Hormones and Behavior 65 (2014) 273–284

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

# Evidence of lasting dysregulation of neuroendocrine and HPA axis function following global cerebral ischemia in male rats and the effect of Antalarmin on plasma corticosterone level

Patricia B. de la Tremblaye [a], Julie Raymond [a], Marc R. Milot [a], Zul Merali [a,b], Hélène Plamondon [a,*]

[a] Behavioural Neuroscience Group, School of Psychology, University of Ottawa, 136 Jean-Jacques Lussier, Ottawa, ON, K1N 6N5, Canada
[b] University of Ottawa Institute of Mental Health Research (IMHR), 1145 Carling Avenue Ottawa, Ontario K1Z 7K4, Canada

ARTICLE INFO

*Article history:*
Received 10 October 2013
Revised 9 January 2014
Accepted 10 January 2014
Available online 17 January 2014

*Keywords:*
HPA dysregulation
Corticotropin releasing hormone
Glucocorticoid receptors
CRHR1
Immunohistochemistry
Global cerebral ischemia
Rat

ABSTRACT

Abnormal function of the neuroendocrine stress system has been implicated in the behavioral impairments observed following brain ischemia. The current study examined long-term changes in stress signal regulation 30 days following global cerebral ischemia. Experiment 1 investigated changes in the expression of corticotropin releasing hormone (CRH) and its subtype 1 receptor (CRHR1), glucocorticoid receptors (GR) in the paraventricular nucleus of the hypothalamus (PVN), the central nucleus of the amygdala (CeA), and the CA1 subfield of the hippocampus. Tyrosine hydroxylase (TH) was determined at the locus coeruleus (LC). Experiment 2 investigated the role of central CRHR1 activation on corticosterone (CORT) secretion at multiple time intervals following global ischemia after exposure to an acute stressor. Findings from Experiment 1 demonstrated a persistent increase in GR, CRH and CRHR1 immunoreactivity (ir) at the PVN, reduced GR and CRHR1 expression in pyramidal CA1 neurons, and increased LC TH expression in ischemic rats displaying working memory errors in the radial arm Maze. Findings from Experiment 2 revealed increased CORT secretion up to 7 days, but no longer present 14 and 21 days post ischemia. However upon an acute restraint stress induced 27 days following reperfusion, ischemic rats had increased plasma CORT secretions compared to sham-operated animals, suggesting HPA axis hypersensitivity. Antalarmin (2 μg/2 μl) pretreatment significantly attenuated post ischemic elevation of basal and stress-induced CORT secretion. These findings support persistent neuroendocrine dysfunctions following brain ischemia likely to contribute to emotional and cognitive impairments observed in survivors of cardiac arrest and stroke.



## Introduction

The effects of transient global cerebral ischemia on rat behavior such as long-lasting spatial learning and memory deficits are commonly associated with selective neuronal damage of the CA1 hippocampal region (Hartman et al., 2005; Lehotsky et al., 2009). However, increasing evidence supports the involvement of stress regulators in the pathophysiology of cerebral ischemia, which could contribute to behavioral impairments. For example, corticotropin releasing hormone (CRH) release, protein and mRNA expression as well as corticosterone (CORT) levels remain elevated for days in discrete brain regions and in the periphery following cerebral ischemia (Hwang et al., 2006; Khan et al., 2004; Wong et al., 1995). Past studies have also shown that plasma levels of norepinephrine (NE) are up-regulated during (Gustafson et al., 1991), in the hours (Globus et al., 1989), and even weeks (Pich et al., 1993) after brain ischemia. These findings suggest that global ischemia represents a potent physiological stressor. If the effects of such a stressor persist over time, the allostatic load may result in maladaptive physiological and behavioral responses (Beauchaine et al., 2011).

Upon exposure to physiological or psychogenic stressors, the HPA axis initially responds via increased synthesis and release of CRH from the paraventricular nucleus of the hypothalamus (PVN). PVN CRH neurons project to the median eminence where CRH is released and binds to CRHR1 receptors in the anterior pituitary gland inducing the release of adrenocorticotropic hormone (ACTH) into the blood stream. ACTH subsequently triggers secretion of glucocorticoids from the adrenal cortex. A negative feedback mechanism by glucocorticoids then acts to inhibit HPA axis activation via mineralocorticoid (MR) and glucocorticoid (GR) receptors present at the pituitary, hypothalamus and hippocampus (Reul and de Kloet, 1985), which regulate HPA activity in a complementary fashion; MR regulate CORT secretion under basal conditions and are activated in the initial phase of the stress reaction, followed by GR activation to terminate the stress response (Joels et al., 2008). Both MR and GR are highly abundant in CA1 pyramidal neurons of the

*Abbreviations:* HPA, axis dysregulation post-ischemia.

* Corresponding author at: University of Ottawa, School of Psychology, Behavioral Neuroscience Group, 136 Jean-Jacques Lussier, Vanier Bldg, Rm 2082, Ottawa, Ontario K1N 6N5, Canada. Fax: +1 613 562 5147.
*E-mail address:* hplamond@uottawa.ca (H. Plamondon).

0018-506X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yhbeh.2014.01.003

hippocampus, a brain structure known to inhibit HPA activity as well as for its importance in learning and memory (Sarabdjitsingh et al., 2009). In response to stress, CRH also activates locus coeruleus (LC) neurons to stimulate the release of NE in forebrain terminal projections, which in turn stimulates the release of CRH at the PVN and central nucleus of the amygdala (CeA), indicating a strong relationship between NE and CRH in modulating HPA activation (Koob, 1999; Reyes et al., 2008; Roozendaal et al., 2008). Among other brain regions, the amygdala has been shown to facilitate adrenocortical responses through interactions with the PVN (Feldman et al., 1995; Van de Kar and Blair, 1999). The lateral part of the CeA contains the highest density of CRH cell bodies within the amygdaloid complex while a more modest amount is found within the basolateral nucleus of the amygdala (BLA) (Hsu et al., 1998; Pitts et al., 2009). It is worthy of note that exposure to various stressors as well as ischemia increase extracellular CRH release and/or mRNA expression at the CeA (Hsu et al., 1998; Merali et al., 1998; Merlo Pich et al., 1995).

