Dexmedetomidine, an α-2a adrenergic agonist, promotes ischemic tolerance in a murine model of spinal cord ischemia-reperfusion

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Objective: Dexmedetomidine, an α-2a adrenergic agonist, given pre- and postoperatively was previously shown to attenuate neuronal injury in a murine model of spinal cord ischemia-reperfusion. In the brain, α-2 agonists have been shown to induce the phosphorylation of cyclic AMP response-element binding protein (CREB), a transcription factor necessary for neuron survival. We hypothesized that the α-2a adrenergic agonist given preoperatively increases CREB-mediated neuroprotective proteins, attenuating neuronal injury and cytoarchitectural decay.

Methods: Mice (ie, C57BL/6 mice) underwent 5 minutes of aortic occlusion via median sternotomy. Mice received 25 μg/kg dexmedetomidine or equivalent normal saline at 24 hours, 12 hours, and 30 minutes preoperatively. Functional outcomes were recorded at 6 to 48 hours postoperatively when spinal cords were removed for histologic analysis. Spinal cords were examined for protein kinase B, CREB, B-cell lymphoma 2, and brain-derived neurotrophic factor following treatment alone or ischemia-reperfusion surgery.

Results: Following aortic occlusion, mice in the treatment group had preserved neurologic function at all time points ($P < .05$). Histologic analysis showed preserved cytoarchitecture and decreased neuronal injury in the treatment group when compared with ischemic controls. Additionally, analysis of spinal cord homogenate following surgery and pretreatment revealed a significant ($P < .05$) increase in B-cell lymphoma 2 and brain-derived neurotrophic factor expression and protein kinase B and CREB phosphorylation with α-2a adrenergic agonist pretreatment.

Conclusions: Pretreatment with the α-2a agonist dexmedetomidine preserved neurologic function and attenuated neuronal injury following thoracic aortic occlusion in mice. This relationship was associated with an increased phosphorylation of protein kinase B and CREB and subsequent up-regulation of antiapoptotic factor B-cell lymphoma 2 and brain-derived neurotrophic factor. Thus, α-2a receptor agonism-induced CREB phosphorylation and contributes to dexmedetomidine's protective mechanism in the spinal cord following ischemia. (J Thorac Cardiovasc Surg 2014;147:500-7)

The ischemic insult to the spinal cord during thoracoabdominal aortic interventions continues to have devastating consequences in up to one fifth of high-risk patients.[1] Surgical adjuncts such as hypothermic circulatory arrest and cerebrospinal fluid drainage have reduced the incidence of the complication; however, implementation of these measures has only been met with a partial reduction of postoperative paraplegia.[2]

Ischemia-reperfusion (IR) injury is complex and poorly understood. Neurons are the most vulnerable cells to ischemia and coupled with their inability to regenerate, spinal cord IR is a significant threat to patients. Pharmacologic adjuncts that increase ischemic tolerance by neurons would serve as an ideal adjunct to further reduce the risk of this complication.

The neuroprotective effects of α-2 receptor agonists have been studied for more than 20 years[3]; however, their mechanisms of protection from ischemia-reperfusion remain unknown. In cerebral neurons, clonidine and other α-2 agonists have been shown to promote phosphorylation of neuroprotective pathways.[4] One such pathway converges at cyclic AMP response-element binding protein (CREB).[5,6] CREB is a transcription factor that is critical for neuron survival after ischemia supporting its role in the induction of ischemic tolerance.[7] Among other functions, CREB induces expression of prosurvival proteins such as B-cell lymphoma 2 (BCL-2),[8] brain derived neurotrophic factor (BDNF),[9,10] and a variety of free radical scavengers.[11,12]

Dexmedetomidine is a newer and more specific α-2a agonist. It is commonly used in operating rooms and in

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ETABS

Abbreviations and Acronyms
BCL-2 = B-cell lymphoma 2
BDNF = brain-derived neurotrophic factor
CREB = cyclic AMP response-element binding protein
IR = ischemia-reperfusion
PBS = phosphate buffered saline

intensive care units. It has gained widespread attention for its neuroprotective properties in the brain.[13,14] We therefore postulated that it may have neuroprotective properties in the spinal cord. We hypothesized that pretreatment with dexmedetomidine will preserve neurologic function and neuron viability following thoracic aortic occlusion by promoting ischemic tolerance in the spinal cord through an induction of CREB-mediated neuroprotective proteins.

METHODS

Animal Procedures

The Animal Care and Use Committee at the University of Colorado at Denver Health Sciences Center approved all experiments, and this investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (www.nap.edu/catalog/5140.html). Male C57BL/6 mice between ages 10 and 20 weeks were used for all experiments.

