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Research Report

The function of the adrenocortical axis in permanent middle cerebral artery occlusion: Effect of glucocorticoids on the neurological outcome

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ABSTRACT

We characterized the effect of acute ischemic stroke on the activation of the hypothalamic–pituitary–adrenal (HPA) axis and evaluated the role of glucocorticoids (GC) in the clinical outcome following ischemic stroke. Male spontaneous hypertensive rats underwent permanent middle cerebral artery occlusion (PMCAO) and developed a cortical infarct. At 4 h post-PMCAO or sham operation, serum levels of ACTH and corticosterone (CS) were elevated 5 and 4 fold respectively as compared to controls and then returned to basal levels at 24 h post surgery. In these experimental groups we found also a significant depletion of median eminence (ME)-CRH_{41}. In adrenalectomized (Adx) rats that underwent PMCAO the degree of motor disability and infarct volume was similar to that of intact rats. Administration of dexamethasone (Dex) to Adx-PMCAO rats significantly improved the motor disability and decreased the infarct volume. However, in sham-Adx with PMCAO, Dex had no effect on these two parameters. In rats with PMCAO or sham-PMCAO, brain production of PGE_2 was significantly increased. This effect was further enhanced in Adx-PMCAO rats and significantly inhibited by Dex. In conclusion, activation of the HPA axis following PMCAO is due to stress induced by surgery. This activation is mediated by hypothalamic CRH_{41}. Absence of endogenous GC or administration of Dex in naïve rats does not alter motor and pathological parameters in the acute stage following PMCAO. In contrast, administration of Dex significantly improved the outcome following cerebral ischemia in Adx rats which may be due to increased glucocorticoid receptors. Brain production of PGE_2 does not play an important role in the pathophysiology of the acute phase of cerebral ischemia.

1. Introduction

Activation of the hypothalamic–pituitary–adrenal (HPA) axis is a major response to a variety of stress signals including neural, metabolic and immune stimuli (Feldman et al., 1995; Herman et al., 2003). The HPA axis consists of hypothalamic neurons located in the paraventricular nucleus (PVN) that contain corticotrophin-releasing hormone (CRH_{41}) which is the major ACTH secretagogue (Herman et al., 2003). In response to stress stimuli, CRH_{41} is released from ME and reaches the anterior pituitary stimulating ACTH secretion which in turn stimulates the secretion of GC from the adrenals. A large body of evidence

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indicates that extra-hypothalamic brain structures regulate the HPA axis responses via specific neural pathways and neurotransmitters. For example, discrete norepinephrine or serotonin releasing brain stem nuclei, which project into the PVN, activate the HPA axis responses (Feldman et al., 1995). Also, limbic structures such as the amygdala and the hippocampus are known to facilitate or inhibit, respectively, the adrenocortical responses (Feldman and Weidenfeld, 1995; Van de Kar and Blair, 1999; Weidenfeld et al., 2005). The HPA axis responses to stress are also regulated by a negative feedback exerted by circulating GC via the mediation of specific intracellular type I and type II GC receptors (de Kloet et al., 1993; Feldman et al., 1995).

Cerebral ischemia leads to brain damage caused by several pathological mechanisms. These include excitotoxicity, free radical formation, inflammatory cytokines, prostaglandins and apoptosis (Danton and Dietrich, 2003; Leker and Shohami, 2002). The effect of brain ischemia on the HPA axis responses and the role of GC in the pathologic processes are not fully elucidated. In the present study we used an experimental animal model for focal brain ischemia to study its effect on the HPA axis responses. We also examined the role of endogenous and exogenous GC and GC receptors on the neurological and pathological outcome following experimental brain ischemia.

2. Results

SHR rats underwent PMCAO or sham surgery as detailed below and serum levels of ACTH, CS and ME CRH_{41} content were measured (Fig. 1). Four hours following sham PMCAO, ACTH and CS markedly increased by 3 and 6 fold respectively as compared to naïve non operated rats. At 24 h post surgery, with or without PMCAO the levels of these hormones significantly decreased but remained slightly elevated above basal levels.

Next we examined the role of ME CRH_{41}, the major secretaguoge of ACTH in mediating the hormonal response to PMCAO. At 4 h following PMCAO or sham, the content of ME CRH_{41} decreased significantly by 40% as compared to naïve rats. This decrease possibly reflects the secretion of CRH_{41} from the ME neuronal terminals to the anterior pituitary via the portal system. At 24 h ME CRH_{41} levels returned to levels found in naïve control rats.

