Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 384 (2005) 277–281
Neuroscience Letters
www.elsevier.com/locate/neulet

Bongkrekic acid ameliorates ischemic neuronal death in the cortex by preventing cytochrome *c* release and inhibiting astrocyte activation

Marianna Muranyi [d], Ping-An Li [a,b,c,*]

[a] *Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii at Manoa, 1951 East-West Road, Auxiliary Building Room 200, Honolulu, HI 96822, USA*
[b] *Department of Medicine, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96822, USA*
[c] *Department of Complementary and Alternative Medicine, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96822, USA*
[d] *Cardiovascular Research Center, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96822, USA*

Received 29 March 2005; received in revised form 3 May 2005; accepted 3 May 2005

Abstract

Mitochondrial release of cytochrome *c* (cyt-*c*) plays a critical role in initiating cell death after cerebral ischemia. The objective of this study was to determine whether bongkrekic acid (BKA) ameliorates ischemic neuronal damage by inhibiting the release of cyt-*c*. These results showed that a 10 min period of global ischemia caused neuronal death, increased the release of cyt-*c* and activated astrocytes in the cortex and CA1. BKA treatment reduced ischemic-induced neuronal death, prevented cyt-*c* release and inhibited astrocyte activation in the cortex, but not in the CA1. These results suggest that the neuroprotective effect of BKA is associated with its ability to prevent cyt-*c* release and to inhibit astrocyte activation.

Keywords: Cerebral ischemia; Bongkrekic acid; Neuronal death; Cytochrome *c*; Astrocyte; MPT

Transient global cerebral ischemia induces delayed neuronal death in the CA1 sector of the hippocampus, neocortex, and caudate putamen [12]. Previous studies have suggested that cerebral ischemia and reperfusion injury may cause the mitochondria to form a permeability transition (MPT) pore that leads to release of cytochrome *c* (cyt-*c*), activation of a cell death signaling cascade, and eventually neuronal cell death [4]. Inhibition of MPT by cyclosporin A or its analog has been shown to markedly prevent neuronal death caused by both global and focal ischemia [17]. Bongkrekic acid (BKA), a branched unsaturated tricarboxylic acid from *Pseudomonas cocovenans*, inhibits adenine nucleotide translocator (ANT), thus preventing the opening of the MPT and release of procaspase-9 in vivo [3]. Although the effects of BKA on ANT inhibition have been well characterized in vitro, only one study has been published on the effect of this MPT blocker in vivo after focal ischemia [3]. The objective of this study was to explore if BKA ameliorates ischemic neuronal damage by inhibiting the release of cyt-*c* from the mitochondria in vivo after transient global cerebral ischemia.

Sixty male Wistar rats (Simonden Laboratory, Gilrey, CA, USA), weighing 280–320 g, were used. All animal use procedures were performed in strict accordance with the *NIH Guide for Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of Hawaii. BKA (4 μg/kg, 8 μg/kg or 16 μg/kg) was injected intracerebroventricularly (i.c.v.) 30 min before ischemia followed by intraperitoneal (i.p.) injections daily for 3 days after reperfusion. i.c.v. injections were performed using a 10 μl Hamilton syringe (needle outer diameter 450 μm). Eight microliters of BKA solution was stereotactically de-

* Corresponding author. Tel.: +1 808 956 6464; fax: +1 808 956 7316.
E-mail address: pali@pbrc.hawaii.edu (P.-A. Li).

0304-3940/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2005.05.011

posited in the right ventricule, at −0.8 mm caudal to bregma, 1.4 mm lateral to midline and 3.5 mm ventral to dura. Artificial cerebrospinal fluid was injected in control animals. Ten minutes global ischemia was induced as described before [7,15].

