Published in final edited form as:

J Neuroimmunol. 2009 December 10; 217(1-2): 14–19. doi:10.1016/j.jneuroim.2009.06.001.

Ischemic preconditioning-induced neuroprotection is associated with differential expression of IL-1 β and IL-1 receptor antagonist in the ischemic cortex

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Abstract

Ischemic preconditioning (IP) is a phenomenon that organs develop a tolerance toward subsequent lethal ischemic insults. Among the factors that are involved in IP, IL-1 β and its endogenous receptor antagonist IL-1ra have been identified as important players in the induction of IP. The present study investigated whether IP affects the levels of these two antagonistic proteins during tolerance and reperfusion periods after ischemic stroke. The IP 24 h prior to ischemic stroke resulted in neuroprotection in the cortex. IP-induced protection is accompanied by increased IL-1 β gene and IL-1ra gene and protein levels during the tolerance period. In the post-ischemic cortex, IP resulted in the suppression of IL-1 β mRNA and protein levels at 6 h without affecting IL-1ra expression and the up-regulation of IL-1ra protein at 24 h. These findings demonstrate that IP differentially regulates cortical IL-1 β and IL-1ra expression before and after ischemic stroke and suggest that the shift toward an anti-inflammatory state in the post-ischemic cortex may contribute to IP-induced neuroprotection.

Keywords

IL-1 β ; IL-1 receptor antagonist; ischemic preconditioning; bilateral common carotid artery occlusion; middle cerebral artery occlusion

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1. Introduction

Ischemic preconditioning (IP) triggered by a sub-lethal stimulus induces tissue tolerance toward subsequent full insult through modulation of endogenous molecules (Swain et al., 1984; Murry et al., 1986; Dirnagl et al., 1999; Gidday, 2006). The IP-induced protective effect against ischemic and reperfusion injuries has been demonstrated in a variety of organs including heart, lung, liver and brain (Kitagawa et al., 1990; Barrier et al., 2005; Cho et al., 2005; Pasupathy and Homer-Vanniasinkam, 2005). In the brain, brief global or focal ischemic stimuli several minutes to hours before serious ischemic injury attenuate neuronal cell death and infarct volumes in animal models of stroke (Ohtsuki et al., 1996; Barone et al., 1998; Cho et al., 2005). Although multiple protective mechanisms involving neurotransmitters, kinases, transcription factors and cytokines have been suggested for the induction of ischemic tolerance, the molecular mechanism that govern IP-induced neuroprotection and effector modules that account for the protection is not fully defined (Kirino, 2002; Dirnagl et al., 2003; Dirnagl and Meisel, 2008).

Interleukin-1 β (IL-1 β), a pro-inflammatory cytokine, has been implicated in ischemic brain injury. For instance, exogenous administration of IL-1 β increases brain injury while immunoneutralization of IL-1 β reduces ischemic brain damages (Yamasaki et al., 1995; Loddick and Rothwell, 1996). The role of IL-1 β in ischemic brain is further supported by showing that its endogenous receptor antagonist (IL-1ra), which block IL-1 mediated signaling, was neuroprotective (Relton and Rothwell, 1992; Betz et al., 1995). Literature shows that exogenously administration of IL-1ra or overexpression of IL-1ra gene in mice is protective in cerebral ischemia (Relton and Rothwell, 1992; Betz et al., 1995) and deletion of the IL-1ra gene was shown to increase neuronal injury (Pinteaux et al., 2006). These studies suggest that repression of IL-1 β and/or increase of IL-1ra can be a useful therapeutic target for ischemic stroke.

