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DOR activation inhibits anoxic/ischemic Na^+ influx through Na^+ channels via PKC mechanisms in the cortex

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ABSTRACT

Activation of delta-opioid receptors (DOR) is neuroprotective against hypoxic/ischemic injury in the cortex, which is at least partially related to its action against hypoxic/ischemic disruption of ionic homeostasis that triggers neuronal injury. Na^+ influx through TTX-sensitive voltage-gated Na^+ channels may be a main mechanism for hypoxia-induced disruption of K^+ homeostasis, with DOR activation attenuating the disruption of ionic homeostasis by targeting voltage-gated Na^+ channels. In the present study we examined the role of DOR in the regulation of Na^+ influx in anoxia and simulated ischemia (oxygen-glucose deprivation) as well as the effect of DOR activation on the Na^+ influx induced by a Na^+ channel opener without anoxic/ischemic stress and explored a potential PKC mechanism underlying the DOR action. We directly measured extracellular Na^+ activity in mouse cortical slices with Na^+ selective electrodes and found that (1) anoxia-induced Na^+ influx occurred mainly through TTX-sensitive Na^+ channels; (2) DOR activation inhibited the anoxia/ischemia-induced Na^+ influx; (3) veratridine, a Na^+ channel opener, enhanced the anoxia-induced Na^+ influx; this could be attenuated by DOR activation; (4) DOR activation did not reduce the anoxia-induced Na^+ influx in the presence of chelerythrine, a broad-spectrum PKC blocker; and (5) DOR effects were blocked by PKCβII peptide inhibitor, and PKCθ pseudosubstrate inhibitor, respectively. We conclude that DOR activation inhibits anoxia-induced Na^+ influx through Na^+ channels via PKC (especially PKCβII and PKCθ isoforms) dependent mechanisms in the cortex.

Introduction

Finding new strategies against hypoxic/ischemic injury has been a long-term battle and attracted much attention from both scientists and clinicians in diversified fields (Fan et al., 2006; Fazzina et al., 2010; Jin et al., 2008; Lin et al., 2009; Ma et al., 2005; Pallast et al., 2010; Sun et al., 2008). One of the most important notions is that ionic disruption may initiate hypoxic/ischemic injury. Excessive disruption of ionic homeostasis is the initial and key step in hypoxic/ischemic neuronal injury and death (Chao and Xia, 2010; Sung et al., 2008). Large Na^+ influx induces cellular injury manifested as acute functional and morphological changes, e.g., loss of electrophysiological response to stimulus, cell swelling, bleb formation and membrane injury. Removal of extracellular Na^+ or blockade of Na^+ entry prevents hypoxic/ischemic neuronal damage and death (Chao and Xia, 2010). Our previous work has demonstrated that delta-opioid receptor (DOR) activation is neuroprotective against hypoxic/ischemic stress (see review by Chao and Xia, 2010); this finding is also supported by several lines of new evidence from many independent laboratories (Borlongan et al., 2009; Gao et al., 2010; Gwak et al., 2010; Turner and Johnson, 2011; Yang et al., 2011). In our mechanistic exploration, we found, besides the increase in pro-survival signaling and the enhanced anti-oxidative capacity (Feng et al., 2009), the stabilization of disrupted ionic homeostasis via the interaction of DOR with voltage-gated Na^+ channels in the early phase of the stress underlies the DOR neuroprotection against hypoxia/ischemia (Chao et al., 2008, 2009; Kang et al., 2009). For example, we observed that after being exposed to prolonged hypoxia during postnatal development, cortical neurons are more sensitive

Abbreviations: $[Na^+]o$, extracellular Na^+ concentrations; ACSF, artificial cerebrospinal fluid; CHEL, chelerythrine; DAG, diacylglycerol; DOR, delta-opioid receptors; MOR, μ-opioid receptors; NTI, naltrindole; OGD, oxygen-glucose deprivation; PKC, protein kinase C; PKCβII PI, protein kinase βII peptide inhibitor; PKCθ PI, protein kinase Cθ pseudosubstrate inhibitor; TTX, tetrodotoxin.