To evaluate the effect of GC on the neurological and pathological outcome following PMCAO, we used Adx and sham-Adx rats treated with Dex. Our results show that removal of circulating GC by Adx prior to PMCAO had no effect on both the degree of motor disability score or on the infarct volume when compared to the respective sham Adx-PMCAO rats (Fig. 2). Administration of Dex to sham-Adx-PMCAO rats had no effect on the motor disability score as well as on the infarct volume as compared to sham-Adx group treated with vehicle. In contrast, administration of Dex to Adx-PMCAO rats significantly reduced the motor disability score and the infarct volume by 50%. Representative stained sections with infarcts of brains from rats with PMCAO and PMCAO-Adx rats pretreated with Dex are shown in Fig. 3.

Next we evaluated the effect of PMCAO and GC on the ex-vivo production of PGE_2 by lateral cortical tissue. Sham PMCAO increased PGE_2 production by twofold as compared to intact rats. Adx further increased PGE_2 by 3 fold. PMCAO caused a marked 5 fold increase in PGE_2 as compared to intact controls. This effect was significantly enhanced following Adx. Administration of Dex significantly reduced PGE_2 production in sham Adx rats and Adx PMCAO rats (Table 1).

Finally we tested a possible mechanism which may involve in the beneficiary effect of Dex in Adx-PMCAO rats. To this aim we measured Adx-induced changes in the binding of Dex to brain glucocorticoid receptors (GR). Table 2 showed that Adx caused a marked gradual increase in Dex binding capacity to cytosolic GR in the frontal cortex. At 4 days and 10 days following Adx the binding capacity of Dex increased by

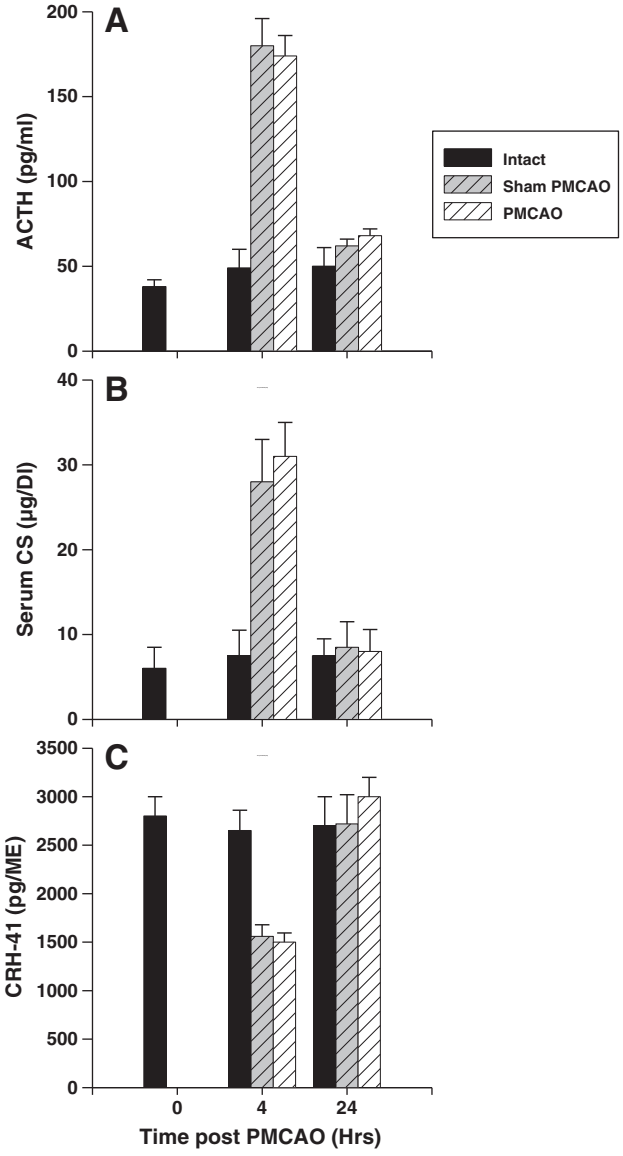

Fig. 1 – Serum levels of ACTH (A), CS (B) and ME content of CRH_{41} (C), in intact (black), sham-PMCAO (hatched-gray) and rats that underwent PMCAO (hatched-white). Hormones levels were determined at 4 and 24 h post PMCAO or sham-PMCAO. Each value represents the mean ± SEM of 8 rats. (a) $p < 0.05$ as compared to the values of the intact group and to the values of 24 h post PMCAO.