In spite of the contributing role of IL-1 β in ischemic injury, low levels of IL-1 β are reported to play a key role in the induction of ischemic tolerance. A study showed that global IP in gerbil increased IL-1 β levels in the blood and that systemic IL-1 β administration prior to ischemia protected CA1 neurons, which was disappeared by co-administration of IL-1ra (Ohtsuki et al., 1996). Similarly, a preconditioning by brief (15 min) middle cerebral artery occlusion (MCAO) in spontaneously hypertensive rats induced low-level IL- β expression in the cortex during the tolerance period, indicating a contribution to ischemic tolerance by IL-1 β (Wang et al., 2000). In the same model, Barone and the colleagues reported an increase in IL-1ra expression in the cortex during the tolerance period (Barone et al., 1998). Considering that IL-1ra blocks biological activity of IL-1 β , it is difficult to address the roles for these antagonistic molecules in IP-induced neuroprotection. Since the balance between IL-1 β and IL-1ra affects the development of brain diseases (Arend, 2002), evaluation on the simultaneous, not individual, changes of IL-1 β and IL-1ra would provide a better understanding in their roles in IP mechanisms. In addition, IP might have an impact on the ischemia-induced expression of IL-1 β or IL-1ra because these molecules are key mediators determining the extent of ischemic injury (Allan et al., 2005). The compounding effects of ischemic stroke on IP-induced changes in IL-1 β and IL-1ra expressions thus require an investigation on the temporal and relative changes of both IL-1 β and IL-1ra levels during the tolerance and post-ischemic reperfusion periods to define their roles in IP-induced protection.

We and others established repetitive brief bilateral common carotid artery occlusion (BCCAO) 24 h before transient MCAO as a late preconditioning paradigm that develops tolerance within hours to days after stimulus and requires gene activation and protein synthesis (Kitagawa et al., 1990; Cho et al., 2005). Using the repetitive BCCAO

preconditioning paradigm, the present study investigates whether IP-induced neuroprotection is associated with temporal changes of both IL-1 β and IL-1ra expression during the tolerance and post-ischemic reperfusion periods.

2. Materials and Methods

Experiments were performed in male C57BL/6 mice (age 10 to 11 weeks weighing 24–26 g, Koatech, Republic of Korea). All procedures were approved by the Institutional Animal Care and Use Committee at the Medical School of Ewha Womans University and confirmed to international guidelines on the ethical use of animals. The number of animals used for the study was minimized to reduce animal suffering.

2.1. Ischemic preconditioning by bilateral common carotid artery occlusion (BCCAO)

Procedures for BCCAO preconditioning have been established and published previously (Cho et al., 2005). Briefly, mice were anesthetized with isoflurane (1.6–2%) and a fiber optic probe was glued to the right parietal bone (2 mm posterior and 5 mm lateral to bregma) and connected to a laser-Doppler flowmeter (Periflux System 5010, Perimed, Sweden). Cerebral blood flow (CBF) was continuously recorded using a computer-based data acquisition system (Perisoft). Ischemic preconditioning was induced by 3 episodes of 1 min occlusion of both common carotid arteries, each followed by 5 min of reperfusion (IP group, Fig. 1B). Wounds were closed and animals were returned to their cages. In the sham-operated mice, the carotid arteries were exposed for 15 min without occlusion (sham group). Control animals did not receive any surgical preconditioning stimulus (control group).

2.2. Transient middle cerebral artery occlusion (MCAO)

Time window of BCCAO IP paradigm for ischemic stroke was previously defined by Cho et al. (2005). The tolerance was induced specifically at 24 h, but not 3 h or 72 h after BCCAO. Therefore, we confirmed the tolerance against ischemic stroke at 24 h after BCCAO IP in this study (Fig. 1A). Mice were reanesthetized with isoflurane 24 h after BCCAO or sham operation, and the fiber optic probe was reattached to the right parietal bone (2 mm posterior and 5 mm lateral to bregma) and connected to a laser-Doppler flowmeter. CBF was continuously recorded during MCAO and reperfusion periods (Fig. 1C). Also, some mice without BCCAO or sham manipulation underwent MCAO (control +MCAO group).

Techniques for transient MCAO with an intravascular suture have been described previously (Park et al., 2004; Cho et al., 2005). Briefly, a 6-0 silicon-coated black monofilament surgical suture (Doccol Cooperation, Redlands, CA, USA) was inserted into the exposed right external carotid artery, advanced into the internal carotid artery, and wedged into the circle of Willis to obstruct the origin of the MCA. The filament was left in place for 30 minutes and then withdrawn to re-establish CBF. Only animals that exhibited a reduction in CBF >85% during MCA occlusion and in which CBF recovered by >80% after 10 min of reperfusion were included in the study. Rectal temperature was maintained at 37.0 $\pm$ 0.5°C using a thermostatically controlled heating pad, both during surgery and during the recovery period until the animal regained consciousness.

