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Research Report

Cerebral preconditioning using cortical application of hypertonic salt solutions: Upregulation of mRNAs encoding inhibitors of inflammation

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ABSTRACT

Previous studies have demonstrated that local application of hypertonic KCl or NaCl to the cerebral cortex induces tolerance to a subsequent episode of ischemia. The objective of the present study was to determine whether application of these salts increases the levels of mRNAs encoding inhibitors of inflammation. Hypertonic KCl or NaCl was applied for 2 h to the frontal cortex of Sprague–Dawley rats. After recovery periods up to 24 h, levels of selected mRNAs were measured in samples from frontal and parietal cortex using Northern blots. Application of hypertonic KCl caused a rapid and widespread increase in the levels of mRNA coding for tumor necrosis factor (TNF), tristetraprolin (TTP), suppressor of cytokine signaling-3 (SOCS3), and brain-derived neurotrophic factor (BDNF), and a 24-h delayed induction of ciliary neurotrophic factor (CNTF) mRNA. Application of hypertonic NaCl caused alterations in mRNA levels that were restricted to the frontal cortex. In this region, application of NaCl rapidly increased levels of mRNA encoding TNF, TTP, and SOCS3, but not BDNF, and caused a delayed induction of CNTF mRNA. These results raise the possibility that upregulation of inhibitors of inflammation after preconditioning may contribute to the induction of tolerance to ischemia.

1. Introduction

Preconditioning the brain with a variety of sublethal stimuli induces profound tolerance to a subsequent episode of ischemia (Dirnagl et al., 2003; Kirino, 2002). One of the preconditioning stimuli that has been employed is cortical spreading depression (CSD) (Kawahara et al., 1995; Kobayashi et al., 1995; Matsushima et al., 1996). In experimental models of preconditioning, CSD is commonly evoked by applying a high concentration of KCl to the cerebral cortex for a period of 1–2 h. Application of KCl not only triggers multiple episodes of CSD, but also produces a small cortical lesion at the application site (Kobayashi et al., 1995). Thus, the induction of tolerance to ischemia following application of KCl may be a consequence of CSD, the cortical lesion, or both. Recently, cortical application of hypertonic NaCl, like KCl, was shown to cause a small cortical lesion and induce tolerance to ischemia (Muramatsu et al., 2004). Importantly, application of NaCl, unlike KCl, failed to evoke CSD. Thus, the presence of a cortical lesion by itself appears to be sufficient to induce tolerance to ischemia. The molecular mechanisms by which application of hypertonic salt solutions trigger neuroprotective pathways, however, remain poorly understood.

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Application of KCl to the cerebral cortex has previously been shown to increase the expression of proinflammatory cytokines, including tumor necrosis factor (TNF) and interleukin-1β (IL-1β) (Jander et al., 2001). Expression of these cytokines has been linked to ischemic tolerance in other models of cerebral preconditioning (Tasaki et al., 1997; Wang et al., 2000). Indeed, direct administration of TNF or IL-1β has been shown to induce tolerance to ischemia (Nawashiro et al., 1997; Ohtsuki et al., 1996), suggesting that inflammatory cytokines might mediate neuroprotection in experimental models of preconditioning. Inflammatory mediators could induce protective preconditioning by initiating counter-regulatory mechanisms that reduce inflammation caused by a subsequent insult (Mantovani et al., 2004). Feedback inhibitors of inflammatory cytokines and Toll-like receptors (TLRs) have been shown to limit the degree, duration, and spatial dissemination of inflammation. The counter-regulatory mechanisms include upregulation of anti-inflammatory cytokines, decoy receptors, and intracellular feedback inhibitors (Kariko et al., 2004). Recent studies have identified a number of intracellular feedback inhibitors that suppress the inflammatory response to harmful stimuli.

Tristetraprolin (TTP) promotes degradation of mRNAs encoding proinflammatory cytokines such as TNF, thus limiting their expression (Carballo et al., 1998). In a canine model of cardiac ischemia–reperfusion, TTP was identified as a potential mediator of protection following ischemic preconditioning (Zubakov et al., 2003).