Aortic Crossclamping: IR Surgery

Mice were anesthetized using 2% isoflurane and placed in the supine position. Surgery was performed under normothermic conditions. The core body temperature was maintained at 36.5°C ± 0.5°C using a rectal temperature probe and automatic temperature adjusting bed (Vestavia Scientific, Birmingham, Ala). The aortic arch was exposed using a cervicothoracic approach as previously described.[15] Disruption of arterial flow to the spinal cord was achieved by placing vascular clamps on the aortic arch distal to the left common carotid artery and the subclavian artery for 5 minutes. To confirm at least 90% reduction in distal flow, a laser Doppler blood flow monitor (Moor Instruments, Devon, United Kingdom) was placed over the left femoral artery.

Medication Administration

Mice in the treatment group received intraperitoneal injections of 25 μg/kg dexmedetomidine (n = 8) based on previous studies[13] or normal saline (n = 8) at 24 hours, 12 hours, and 30 minutes before IR. Additionally, 3 mice underwent sham surgery with sternotomy and dissection of the aorta and subclavian artery with no occlusion. To compare effects of medication administration alone, 4 treated mice and 4 mice given normal saline injections at time points above had spinal cords removed for protein and messenger RNA studies without IR surgery.

Functional Scores

The Basso Mouse Scale for locomotion,[16] which ranges from a score of 0 for complete paraplegia to a score of 9 for normal function, was used to quantify hind-limb function in mice after ischemia. Function was scored at 12, 24, 36, and 48 hours after reperfusion.

Histologic Analysis

After 48 hours of reperfusion, the animals were sacrificed. The vertebral column was removed en bloc from T8-L3. Spinal cords were removed from the vertebral column by injection of phosphate buffered saline (PBS) (pH 7.4) into the spinal column. Spinal cords were then transferred to 4% formalin where they remained for at least 24 hours before paraffin embedding, sectioning, and hemotoxylin and eosin staining.

Quantification of Neuron Degeneration

Spinal cords were placed into paraffin block following 48 hours of reperfusion. Sections 5-μm thick were placed on poly-lysine slides. Paraffin was removed by placing slides in 100% xylene followed by washes with 100% ethanol and 70% ethanol deionized water. Slides were then blocked with a solution of 0.06% potassium permanganate (Sigma Aldrich, St Louis, Mo) and stained with Flouro Jade-B stain (EDM Millipore, Billerica, Mass), a marker for neuron degeneration.[17] Stock solution was diluted in 0.1% acetic acid a final concentration of 0.0004% Flouro Jade-B and applied for 30 minutes. Slides were then rinsed in deionized water and placed in the dark to air-dry overnight. Once dry, 100% xylene was applied and slides were mounted with DPX (Sigma Aldrich, St Louis, Mo). Quantification of degenerating neurons was performed by a blinded observer and the number and expressed as number of degenerating neurons per high power field.

Immunoblotting

Spinal cords were removed following pretreatment or IR surgery (Figure 1). Protein extracts were obtained using M-lysis buffer (Roche, Indianapolis, Ind) per the manufacturer's instructions and were placed in a 4X Laemmmli buffer with β-mercaptoethanol and boiled for 10 minutes at 100°C, loaded into a 15-well 4% to 20% gradient-ready gels (Bio-Rad, Hercules, Calif), and run at 160 V for 450 minutes. The gels were transferred to nitrocellulose membranes at 100 V for 60 minutes and then cross-linked using a UV Stratalinker (Stratagene, La Jolla, Calif). The membranes were blocked in 5% dry milk in 0.1% Tween with PBS, and rinsed 3 times in 0.1% Tween with PBS. The blocked membranes were incubated with primary antibodies overnight at 4°C (5% bovine serum albumin in 0.1% Tween with PBS). The membranes were washed in 0.1% Tween with PBS 3 times and incubated in appropriate horseradish peroxidase-conjugated secondary antibodies diluted to 1:5000 in 5%

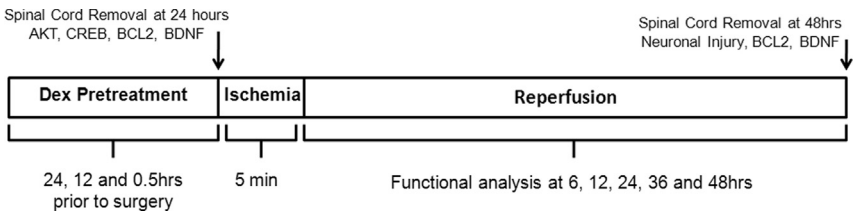

FIGURE 1. Graphic representation of the study design. *AKT*, Protein kinase B; *CREB*, cyclic AMP response-element binding protein; *BCL2*, B-cell lymphoma 2; *BDNF*, brain-derived neurotrophic factor; *Dex*, dexmedetomidine.