2.3. Infarct volume measurement

Infarct volume was measured according to the procedures described previously (Lin et al., 1993; Park et al., 2004). Mice were sacrificed 72 h after MCA occlusion and brains were removed, frozen and sectioned (30 μ m thicknesses) in a cryostat. Brain sections were collected serially at 600 μ m intervals, and stained with cresyl violet. Infarct volume was

determined using an image analyzer (Axiovision LE 4.1, Carl Zeiss, Germany). Values were reported after they were corrected for post-ischemic swelling.

2.4. Real-time PCR analysis

Total RNA was prepared from the cortex of ipsilateral hemisphere using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). Samples were sonicated in 0.5 ml Trizol, purified using chloroform, precipitated with isopropanol, and dissolved with diethylpyrocarbonate-treated water (iNtRON Biotechnology, Republic of Korea). First-strand cDNA was synthesized with 1 µg of total RNA from each sample and 1 µl of oligo (dT)₁₅ (Bioneer, Republic of Korea) using the Power cDNA Synthesis kit (iNtRON Biotechnology). 2 µl of diluted cDNA (1:10) was amplified with SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) in a final volume of 20 µl. The PCR reaction was performed using ABI prism 7000 sequence detector (Applied Biosystems). The PCR program consisted of initial denaturation at 95 °C for 5 min and 40 cycles at 95 °C for 30 sec, 52 °C for 30 sec, and 72 °C for 45 sec. The primers for cyclophilin (forward, 5'-TGAAGTTGTCCACAGTCAGC-3', and reverse, 5'-TTCATCTGCACTGCCAAGAC-3'), IL-1β (forward, 5'-AATGATCTGTTCTTTGAGGCTGAC-3', and reverse, 5'-CGAGATGCTGCTGTGAGATTTGAAG-3') and IL-1ra (forward, 5'-GACCTTCTACCTGAGGAACAACCAG-3', and reverse, 5'-AAGAACACATTCCGAAAGTCAATAGG-3') were purchased from Bioneer. Cycling threshold (Ct) values of IL-1β and IL-1ra were normalized to Ct values of cyclophilin and relative expression levels were calculated by $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001). All samples were run in triplicate and control animals without MCAO (n = 3) served as controls.

2.5. Western blot analysis

The cortical tissue obtained from the ipsilateral hemisphere was lysed in sodium dodecyl sulfate (SDS)-buffer (62 mM Tris-HCl, 1 mM ethylenediamine tetraacetic acid, 2% SDS, pH 6.8–7.0) containing one tablet of a protease inhibitor cocktail (Complete Mini, Boehringer Mannheim, Germany) per 10 ml of solubilizing buffer then incubated for 20 min on ice, and centrifuged at 1300 rpm for 10 min at 4 °C. The protein concentration of the supernatant was determined (Bio-Rad Laboratories, Hercules, CA, USA) and 100 µg of protein was loaded for SDS-polyacrylamide gel electrophoresis. Proteins were transferred onto Immobilon-P transfer (Millipore Corporate, Billerica, MA, USA) membranes using an electroblotting apparatus. Membranes were blocked overnight in Tris-buffered saline (TBS) containing 0.1% Tween-20 and 5% dry milk, incubated with anti-IL-1β (17 kDa, 1:100, R&D, Minneapolis, MN, USA) or -IL-1ra (17 kDa, 1: 200, R&D) antibody overnight and washed three times (30 min each) with TBS containing 0.1% Tween-20. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h each and then washed three times (30 min each) with TBS containing 0.1% Tween-20. Protein bands were visualized with the ECL Plus Western blotting Luminol Reagent (Santa Cruz Biotechnology, CA, USA). The membrane was stripped with antibody stripping solution (Chemicon International, Temecula, CA, USA) according to the manufacturer's instruction and reblotted to visualize actin (1:1000; Santa Cruz Biotechnology). For quantification, densities of IL-1β and IL-1ra were normalized to corresponding blots for actin. To determine the physiological relevance of these proteins, the levels of IL-1β and IL-1ra in the brain tissue were analyzed against the known concentration of recombinant IL-1β (17 kDa, cat. No. 481-ML/CF, R&D) and IL-1ra (17 kDa, cat. No. 480-RM/CF, R&D) proteins by Western blot.

