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Involvement of IL-1β in acute stress-induced worsening of cerebral ischaemia in rats

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Abstract Stress is known to be one of the risk factors of stroke. Most of the knowledge on the effects of stress on cerebrovascular disease in humans is restricted to catecholamines and glucocorticoids effects on blood pressure and/or development of atherosclerosis. However, few experimental studies have examined the possible mechanisms by which stress may affect stroke outcome. We have used an acute stress protocol consisting of the exposure of male Fischer rats to an acute, single exposure immobilisation protocol (6 h) prior to permanent middle cerebral artery occlusion (MCAO), and we have found that stress worsens behavioural and neurological outcomes and increased infarct size after MCAO. The possible regulatory role of the TNFα and IL-1β was studied by looking at the release of these cytokines in brain. The results of the present study showed an increase in IL-1β release in cerebral cortex after exposure to acute stress. Brain levels of IL-1β are also higher in previously stressed MCAO rats than in MCAO animals without stress. Pharmacological blockade of IL-1β with an antibody anti-IL-1β led to a decrease in the infarct size as well as in neurological and behavioural deficits after MCAO. In summary, our results indicate that IL-1β, but not TNFα, accounts at least partly for the worsening of MCAO consequences in brain of rats exposed to acute stress.

1. Introduction

Apart from the changes induced by stress on vascular function (rev. in Taylor and MacQueen, 2006), less is known about the effects of previous stress or mood disorders on the outcome of a subsequent stroke episode. Whereas several epidemiological studies have reported positive associations between depressive symptoms and psychological stress and stroke risk (Simons et al., 1998; Jonas and Mussolino, 2000; Larson et al., 2001; Ohira et al., 2001), few studies have examined the influence of psychological distress on stroke outcome in humans, and the results are controversial (Colantonio et al., 1992; Everson et al., 1998). In the experimental field, some studies have shown an exacerbation of stroke outcome in animal models of social stress (DeVries et al., 2001; Sugo et al., 2002), whereas others indicate that stress might protect against ischaemic damage (Thoresen et al., 1996), particularly in chronic predictable stress models (Madrigal et al., 2003).

Exposure of rats to immobilisation stress, an animal paradigm of psychological stress (Magariños et al., 1997) causes expression and/or release of proinflammatory and

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inflammatory mediators, including cytokines, and oxidative stress (rev. in Madrigal et al., 2004). Two of the main proinflammatory cytokines, tumour necrosis factor-alpha (TNFα) and interleukin-1 (IL-1) are rapidly produced in the brain in response to tissue injury, both in ischaemia (Liu et al., 1994; Wang et al., 1997) and after stress exposure (Dunn and Wang, 1995; Holden and Pakula, 1999; Lee et al., 2006; rev. in Black, 2002). These two mediators regulate the immune early responses to inflammation, both in brain and peripheria (rev. in Watkins and Maier, 1998). In an attempt to elucidate their role in stressed animals suffering stroke, we have recently shown that the increase in TNFα- but not IL-1β-expression and release after subacute, repeated stress, as well as the expression of one of its receptors (TNFR1) accounts for the increase in infarct size and accumulation of oxidative/nitrosative parameters after ischaemia (Caso et al., 2006).

The aim of the present study was to investigate further the mechanisms by which a different stress paradigm, acute immobilisation stress, worsens cerebral ischaemia outcome and the possible implication of TNFα and IL-1β.

2. Experimental procedures

2.1. Animals

Adult male Fischer rats weighing 225–250 g were used. All experimental protocols adhered to the guidelines of the Animal Welfare Committee of the Universidad Complutense (following DC 86/609/EU). Rats were housed individually under standard conditions of temperature and humidity and a 12 h-light/dark cycle (lights on at 08:00) with free access to food and water. All animals were maintained under constant conditions for 4 days prior to stress.

2.2. Immobilisation stress

Rats were exposed to stress between 9:00 and 15:00 h in a room adjacent to the animals homeroom. The protocol of stress used was an acute model consisting of 6 h immobilisation (S6h). Immobilisation was performed using a conical plastic rodent restrainer that allowed for a close fit to rats.

2.3. Experimental groups

Several groups (n=8–10 in each group) were used: (1) a control group (animals were handled for few seconds once at 9:00 h); (2) an acute stress group sacrificed 24 h after immobilisation (S6h); (3) a permanent middle cerebral artery occlusion (MCAO) group, operated at 15:00 h and sacrificed 24 h after operation; (4) a MCAO group with prior exposure to stress, operated after immobilisation, sacrificed 24 h after operation (S6h+MCAO).

