

Regional Variation in the Activation Threshold for 1,3-DNB-Induced Mitochondrial Permeability Transition in Brainstem and Cortical Astrocytes

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Abstract

1,3-Dinitrobenzene (DNB) produces edematous, glio-vascular lesions in brainstem nuclei with high energy demands. Astrocytes in vulnerable brainstem nuclei appear to be an early and selective target of DNB and other nitroaromatic compounds, though the molecular basis of this susceptibility is poorly understood. It has been postulated that mitochondria are a principal target of DNB in sensitive cell types [Neuropathol. Appl. Neurobiol. 13 (5) (1987) 371], where redox-cycling of DNB increases levels of reactive oxygen species and disrupts cellular energy metabolism. The present study investigates the role of regional differences in activation of the mitochondrial permeability transition pore (mtPTP) by DNB in brainstem and cortical astrocytes and examines the expression of Bcl-2 proteins as potential regulators of mtPTP function. Neonatal rat astrocytes were cultured from both DNB-sensitive (brainstem) and insensitive (cortex) brain regions and evaluated for DNB-induced alterations in cell morphology and mitochondrial function. Exposure to DNB resulted in rapid changes in the morphology of brainstem astrocytes consistent with loss of ion homeostasis and initiation of necrotic cell death. These changes were not observed in cortical astrocytes at corresponding concentrations of DNB and were prevented in brainstem astrocytes by the mtPTP inhibitor, bongkreikic acid, suggesting that mitochondrial dysfunction is involved in DNB-induced morphological changes in brainstem astrocytes. Mitochondrial depolarization in brainstem astrocytes was observed at DNB concentrations as low as 10 μM , whereas no loss of mitochondrial membrane potential ($\Delta\Psi_{\text{mi}}$) occurred in cortical astrocytes at less than 100 μM DNB. DNB-induced loss of $\Delta\Psi_{\text{mi}}$ followed apparent first-order kinetics, with EC_{50} -values for half-maximal rates of mitochondrial depolarization of ~ 23 and ~ 290 μM in brainstem cortical astrocytes, respectively. DNB-induced mitochondrial depolarization was prevented by pretreatment with bongkreikic acid, indicating that loss of $\Delta\Psi_{\text{mi}}$ was mediated by activation of the mtPTP. Inhibition of succinate dehydrogenase (SDH) activity occurred in astrocytes from both brain regions exposed to DNB and was blocked in brainstem, but not cortical, astrocytes by bongkreikic acid. Constitutive expression of Bcl-X_L was high in cortical tissue and astrocytes, whereas Bax expression was low. However, Bax was highly expressed in brainstem tissue and astrocytes and Bcl-X_L expression was markedly lower. The expression of Bcl-2 was similar in both brain regions. These data suggest that the selective vulnerability of brainstem astrocytes to DNB is due to a lower threshold for activation of the mtPTP that is mediated, in part, by distinct expression patterns of Bcl-2 proteins rather than by intrinsic differences in susceptibility of the electron transport chain.

Keywords: 1,3-Dinitrobenzene; Astrocytes; Mitochondrial permeability transition pore; Bcl-2 proteins