2.6. Statistical analysis

The data are expressed as mean $\pm$ SEM. Multiple comparisons were evaluated by the analysis of variance and followed by post hoc Fisher's PLSD tests using Statview (Statview version 5, SAS Institute, USA). Differences were considered significant at $p < 0.05$.

3. Results

3.1. Neuroprotective effects in the cortex by ischemic preconditioning

The experimental timeline for BCCAO IP, subsequent MCAO, and outcome measurement was shown in Fig. 1A. Three intermittent BCCAOs induced more than 80% CBF reduction and immediate reperfusion upon release of both common carotid arteries (Fig. 1B). There was similar degree of % CBF reduction and reperfusion during and after MCAO in all three groups (Fig. 1C). To confirm that BCCAO IP paradigm results in ischemic protection, infarct volume was determined 3 days after MCAO. In agreement with previous reports (Cho et al., 2005), infarct size was markedly reduced in the IP group compared to that in control and sham groups (-45% , Fig. 2). The reduction of ischemic injury occurred predominantly in the cortex (-48%) but not in the striatum (Fig. 2B). Furthermore, there was no difference in infarct volume in control and sham groups, excluding possible protective effects of anesthesia. These findings suggest that the neuroprotection by BCCAO IP involves factors other than CBF and anesthesia.

3.2. Ischemic preconditioning increases IL-1 β and IL-1ra levels in the cortex before MCAO

Because the protective effect of IP was limited in the cortex, the rest of the study used cortical tissues to determine gene and protein expressions. To test whether protective effects of IP in the cortex are associated with IL-1 β and IL-1ra expressions, temporal changes of IL-1 β and IL-1ra levels between 6 h and 48 h after IP were assessed. BCCAO IP induced significant IL-1 β gene expression (2.4-fold) compared to those of the sham group (1.8-fold) at 24 h (Fig. 3B). Compared to the sham group, IL-1ra mRNA level in the IP group was significantly increased at 6 h (1.7-fold) and 24 h (3.5-fold; Fig. 3B). In the sham group, neither IL-1 β nor IL-1ra mRNA levels differed at any time point compared to the control group. These findings suggest that both IL-1 β and IL-1ra mRNA levels are maximally increased in the cortex at 24 h after IP.

We further determined the protein levels in the cortex at 24 h and 48 h after IP. The concentrations of IL-1 β and IL-1ra proteins estimated against known recombinant standards fell into several pg/100 μ g of total protein of the cortex, indicating physiological relevant concentrations as these proteins were shown to present in pg ranges in the stimulated brain (O'Connor et al., 2003; Palin et al., 2004; Valero et al., 2007). Protein expressions of IL-1 β and IL-1ra at 24 h in both sham and IP groups were significantly increased compared to those in the control group (1.8-fold and 2.3-fold for IL-1 β , 1.2-fold and 1.5-fold for IL-1ra, respectively; Fig. 4). Unlike mRNA levels, a significant difference was observed only in IL-1ra protein level compared to the sham group. At 48 h, IL-1 β levels were increased in both sham and IP groups compared to the control group, but no statistical difference was observed between sham and IP groups. These findings suggest that IP selectively increases IL-1ra protein level at 24 h.

