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Changes in HPA reactivity and noradrenergic functions regulate spatial memory impairments at delayed time intervals following cerebral ischemia

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ABSTRACT

This study investigates the association of ischemia-induced spatial memory impairment to alterations of the HPA axis and noradrenergic activation post insult. Experiment 1 characterized the effects of 10 min forebrain ischemia on corticosterone (CORT) secretion following ischemia and in response to spatial memory assessment in the Barnes maze, as well as the impact of pre-ischemia treatment with the glucocorticoid inhibitor metyrapone (175 mg/kg; s.c.). The results showed that cerebral ischemia represents a significant physiological stressor that upregulated CORT secretion 1, 24 and 72 h post-ischemia but not at 7 days. In response to testing in the Barnes maze ischemic animals showed elevated CORT secretion simultaneously with spatial memory deficits. The single dose of metyrapone attenuated the ischemia-induced adrenocortical hyper-responsiveness and subsequent memory deficits despite not providing neuroprotection in the hippocampal CA1 pyramidal cells. To complement these findings, we examined whether norepinephrine which provides positive feedback to the HPA axis and is upregulated following brain ischemia could influence memory performance at delayed intervals after ischemia. Experiment 2 demonstrated that pre-testing administration of the alpha2-adrenoceptor agonist clonidine (.04 mg/kg, s.c.) attenuated ischemia-induced working memory impairments in a radial maze while opposite effects were obtained with the antagonist yohimbine (.3 mg/kg, s.c.). Post-testing administration of clonidine produced spatial reference memory impairments in ischemic rats. The findings from the current study demonstrate increased sensitization and responsiveness of systems regulating stress hormones at long intervals post ischemia. Importantly, we demonstrate that these effects contribute to post ischemic cognitive impairments which can be attenuated pharmacologically even in the presence of hippocampal degeneration at time of testing.

Introduction

Degeneration of hippocampal CA1 pyramidal cells represents a hallmark of forebrain ischemia in rats and humans, which is thought to directly contribute to cognitive deficits, particularly spatial memory impairments (Gionet et al., 1991; Moulaert et al., 2009; Olsen et al., 1994; Petito et al., 1987; Volpe et al., 1992). Nonetheless, spatial memory impairments (Iwasaki et al., 2006) and other behavioral impairments such as locomotor hyperactivity (Kuroiwa et al., 1991) in ischemic rodents have been described prior to CA1 neuronal degeneration. Further, intact spatial memory and locomotor functioning in ischemics has been demonstrated despite extensive CA1 injury (Bueters et al., 2008; Farrell et al., 2001; Gobbo and O'Mara, 2004; Roberge et al., 2008). Thus, the causes of ischemia-induced behavioral impairments are not fully established despite the historical focus on hippocampal damage.

Different studies have showed potent effects of cerebral ischemia on stress hormones and associated regulatory systems. For example, corticotropin-releasing hormone (CRH) and corticosterone (CORT) levels remain elevated for days in discrete brain regions and in the periphery following cerebral ischemia (Chen et al., 1998; Hwang et al., 2006; Khan et al., 2004; Wong et al., 1995) and upregulation of norepinephrine secretions has been documented in brain tissue during (Bentue-Ferrer et al., 1986; Gustafson et al., 1991) and for weeks after ischemia (Pich et al., 1993), indicating that it represents a potent physiological stressor.

To date, characterization of the effects of ischemia-induced CRH and CORT secretions have been restricted to neuronal degeneration (Charron et al., 2008; Sapolsky and Pulsinelli, 1985; Stevens et al., 2003), and little is known concerning a protracted role of upregulated secretions of these stress signals or of norepinephrine release on behavioral and/or cognitive dysfunctions post ischemia. This is an intriguing question considering that neuroendocrine dysfunction/sensitization can be observed at delayed time intervals after a single exposure to an acute stressor (Belda et al., 2008a,b), and that single stressors, if significant enough, can have lasting impacts on cognitive functioning (El Hage et al., 2006). It therefore appears possible that a

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physiological stressor such as cerebral ischemia produces lasting disruptions in neuroendocrine reactivity and differential arousal/reactivity contributing to spatial memory deficits. In this context, locomotor hyperactivity in the open-field (Colbourne et al., 1998; Green et al., 1995; Yan et al., 2007), its modulation by illumination (Milot and Plamondon, 2008) and heightened acoustic startle responses (Hickey et al., 1996) after brain ischemia are behaviors suggestive of underlying alterations in emotional reactivity associated with upregulated neuroendocrine reactivity and release of stress hormones (Smee et al., 1975; Veldhuis and De Wied, 1984).

The hypothalamic–pituitary–adrenal (HPA) axis and noradrenergic systems act synergistically in mediation of spatial memory performance in non-ischemic animals (Arnsten et al., 1999; de Quervain et al., 1998; Schwabe et al., 2010; Zhang and Cai, 2005). The effects of CORT on memory functioning depend on co-activation of the noradrenergic system which provides positive feedback to the HPA axis (Feldman and Weidenfeld, 1996; Ziegler et al., 1999), and its effects on spatial memory can be prevented by blocking the effect of norepinephrine (Roozendaal et al., 2003; Roozendaal et al., 2004). The effects (both genomic and non-genomic) of heightened CORT on behavior and on spatial memory can be elicited after a 15–30 min delay (McEwen and Sapolsky, 1995; Roozendaal, 2002; Sajadi et al., 2006). Norepinephrine has much more rapid non-genomic action (McEwen and Sapolsky, 1995) and excessive release of this neurochemical can also disrupt spatial memory functioning in animals (Arnsten et al., 1999; McAllister, 2001; Zhang and Cai, 2005).