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to subsequent stress, which is largely attenuated by Na^+ channel blocker tetrodotoxin (TTX). Intriguingly, an increased Na^+ channel density/current (Gu and Haddad, 2003; Gu et al., 2008; Xia et al., 2000) and decreased DOR density (Xia et al., 2001) occurred in the exposed brain. Furthermore, we observed that in a mutant brain with epileptic seizures, cortical neurons are hyper-excitable with an up-regulation of voltage-gated Na^+ channels (Xia et al., 2003) and down-regulation of DOR expression (Zhao et al., 2005). Our preliminary study directly showed that DOR protects against anoxic disruption of Na^+ homeostasis in the cortex via Na^+ channel regulation (Kang et al., 2009). However, it is unknown if DOR activation can inhibit Na^+ influx induced by ischemic stress or other factors that lead to Na^+ influx without hypoxic/ischemic stress. This is an important issue to address for a better understanding of DOR's role in the regulation of ionic homeostasis and for potential applications of DOR-mediated neuroprotection in clinical settings.

Moreover, the mechanisms underlying how DOR regulates Na^+ homeostasis in the cortex is unknown. We previously observed that protein kinase C (PKC) is involved in DOR protection against neuronal injury in prolonged hypoxia (Ma et al., 2005) and against anoxic K^+ derangement in short-term anoxia (Chao et al., 2007b). It is unclear, however, if the same mechanism mediates the regulation of Na^+ homeostasis under hypoxic/ischemic stress. More importantly, there is no information available for PKC isozymes involved in the DOR neuroprotection. PKC represents a family of at least 10 different ubiquitous serine/threonine isozymes that are expressed in the brain and regulate a broad spectrum of cellular functions. PKC has been implicated in several nervous system diseases, including cerebral ischemia (Battaini, 2001; Bright and Mochly-Rosen, 2005; Chou and Messing, 2005). Various PKC isozymes mediate different and sometimes opposing functions after activation by the same stimulus (Chen et al., 2001; Wang et al., 2004). PKC isozymes such as PKCβII and PKCθ belong to different subgroups of PKC, and some of them have been linked with the functioning of Na^+ channel (Vijayaragavan et al., 2004). The expression/activities of these isozymes during hypoxia/ischemia are different (Krupiński et al., 1998; Li et al., 2006; Selvatici et al., 2003, 2006; Sieber et al., 1998), and they may play different roles in cell protection. For example, PKCβII has been shown to be neuroprotective (Cordey and Pike, 2006; Li et al., 2005), while little information is available as for the role of PKCθ in neuroprotection (Fauconnier et al., 2011). DOR belongs to a family of G-protein-coupled metabotropic receptors, whose effect is mediated by G proteins and G-protein-dependent cytoplasmic second messengers involving protein kinases (Law et al., 2000; Ma et al., 2005; Narita et al., 2006). Voltage-gated Na^+ channels are important targets modulated by metabotropic receptors via G protein/protein kinases, including PKC (Chen et al., 2006; Dascal and Lotan, 1991; West et al., 1991). Therefore, it is possible that PKC functions as a "signal bridge" between DOR and Na^+ channels, and mediates the effects of DOR on Na^+ channels in hypoxia/ischemia.

In the present study, we investigated if DOR activation inhibits Na^+ influx induced by anoxia, simulated ischemia (oxygen-glucose deprivation, OGD) and by veratridine, a Na^+ channel opener without hypoxia/ischemia and if PKC, especially PKCβII and PKCθ isoforms, is involved in DOR protection against the disruption of Na^+ homeostasis.