3.3. Ischemic preconditioning reduced expression of IL-1 β in the cortex after MCAO

To investigate the effect of BCCAO IP on ischemia-induced IL-1 β expression in the cortex, IL-1 β mRNA and protein levels were determined in the ipsilateral side after MCAO. Compared to the control group, IL-1 β mRNA levels were markedly increased in the sham group at 6 h and 24 h after MCAO (7.9- to 8.0-fold, Fig. 5A). The increase in IL-1 β gene expression was significantly repressed in the IP group especially at 6 h (0.4-fold compared

to the sham group, Fig. 5A). The level of IL-1 β gene went down at 72 h but it was still higher in the IP group compared to the sham group (1.5-fold). For protein expression, a significant increase of IL-1 β was shown in the sham group compared to control (4.1-fold) at 6 h. Similar to the mRNA levels, the increase was repressed in the IP group (0.6-fold, Fig. 5B). At 24 h and 72 h, IL-1 β expression was weak and there was no difference between groups. Taken together, the findings suggest that BCCAO IP reduces MCAO-induced IL-1 β expression at early reperfusion period in the cortex.

3.4. Ischemic preconditioning increased expression of IL-1ra in the cortex after MCAO

We next determined the effect of BCCAO IP on ischemia-induced IL-1ra expression in the cortex. In the sham group, IL-1ra mRNA level was increased at 6 h (64.6-fold) and 24 h (263.6-fold); at 72 h the level was decreased but still remained increased compared to the control group (23.8-fold, Fig. 6A). IP further increased IL-1ra gene expression at 24 h, but there was no statistically significant difference between sham and IP groups. Similar to mRNA changes, IL-1ra protein levels were increased at 6, 24 and 72 h in the sham group compared to the control group (1.9-, 2.2- and 1.3-fold, respectively, Fig. 6B). At 24 h reperfusion, IP-induced IL-1ra protein level was significantly different from that of sham group (1.5-fold). The findings suggest that BCCAO IP results in a reciprocal expression of MCAO-induced up-regulation of IL-1ra and down-regulation of IL-1 β during reperfusion.

4. Discussion

In the present study, BCCAO IP paradigm significantly attenuated ischemic injury in the cortex only. Since the striatum represents the ischemic core, IP-induced protection may be less effective in rescuing the necrotic core. However, the observation of reduced infarct size in the cortex is consistent with the idea that the penumbra is the area that can be rescued. To understand the mechanism by which IP-induced protection occurs, the current study determined the expression of IL-1 β and its endogenous antagonist IL-1ra gene and protein during the induction of tolerance and after MCAO in the brain. We report that BCCAO IP differentially regulates IL-1 β and IL-1ra gene and protein expressions. Specifically, IL-1 β protein level was mildly increased during a tolerance period but down-regulated after MCAO in the preconditioned cortex. On the other hand, IP-induced IL-1ra protein level was further up-regulated following MCAO. The findings of elevated IL-1ra with repressed IL-1 β expression suggest that BCCAO IP paradigm causes a shift of IL-1 β and IL-1ra gene/protein expression toward an anti-inflammatory state in the post-ischemic cortex.

IL-1 β is thought to be a preconditioning stimulus as systemic administration of IL-1 β prior to ischemic insults reduces neuronal injury, which is reversed by co-administration with IL-1ra (Ohtsuki et al., 1996). In addition, increased expression of IL-1 β protein in glial cells in the cortex after IP was demonstrated during tolerance periods (Wang et al., 2000). In our BCCAO IP model, we showed increases in IL-1 β mRNA expression levels at the time of tolerance period but its protein level did not reach the statistical significance. Mild increase of IL-1 β by BCCAO IP compared to the reports of previous studies may result from the difference of IP paradigm in duration and severity of sublethal stimulus. It has been reported that up-regulation of IL-1ra expression after brain injury usually follows IL-1 β expression (Legos et al., 2000; Loddick et al., 1997). Interestingly, IL-1ra gene expression preceded IL-1 β expression after BCCAO IP and its protein level was significantly increased (Fig. 3 B & 4B). The finding indicates that up-regulation of IL-1ra expression is an independent mechanism triggered by IP and not a result to offset IL-1 β expression/activity. Consistent with our result, increased level of IL-1ra was reported in neuron-like cell in the preconditioned cortex during tolerance periods (Barone et al., 1998). We also found that IL-1ra protein level was maximally increased at 24 h and decreased at 48 h. The selective induction of tolerance at 24 h in our BCCAO IP model made us to focus to determine a

correlation between IL-1ra level and IP-induced protection. However, because inhibition of IL-1R by IL-1ra nullified IP effects (Ohtsuki et al., 1996), the possibility that low level of IL-1 β induced by IP might be involved in neuroprotection through IL-1R signaling cannot be ruled out.