For additional experiments requiring intracerebroventricular (i.c.v.) administration, other eight groups were used: (5) animals without stress or MCAO, i.c.v. injected with buffer phosphate (bp); this was used as control group (C bp); (6) animals without stress or MCAO, i.c.v. injected with anti-IL-1β (C a-IL-1β); (7) i.c.v. buffer phosphate (bp) 1 h previous to immobilisation (S6h bp); (8) i.c.v. anti-IL-1β 1 h previous to immobilisation (S6h a-IL-1β); (9) i.c.v. injection of bp 6 h previous to MCAO (MCAO bp); (10) i.c.v. injection of anti-IL-1β 6 h prior to MCAO (MCAO a-IL-1β); (11) acute stress with i.c.v. injection of bp prior to the immobilisation exposure and MCAO after stress (S6h bp+MCAO); (12) acute stress with i.c.v. injection of anti-IL-1β prior to the immobilisation exposure and MCAO after stress (S6h a-IL-1β+MCAO). Groups 5, 7, 9 and 11 were carried out to discard possible effects of i.c.v. procedure (anaesthesia, sterotaxic surgery and volume injected, see below) on the parameters studied.

To avoid circadian rhythm modifications, all animals were sacrificed between 15:00 and 16:00 h as follows: control groups, immediately; stress groups 24 h after the immobilisation session; MCAO (3,9,10) and S6h+MCAO (4, 11, 12) groups: 24 h after MCAO.

All the biochemical analysis were carried out on cerebral cortex from ipsilateral hemisphere (in MCAO groups) or left hemisphere (in non-MCAO groups).

2.4. Permanent focal ischaemia

In stressed animals, permanent middle cerebral artery occlusion (MCAO) was performed immediately after the immobilisation exposure. Permanent occlusion was made in left common carotid artery (CCA) and in the ipsilateral distal middle cerebral artery (MCA), as described previously (Brint et al., 1988; De Cristóbal et al., 2001).

For permanent CCA occlusion a silk ligature was used, whereas MCA was occluded by applying an electrocoagulator tip (Select-Sutter Medizintechnik, Freiburg, Germany) just above the vessel on the wire hook; after transferring heat through the wire, the MCA was cauterized and severed.

2.5. Intracerebroventricular injection

In a set of experiments, aiming to neutralize the effects of IL-1β released during stress after MCAO, one intracerebroventricular injection of anti-IL-1β was made before the immobilisation exposure. Injection consisted of 5 μL of anti-IL-1β (R&D Systems, Abingdon, UK) in a concentration of 2 μg or 5 μL of buffer phosphate as control (Fernández-Alonso et al., 1996).

Briefly, under anaesthesia with 2% isofluorane in a mixture of 70% nitrogen/30% oxygen, an intracerebroventricular 26-gauge cannula (Plastics One, Roanoke, VA, USA) was inserted into the left lateral ventricle by using sterotaxic apparatus (Davis Kopf Instruments, Tujunga, CA, USA). The coordinates were as follows: anteroposterior, −0.8 mm; lateral, 1.0 mm; dorsoventral, 3.6 mm from the bregma, with the incisor bar placed at 3.3 mm below the interaural plane. Cannulas were anchored to the skull by stainless steel screws and dental cement. After a 1-hour recovery period the animals were subjected to immobilisation.

2.6. Infarct size

Infarct volume was measured as previously described (Madrigal et al., 2003). In short, brains were removed 24 h after MCAO, and a series of 2 mm of coronal slices were obtained and stained in 1% TTC (2,3,5-triphenyl-tetrazolium chloride) in 0.1 M phosphate buffer. Infarct volumes were measured by sampling stained sections with digital camera (Nikon Coolpix 990, Nikon Corporation, Tokyo, Japan) and the image of each section was analysed by an image analyser (Scion Image for Windows 2000, Scion Corporation, Frederik, MD, USA). The digitalised image was displayed on a video monitor. With the observer blinded to the experimental conditions, the contralateral hemisphere perimeter was overlapped on the ipsilateral hemisphere to exclude edema, and infarct borders in both the cortex and subcortex (corpus callosum excluded) were delineated using an operator-controlled cursor. The area of infarct, which was not stained, was determined by counting pixels contained within the outlined regions of interest and expressed in square millimetres. Infarct volumes (in mm^3) were integrated from the infarct areas over the extent of the infarct calculated as an orthogonal projection.

2.7. Behavioural characterisation

Prior to sacrifice, animals were tested according to the procedures detailed, modified from Hunter et al. (2000) at the moment of sacrifice. First, assessment of each animal began with observation of