Rats used for histology and immunocytochemistry were perfusion-fixed with 4% paraformaldehyde. Brains were sectioned on a vibrating microtome at 30 μm thickness for histology and immunocytochemistry. Examination of histological outcomes in the cortex and hippocampal CA1 area were performed using Fluoro-Jade (0.001%, Histo-Chem) stained sections at the level of bregma −3.8 mm [14]. Immunocytochemistry of glial fibrillary acid protein (GFAP) was conducted as described before [10]. The primary antibody was a rabbit anti-GFAP polyclonal antibody (1:300, DAKO) and secondary antibody was rhodamine-conjugated

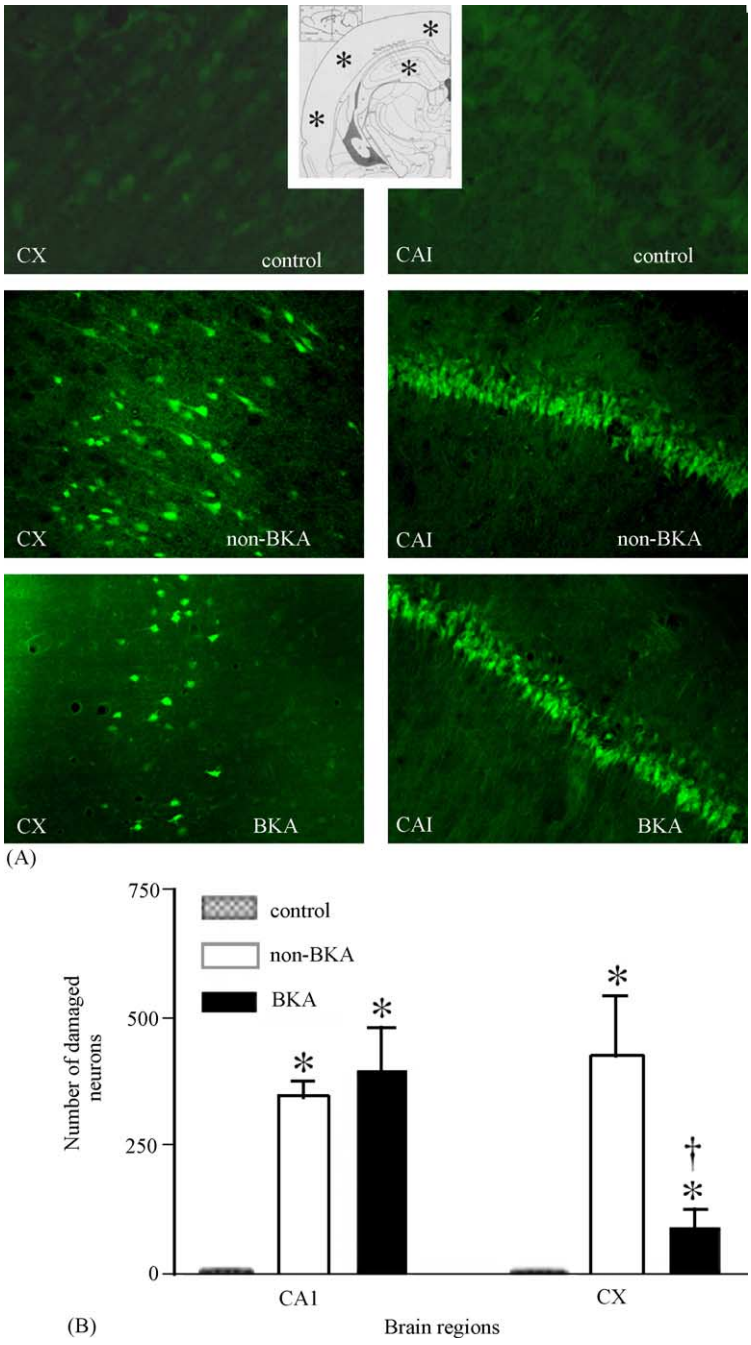

Fig. 1. (A) Photomicrographs showing brain damage in the cortex (CX) and CA1 region of the hippocampus (CA1) after 7 days of recovery in sham-operated controls, vehicle- and BKA-treated animals. Insert: Rat brain coronal section at the level of −3.8 mm to bregma. Symbol (*) indicates sites of microscopical fields (200×) where Fluoro-Jade-positive cells were counted. (B) Bar graph showing the number of Fluoro-Jade positively stained neurons. Data are mean ± S.D. ($n = 7$ per time point). $^{*}P < 0.05$ vs. sham-operated control and $^{\dagger}P < 0.05$ vs. non-treated samples at an identical time point.