 mg/kg, Intraperitoneally), 30 min before CA and 6hr after resuscitation. Three days after CA, brains were harvested for injury quantification in hippocampal CA1 region (H&E staining, light-microscopy). Statistical analysis was performed using one-way ANOVA or student's t test.

Results: SK2 KO mice showed increased neuronal damage by ~30% (to 50.6±7.3% (n=8) versus 36.9±6.5% (n=10) in controls). In contrast, SK2-OE mice showed decreased neuronal damage by ~70%, to 10.5±5.2% (n=7; P<0.05). Similarly, 1-EBIO treatment significantly reduced CA1 neuronal damage compared with vehicle treatment (33.9±8.9% (n=9) versus 62.4±8.6% (n=9); P<0.05). Physiological parameters, including arterial blood gases, pH, electrolytes, lactate and blood glucose were not significantly affected by 1-EBIO administration.

Conclusions: These data indicate that genetic over-expression or 1-EBIO-mediated pharmacological activation of SK2 channels increase CA1 neuronal survival following CA. Therefore, SK2 channels may represent a new target for neuroprotection following cerebral ischemia.

BACKGROUND

- Cardiac arrest (CA) results in global cerebral ischemia that causes cognitive deficits.
- The hippocampal CA1 neurons are essential for cognitive functions and are vulnerable to cerebral ischemia.
- Cell death following global ischemia is predominantly attributed to glutamate excitotoxicity through NMDA-receptors.
- We have demonstrated that small conductance calcium-activated potassium (SK) channels function as endogenous negative modulators of NMDA-receptors in hippocampal CA1 neurons (1).



HYPOTHESIS

Treatments to activate SK channel protect ischemia-induced damage by decreasing NMDA receptor mediated excitotoxicity.

METHODS

Experimental Groups:

- Adult male wild type mice (WT), SK2 channel knock-out mice (SK2 KO) and SK2 channel over expression mice (SK2OE) were used.
- WT male mice were injected SK channel agonist, 1-EBIO (16 mg/kg, Intraperitoneally), 30 min before CA and 6hr after resuscitation.

Experimental CA/CPR Animal Model:

- Mice were anesthetized with 2% isoflurane, the jugular vein was cannulated, the trachea was intubated and mechanically ventilated (2).
- CA was induced by intravenous (IV) KCl for 8 min
- Temperature control; Cranial temperature 38.8 ± 0.2ºC
  Body temperature 37ºC → 28ºC
- CPR was initiated with IV epinephrine (8 µg), ventilation with 100% oxygen and chest compressions (rate 300/min).

Measurements:

- Three days after CA/CPR, brains were removed for analysis of damaged neurons (H&E staining, light-microscopy). The entire length of the CA1 region was counted in three levels (100 µm apart) beginning from -1.5 mm bregma and the percentage of damaged neurons calculated for each brain.

- A separate group of mice received a femoral artery catheter for continuous monitoring of blood pressure and obtaining blood samples.

RESULTS

Physiological and cardiac arrest related parameters

	EBIO	Vehicle	
	Average±SE	Average±SE	P value
Weight (g)	24.7±0.6	24.5±0.5	0.948
Total CA time (min)	9.7±0.1	9.5±0.1	0.143
Epinephrine (µg)	11.4±0.4	10.5±0.3	0.109
Epinephrine/BW (µg/g)	0.5±0.0	0.4±0.0	0.197

Blood gas data at 30 min after CPR

	EBIO			Vehicle		
	Average		SEM	Average		SEM
pH	7.0153	±	0.025	7.001	±	0.043
PaCO2	48.6	±	1.5	56.0	±	3.3
PaO2	291.2	±	1.1	306.2	±	15.5
HCO3-	12.2	±	0.8	13.7	±	1.3
BE	-18.8	±	1.1	-17.9	±	1.9
Na	149.0	±	1.4	150.1	±	1.3
K	5.7	±	0.4	6.3	±	0.4
Cl	118.0	±	1.2	122.0	±	1.6
Glu	324.3	±	48.2	271.5	±	25.3
Lac	4.2	±	0.9	4.9	±	0.9