Materials and methods

Slice preparation

The experiments were approved by the Animal Care and Use Committee of Yale University School of Medicine, which is accredited by the American Association for Accreditation for Laboratory Animal Care. Slices of the frontoparietal cortex were prepared as described in our previous studies (Chao et al., 2007a,b). Transverse cortical slices (400 μm) were cut from the brains of 24–32 day old male C57BL/6 mice (Charles River Laboratories, Wilmington, MA) with a vibrotome containing carbogen (95% O_2, 5% CO_2) saturated ice-cold standard artificial cerebrospinal fluid (ACSF), and then transferred to an incubation holder placed in a beaker containing 150 ml ACSF vigorously aerated with carbogen at ~35 °C. Standard ACSF consisted of (in mM) NaCl 125, KCl 3.1, $NaHCO_3$ 26, $CaCl_2$ 2.4, $MgSO_4$ 1.3, NaH_2PO_4 1.25, and dextrose 10 at pH 7.4. After an equilibration period of at least 90 min in carbogen saturated ACSF at ~35 °C, slices were used for recording. The recordings were made in the outer layer (corresponding to layer II and III) of the cortex.

Induction of anoxia or oxygen-glucose deprivation (OGD) in cortical slices

A slice was transferred to the recording chamber (Model RC-22C, Warner Instrument Co., Hamden, CT) which was perfused with carbogen saturated ACSF (35.5 ± 0.5 °C) at a flow rate of ~3 ml/min. Slices were completely submerged 0.5–1 mm below the ACSF surface in the tissue chamber and kept under normoxic conditions for at least 15 min at ~35.5 °C before the experimental measurements were taken.

Anoxia was induced by switching from the control superfusate (95% O_2, 5% CO_2) to one continuously bubbled with 95% N_2 and 5% CO_2. Each slice was subjected to a single period of anoxia that continued for about 1.5 min after the onset of anoxic depolarization (as

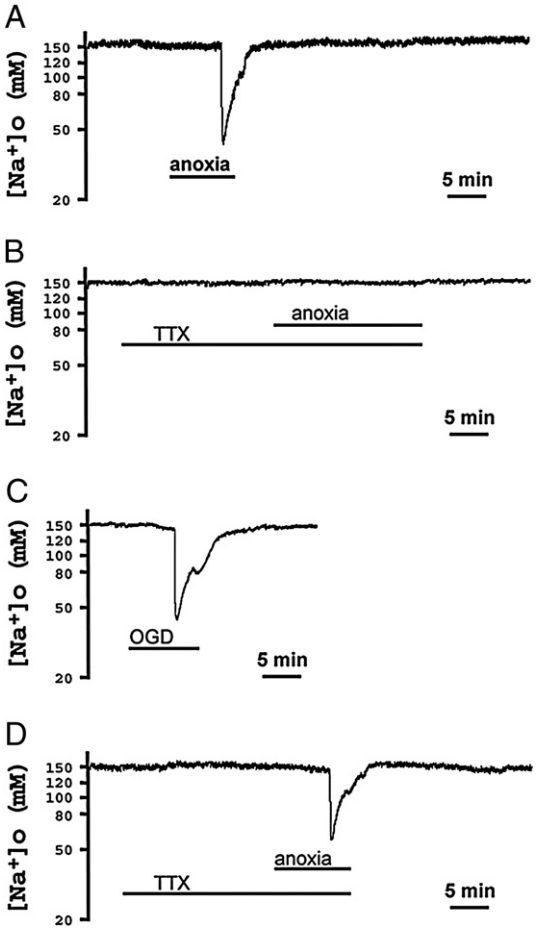

Fig. 1. Effect of Na^+ channel blockade on anoxia-induced Na^+ influx in mouse cortical slices. Anoxia induced a sudden large drop in $[Na^+]o$ (A), which could be completely abolished in most slices (7 out of 11) by voltage-gated Na^+ channel blocker, TTX (B). OGD test showed these slices in B had good viability (C). Of the remaining 4 (36%) TTX-treated slices that still responded to anoxia, two showed a reduced drop in anoxia-induced $[Na^+]o$ (D) and two had no appreciable changes in anoxia-induced $[Na^+]o$ drop. These results suggest that the blockade of voltage-gated Na^+ channels in the cortex largely blocked the anoxia-induced Na^+ influx in the cortex.