Suppressor of cytokine signaling-3 (SOCS3) inhibits signaling through cytokines such as IL-1, IL-6, TNF, and interferon-γ (Kubo et al., 2003), thereby inhibiting inflammation (Alexander and Hilton, 2004). Interference with SOCS3 synthesis has been shown to exacerbate ischemic damage in rat brain (Rao et al., 2002), suggesting a neuroprotective role for SOCS3.

The inactive kinase IRAK-M (IL-1 receptor-associated kinase M) and the adaptor TOLLIP (Toll-interacting protein) both bind to signaling components of TLRs thereby blocking signal transduction (Kobayashi et al., 2002; Zhang and Ghosh, 2002). The presence of these inhibitors following a preconditioning stimulus would be expected to attenuate inflammation during a subsequent episode of ischemia and, thus, diminish the extent of ischemic injury. However, the induction of these inhibitors of inflammation has not been previously investigated in models of cerebral preconditioning. Thus, the primary objective of the present study was to determine whether preconditioning with hypertonic salts triggers expression of selected inhibitors of inflammation. A secondary objective was to compare the induction of the inhibitors after preconditioning with KCl and NaCl to determine whether CSD is required for their induction. A final objective was to compare the effects of KCl and NaCl on levels of mRNA encoding the trophic factors, brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF), the latter of which has been associated with the induction of SOCS3 and TTP (Kelly et al., 2004).

2. Results

2.1. *Physiologic variables*

Physiologic variables were in the normal range prior to application of KCl or NaCl (Table 1). In animals undergoing application of KCl, the numbers of episodes of CSD detected were 20 ± 3 (mean ± SD), 16 ± 2, 16 ± 6, and 18 ± 4 for the 0 h, 2 h, 4 h, and 24 h groups, respectively (Fig. 1). CSD was not detected in animals undergoing application of NaCl.

2.2. *Application of KCl: Northern blots*

Application of 2 M KCl to the frontal cortex for 2 h caused a rapid and widespread increase in cortical levels of mRNAs encoding TNF, TTP, SOCS3, and BDNF (Fig. 2). The induction of these transcripts was most pronounced in the frontal cortex, which included the KCl application site. However, the levels of these transcripts were also increased in the parietal and occipital cortex (not shown), most prominently at 0 and 2 h of recovery (Fig. 2). In the frontal cortex, levels of TNF and BDNF mRNAs in the ipsilateral hemisphere were significantly higher than those in the contralateral hemisphere up to 4 h before subsiding by 24 h. In the same region, levels of TTP and SOCS3 mRNA were significantly increased in the ipsilateral hemisphere at 0 h and 2 h, and SOCS3 mRNA remained significantly elevated at 24 h. By contrast, levels of IRAK-M and TOLLIP mRNA were not significantly different between hemispheres

Table 1 – Physiologic variables

Salt/Recovery time	Body weight (g)	Arterial pH	Arterial pCO_2 (mm Hg)	Arterial pO_2 (mm Hg)	MABP (mm Hg)	Rectal Temp (°C)
KCl						
0 h	262 ± 14	7.39 ± 0.03	42 ± 5	130 ± 35	90 ± 11	37.3 ± 0.3
2 h	344 ± 43	7.42 ± 0.03	41 ± 4	138 ± 34	63 ± 2	37.4 ± 0.6
4 h	343 ± 71	7.45 ± 0.12	43 ± 9	118 ± 29	82 ± 8	37.7 ± 0.2
24 h	270 ± 28	7.40 ± 0.02	38 ± 5	147 ± 30	86 ± 1	37.2 ± 0.3
NaCl						
0 h	291 ± 78	7.41 ± 0.05	39 ± 7	114 ± 41	80 ± 8	37.4 ± 0.0
2 h	302 ± 36	7.45 ± 0.03	36 ± 2	130 ± 30	88 ± 14	37.5 ± 0.4
4 h	341 ± 53	7.45 ± 0.05	39 ± 2	104 ± 45	78 ± 7	37.5 ± 0.2
24 h	313 ± 27	7.45 ± 0.03	36 ± 2	139 ± 45	70 ± 6	37.2 ± 0.1

Values are means ± SD, n = 4 animals per group.