After MCAO, patterns of IL-1 β and IL-1ra expressions were quite differently regulated by IP. During early reperfusion period (up to 6 h), IL-1 β was suppressed in the preconditioned brain (fig. 5) while IL-1ra level was enhanced at later reperfusion period (at 24 h, fig. 6), indicating that genes and proteins involved in ischemic damage/protection are differentially regulated by IP according to time frames after ischemic/reperfusion injury.

Repressions of IL-1 β mRNA and protein levels by IP at early reperfusion period observed in our study are in agreement with the results of previous studies demonstrating a causal relationship between low level of IL-1 β and smaller infarct size (Yamasaki et al., 1995; Pera et al., 2004; Bowen et al., 2006). The repression of IL-1 β is not likely related with the result of negative feedback through IL-1ra enhancement because IL-1ra was not changed by IP at 6 h after reperfusion. The reduction of IL-1 β level by IP may result from reduced infiltration of neutrophils and macrophages which are major sources of IL-1 β at acute phase in the cortex after ischemic insult (Bowen et al., 2006). However, it was also suggested that suppression of IL-1 β might be selectively regulated by IP regardless of neutrophil infiltration since the level of TNF- α , one of major proinflammatory cytokines, was not changed at the same time point (Pera et al., 2004). At late reperfusion period (72 h), there was a moderate but significant increase in IL-1 β mRNA but not in protein expression compared to sham and control groups (fig. 5A). It is possible that much of IL-1 β mRNA might undergo degradation or did not translated. Alternatively, a sensitivity in Western blot may not sufficient to detect a small difference.

Beneficial roles of IL-1ra in the ischemic brain have been well established. At the gene level, deletion of IL-1ra in the mouse increased infarct volume (Pintaux et al., 2006) while overexpression of IL-1ra gene reduced infarct size (Betz et al., 1995). Recombinant IL-1ra protein administration either prior or after ischemic insult reduced ischemic damage (Martin et al., 1994; Mulcahy et al., 2003) and also some neuroprotective agents against ischemic insults having anti-inflammatory effects act by enhancement of endogenous IL-1ra level (Park et al., 2005; Choi et al., 2008). Therefore endogenous induction of IL-1ra plays a role in the defense mechanism against to ischemic injury. However, expression changes of IL-1ra by IP after ischemic insult have not been reported in the brain. Only in human liver, microarray analysis in ischemic preconditioned liver followed prolonged ischemia showed that the most overexpressed gene was IL-1ra gene and its increased protein expression in ischemic liver after preconditioning was confirmed by immunohistochemistry, suggesting attenuation of inflammation by IP through IL-1ra production (Barrier et al., 2005). Induction of IL-1ra in the ischemic brain was observed from 6 h to 72 h at both gene and protein levels in the present study, consistent with other reports tested in focal stroke model (Legos et al., 2000; Wang et al., 1997). IP-induced IL-1ra protein expression was markedly enhanced 24 h after MCAO, which was a subsequent event to earlier IL-1 β repression at 6 h (Fig. 6B). The enhancement of IL-1ra by IP may counteract IL-1 β activity and further reduce IL-1 β -associated inflammatory responses. Although direct roles for IL-1 β and IL-1ra in IP-induced neuroprotection requires genetic and pharmacological approaches, the current study links the reciprocal stroke-induced IL-1 β and IL-1ra changes in the preconditioned brains to the IP-induced neuroprotection. Future gain/loss of functional studies are warranted to define critical roles of IL-1 β and IL-1ra on IP-induced neuroprotection.

In summary, the present study demonstrates that neuroprotection induced by BCCAO IP is associated with the increased IL-1 β and IL-1ra expression during the tolerance period.