Despite the breadth of research concerning the impact of stress hormones and associated systems on spatial memory functioning, little is known about the functional impact of a dysregulation of these systems following cerebral ischemia. Thus, the current study aims to evaluate the possibility that cerebral ischemia affects arousal and/or release of stress hormones during spatial memory assessment performed at delayed intervals post ischemia. To do so, we assessed HPA reactivity associated with behavioral testing and determined whether norepinephrine availability affects spatial memory performance post ischemia. More specifically, Experiment 1 examines the impact of 10 min forebrain ischemia on basal plasma CORT levels 1, 24, 72 h and 7 days post-ischemia and in response to spatial memory assessment in the Barnes maze (BM). We also determined the impact of inhibition of CORT secretions (via metyrapone administration) post ischemia and in response to behavioral testing. Experiment 2 characterizes the impact of pharmacological manipulation of central NE on spatial memory capabilities after ischemia at time intervals when CA1 damage is complete. Together, these experiments will help clarify the possibility that protracted dysregulation of the adrenocortical and noradrenergic systems induced by cerebral ischemia exert a significant impact on post-ischemic cognitive functioning, and that blocking CORT synthesis during ischemia and/or regulating NE availability during testing regulates spatial memory impairments.

Materials and methods

General methodology

Animals

Male Wistar rats (N = 44 and 70 in behavioral experiments 1 and 2, respectively) weighing between 250 and 320 g at time of surgery were obtained from Charles River Laboratories (Rochefort, Quebec, Canada) and habituated to the housing facility for a minimum of two weeks before surgery. They were individually housed and maintained on a 12 h light/dark cycle (lights on at 7:00 AM) with free access to water and standard rat chow. Room temperature was maintained at 21–23 °C with 60% relative humidity. The experimenter handled all the rats daily for 2–3 min in the four days preceding the first day of surgery, and two days prior to initiation of behavioral testing. A separate group of animals (N = 55) was used to assess CA1 neuronal injury 10 and 30 days post-ischemia at times when spatial memory assessments initially took place in Experiments 1 and 2. All experiments and procedures were in accordance with the guidelines set by the Canadian Council of Animal Care and approved by the University of Ottawa Animal Care Committee.

Surgical procedure

Forebrain ischemia was induced using the four-vessel occlusion model as previously described (Pulsinelli and Brierley, 1979; Pulsinelli et al., 1982). Briefly, rats were anesthetized by inhalation of 1.5% halothane in oxygen. The core temperature was maintained at 37 ± .5 °C throughout surgery and during ischemia by means of a feedback-regulated heating blanket connected to a rectal thermometer. The vertebral arteries were irreversibly occluded by electrocoagulation and a small-diameter silk thread looped around the carotid arteries to facilitate subsequent occlusion. Sham-operated animals underwent anesthesia and received the same dorsal and ventral surgical incisions as the ischemic group without electrocoagulation of the vertebral arteries. Twenty-four hours later rats were briefly anesthetized and carotid arteries re-exposed for clamping. Cerebral ischemia occurred between 7:30 and 9:30 AM. After discontinuing anesthesia and at first sign of wakefulness (sniffing and limb movements) the pair of carotid arteries was occluded with microvascular clamps for a 10 min period, the rats freely ventilating. Only rats that lost the righting reflex over the entire occlusion period and which displayed dilated pupils in response to a beam of light prior to clamp removal were included in the study.

On day 4 post-occlusion the pupillary light reflex was assessed in all animals to determine retinal functioning possibly impacting vision during behavioral testing. Rats were transported to a dark room and left to habituate for at least 15 min. The pupillary reflex was examined by illuminating the rats' dark-adapted eyes with a focused light beam. Following examination of the first eye, chosen at random, an additional 60 s in the dark was imposed prior to examining the second eye. The reflex was considered intact if constriction of both pupils occurred within 10 s of light exposure.

Experiment 1

Animals

Of 31 rats (excluding sham-operated) subjected to ischemia, 14 regained the righting reflex or displayed constricted pupils, and two died due to surgery. A similar percentage of animals were excluded based on the righting reflex/pupil dilation from the vehicle and metyrapone treated ischemic groups indicating no treatment-induced bias on exclusion criteria (Milot and Plamondon, 2010). No significant changes in rectal temperature were observed between any of the groups during ischemia. All ischemic and sham-operated rats displayed a normal pupillary light reflex.

Drug treatment and animal groups after exclusion

Metyrapone, a glucocorticoid synthesis inhibitor, was dissolved in a .9% saline solution and administered subcutaneously (175 mg/kg) to rats 30 min prior to induction of forebrain ischemia (n = 7) or sham surgery (n = 6). This dose is similar to that used in previous studies, which together suggested neuroprotective effects of the compound at short post-ischemic intervals but not delayed ones (Krugers et al., 2000; Risedal et al., 1999; Smith-Swintosky et al., 1996). Vehicle-treated ischemic (n = 8) and sham-operated (n = 7) rats were administered saline.

Blood collection

Animals were habituated to the blood collection procedure prior to surgery. They were transported to a room located a short distance from their home room, placed on a table, covered with a small towel, and tail gently stroked for 1 min. This procedure was performed once daily during the three days preceding surgery.