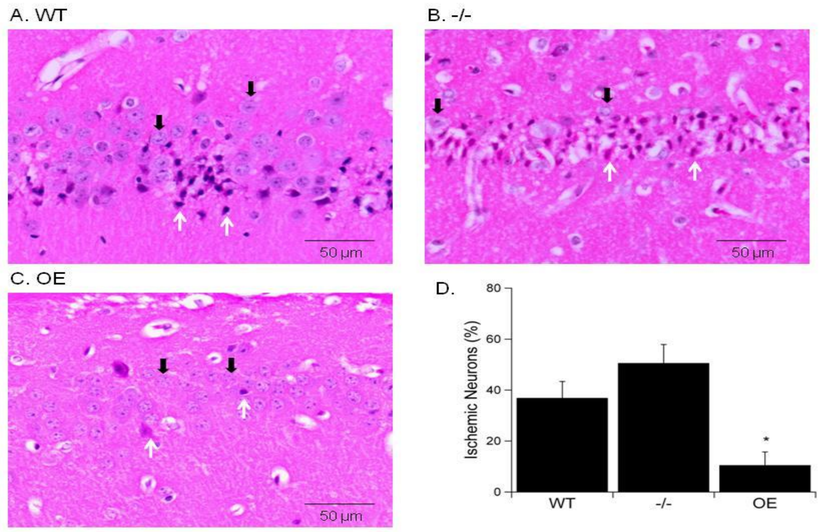


Figure 1.
SK2 KO mice showed increased neuronal damage (50.6±7.3% vs. 36.9±6.5%)
SK2-OE mice showed decreased neuronal damage (10.5±5.2% vs. 36.9±6.5%; P<0.05)

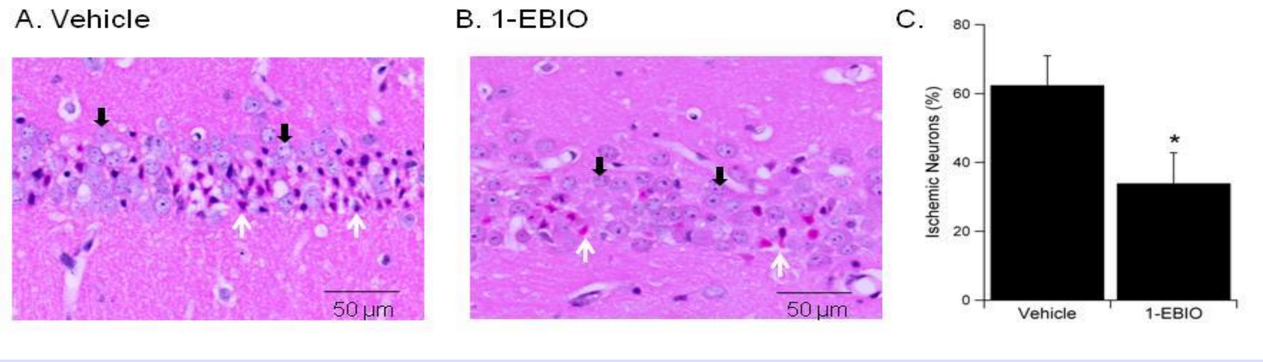


Figure 2.
1-EBIO treatment significantly reduced CA1 neuronal damage compared with vehicle treatment (33.9±8.9% vs. 62.4±8.6%; P<0.05)

CONCLUSIONS

- These data indicate that genetic over-expression or 1-EBIO-mediated pharmacological activation of SK2 channels increase CA1 neuronal survival following CA. Therefore, SK2 channels may represent a new target for neuroprotection following cerebral ischemia.

References

1. Ngo-Anh TJ et al. (2005) *Nat Neurosci* 8:642-649
2. Kofler J et al. (2004) *J Neurosci Methods* 136:33-44