Furthermore, this IP paradigm induces down regulation of IL-1 β and up-regulation of IL-1ra gene/protein expression in the post-ischemic brain. Based on the respective detrimental and protective actions of IL-1 β and IL-1ra, the study suggests that underlying mechanism(s) of IP-induced neuroprotection includes the shift toward an anti-inflammatory state in the post-ischemic cortex.

Acknowledgments

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2006-311-E00434) to EMP and NIH Grant RO1 HL082511 to SC.

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Fig. 1.

Experimental timeline and CBF changes during BCCAO and MCAO. (A) Time interval between IP and MCAO (lower line) and time points for evaluation of IL-1 β and IL-1ra levels after IP or sham with and without MCAO (upper line) were indicated. (B) Ischemic precondition was induced by 3 episodes of 1 min BCCAO with 5 min intervals ($n = 6$). (C) CBF was recorded before, during, and 20 min after 30 min MCAO in control ($n = 5$), sham ($n = 7$) and IP ($n = 6$) groups. Values are expressed as mean $\pm$ SEM (ANOVA with *post hoc* Fisher's PLSD test).

Fig. 2. Infarct volumes of brain subjected to MCAO. (A) Representative brain sections of cresyl violet staining 72 h after MCAO in sham and IP groups. (B) Infarct volumes of total, cortex and striatum in control (n = 5), sham (n = 7) and IP (n = 6) groups. * $P < 0.05$ from control; # $p < 0.05$ from sham (ANOVA with *post hoc* Fisher's PLSD test).

Fig. 3. IL-1 β and IL-1ra mRNA expressions in the cortex primed by ischemic preconditioning. (A) IL-1 β and IL-1ra mRNA levels by RT-PCR in the cortex 24 h after sham operation or IP. (B) Quantitative temporal changes of IL-1 β and IL-1ra mRNA by real-time PCR in the cortex from 6 h to 48 h after sham operation or IP ($n = 3$ in each group). Values are expressed as ratio versus control group (given a nominal value of 1) and represent mean $\pm$ SEM. $*P < 0.05$ from control; $\#p < 0.05$ from sham (ANOVA with *post hoc* Fisher's PLSD test).

Fig. 4.

IL-1 β and IL-1ra protein levels in the cortex primed by ischemic preconditioning. (A) Representative immunoblot of IL-1 β and IL-1ra protein in the cortex 24 h after sham operation or IP. Ten ng of recombinant mouse (rm) IL-1 β and IL-1ra proteins (both 17 kDa) were used for positive controls (+) and IL-1 β and IL-1ra amounts were compared to the respective recombinants protein standards ranging from 0 to 20 ng. (B) Protein density of IL-1 β and IL-1ra in the cortex at 24 h, 48 h and 72 h after sham operation or IP ($n = 4$ in each group). Values are expressed as ratio versus control group (given a nominal value of 1) and represent mean $\pm$ SEM. * $P < 0.05$ from control; # $p < 0.05$ from sham (ANOVA with *post hoc* Fisher's PLSD test).

Fig. 5.

Time course of IL-1 β expression in the cortex after MCAO. (A) Quantitative mRNA expression of IL-1 β at 1 h, 6 h, 24 h and 72 h after MCAO (n = 3–4). (B) Protein levels of IL-1 β at 6 h, 24 h and 72 h after MCAO (n = 3–4). Values are expressed as ratio versus control group without MCAO (given a nominal value of 1). * P < 0.05 from control; # p < 0.05 from sham (ANOVA with *post hoc* Fisher's PLSD test).

Fig. 6.

Time course of IL-1ra expression in the cortex after MCAO. (A) Quantitative mRNA expression of IL-1ra at 1 h, 6 h, 24 h and 72 h after MCAO ($n = 3-4$). (B) Protein level of IL-1ra at 6 h, 24 h and 72 h after MCAO ($n = 4$). Values are expressed as ratio versus control group without MCAO (given a nominal value of 1). * $P < 0.05$ from control; # $p < 0.05$ from sham (ANOVA with *post hoc* Fisher's PLSD test).