Recently, findings from our laboratory (Milot and Plamondon, 2011) demonstrated elevated CORT secretion in ischemic animals in response to Barnes Maze testing simultaneously with spatial memory deficits. A single dose of the glucocorticoid synthesis inhibitor metyrapone prior to vessel occlusion concomitantly attenuated post testing CORT secretion and spatial memory deficits in ischemic animals despite not providing neuroprotection in the hippocampal CA1 area. In addition, pretesting administration of the alpha2-adrenoceptor agonist clonidine (which inhibits NE release) attenuated ischemia-induced working memory impairments while opposite effects were obtained with the antagonist yohimbine. These findings support the notion that global ischemia sensitizes systems regulating stress responses and emotionality even at delayed intervals post-ischemia, effects which, if prevented, can reduce post ischemic memory impairments even in the presence of hippocampal damage.

Given the role of CRH and glucocorticoid in stress-induced emotional and cognitive impairments (Muravieva and Alberini, 2010; Ryan et al., 2010; Vouimba et al., 2007), the current study sought to characterize the impact of 10 min global ischemia on long-term changes in the expression of key neuroregulators involved in the neuroendocrine stress response. Experiment 1 determined post ischemia

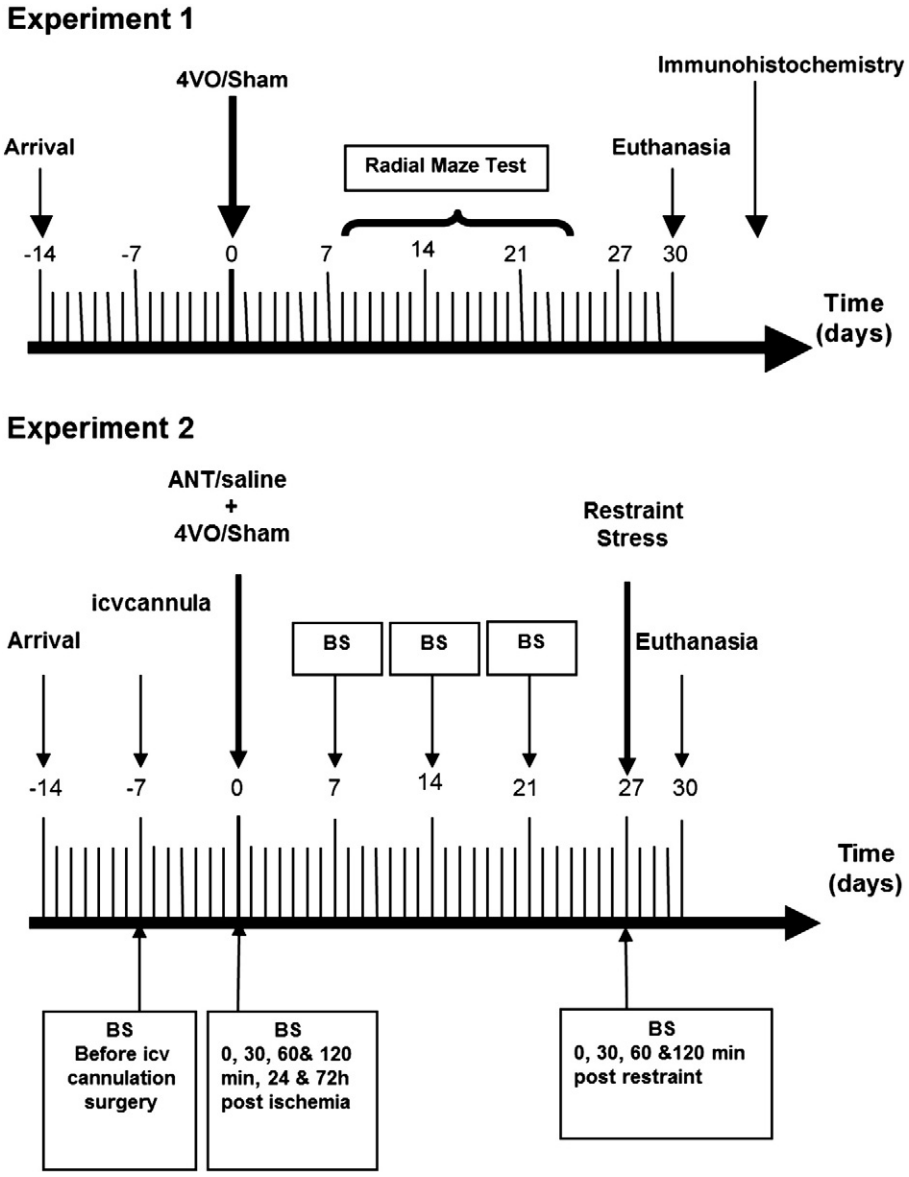


**Fig. 1.** Timeline for Experiment 1 (top panel) and Experiment 2 (lower panel). Day 0 refers to the day of induction of 4 vessel occlusion (4VO), day 8 is the start of Radial Maze testing. In Experiment 2, Antalarmin (2 μg/2 μl) or vehicle was icv administered 30 min before sham or carotid occlusion. Blood Sampling (BS) was performed at various intervals during the experiment. On day 27 post ischemia or sham occlusion, animals were exposed to a 15 min restraint stress.