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POST-STROKE TREATMENT WITH 17β-ESTRADIOL EXERTS NEUROPROTECTIVE EFFECTS IN BOTH NORMOTENSIVE AND HYPERTENSIVE RATS

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Abstract—Although ischemic stroke is a major cause of death worldwide and the predominant cause of acquired disability, the only effective drug therapy that has been developed thus far is reperfusion by tissue plasminogen activator. Since most patients do not qualify for this treatment, new methods have to be developed. It is well known that estradiol (E_2) exerts neuroprotective effects in different models of cerebral ischemia, but post-stroke treatment after an acute stroke has hardly been investigated. As many patients with an acute ischemic stroke have arterial hypertension, it is also of interest to evaluate the influence of this co-morbidity on the treatment efficacy of E_2. The effects of E_2 administered 30 min after a transient middle cerebral artery occlusion (tMCAO) induced by an intracerebral injection of endothelin-1 were assessed in male normotensive Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs). Treatment with E_2 reduced infarct size in both WKY and SHRs and decreased the number of degenerating neurons, indicating that acute treatment with E_2 is indeed neuroprotective. To address the role of glia in neuroprotection, the effects of E_2 on the activation of microglia and astrocytes was determined. It appeared that E_2 had no effect on microglial activation, but reduced the activation of astrocytes in SHRs but not in the normotensive controls. We conclude that post-stroke E_2 treatment in both normotensive and hypertensive rats is neuroprotective. Although the presence of hypertension changed the astrocytic response to E_2, it did not affect treatment efficacy.

Key words: estradiol, neuroprotection, stroke, rat, hypertension.

INTRODUCTION

Stroke is a common cause of death in developed countries and the predominant cause of acquired disability in adults, leaving 50% of stroke survivors permanently disabled (Green, 2008; Di Carlo, 2009). Fifteen percent of the stroke patients suffer from a hemorrhagic stroke whereas, 85% have an ischemic stroke, for which reperfusion by tissue-plasminogen activator is the only approved therapy.

Estradiol (E_2) is one of the many factors shown to be neuroprotective in animal models of ischemic stroke (Koh et al., 2006; Lebesgue et al., 2009; Zheng et al., 2013) and neurodegenerative diseases (Azcoitia et al., 1999; Quesada and Micevych, 2004; Cordellini et al., 2011; Khaksari et al., 2015). Many studies on neuroprotection by E_2 concerned hormone replacement therapy after ovariectomy whereas few studies have focused on acute stroke treatment with E_2 (Yang et al., 2000; McCullough et al., 2001; Liu et al., 2007; Soderstrom et al., 2009; Perez-Alvarez et al., 2012, 2014; Zheng et al., 2013). In most of these studies, modified (often ovariectomized) animals were used and co-morbidities for stroke were not included, which could be one of the reasons why many compounds shown to be neuroprotective in animal models for stroke failed in clinical trials (De Keyser et al., 1999; O'Collins et al., 2006; Green, 2008). Indeed, certain estrogen receptor agonists have been shown to exert neuroprotective actions only in ovariectomized females (Broughton et al., 2014).

Using animal models with hypertension as a co-morbidity can be of relevance since many patients admitted with acute stroke have hypertension. It has been shown that the response of spontaneously hypertensive rats (SHRs) to several treatments (e.g. neurotropic factors, antioxidants and glutamate antagonists) differ from that of normotensive rats (Roussel et al., 1992; Porritt et al., 2010; Zhang et al., 2012; De Geyter et al., 2013). Letourneur et al. showed that the ischemic penumbra was smaller in SHRs when

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Abbreviations: E_2, 17β-estradiol; Et-1, endothelin-1; GFAP, glial fibrillary acidic protein; MAP, mean arterial blood pressure; NDS, neurological deficit score; pMCAO, permanent middle cerebral artery occlusion; SHR, spontaneously hypertensive rats; tMCAO, transient middle cerebral artery occlusion; WKY, Wistar Kyoto.

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compared to Wistar Kyoto (WKY) rats and evolved differently in both strains with an accelerated evolution of penumbral tissue to irreversibly damaged tissue in SHRs (Letourneur et al., 2011). Therefore it is of importance to investigate the effect of hypertension on the efficacy of neuroprotective compounds. However, the results in the literature on neuroprotection by E_2 in hypertensive animals are ambiguous.

Carswell et al. showed that estrogen status can affect the sensitivity to focal cerebral ischemia in stroke-prone SHR, with smaller infarct sizes when endogenous E_2 levels were high (Carswell et al., 2000). Exogenous E_2 pre-treatment however showed no significant effect on infarct size in stroke-prone SHR, but a deleterious effect in WKY rats (Carswell et al., 2004). On the other hand, a study conducted in female SHRs showed a reduction in infarct size after E_2 replacement in ovariectomized rats (Fukuda et al., 2000). These studies highlight the need to further investigate the effects of acute E_2 treatment in normotensive and hypertensive animals as much as possible in the absence of other confounding factors (e.g. anesthetics).

To address the clinical relevance for acute ischemic stroke treatment, the neuroprotective effects of E_2 were investigated in male rats with and without hypertension as a co-morbidity factor. The experiments were performed in the endothelin-1 (Et-1) model which allows induction of ischemic stroke in conscious rats to exclude possible interference with anesthetics. Possible, direct or indirect, targets for E_2 were identified using markers for degenerating neurons and activated glia.

EXPERIMENTAL PROCEDURES

All experiments using animals were approved by the Ethical Committee for Animal Experimentation of the Vrije Universiteit Brussel and performed according to the National Guidelines on Animal Experimentation.

Animals, surgery and induction of an ischemic stroke

Male SHRs from Charles River Laboratories (L'Arbresle Cedex, France) were used as a model for hypertension and albino Wistar Kyoto (WKY, also from Charles River) rats were used as normotensive controls. Animals were always allowed to get used to their new environment for at least one week after transportation. To monitor the mean arterial blood (MAP) pressure, 12-weeks-old WKY and SHR rats were anesthetized by an i.p. injection of 60 mg/kg sodium pentobarbital (Nembutal®; Ceva Sante Animal, Brussels, Belgium) and kept at 37 °C by placing them on a heating pad. The right carotid artery was cannulated for continuous monitoring of MAP using a pressure transducer (HP Hewlett Packard, Boebingen, Germany). After a 30 min equilibration period following surgery, the MAP was registered on-line by the software program VI-Logger (National Instruments, Austin, Texas, USA) for 30 min. These experiments confirmed the difference in MAP at the age of 12 weeks (Fig. 1). The MAP was significantly different between the two strains and there was no overlap between the two groups ($P = 0.0022$). Similar results were found measuring the MAP in a cohort of 69 WKY and 63 SHR rats from Charles River Laboratories in Boston, USA (Brouwers et al., 2015).

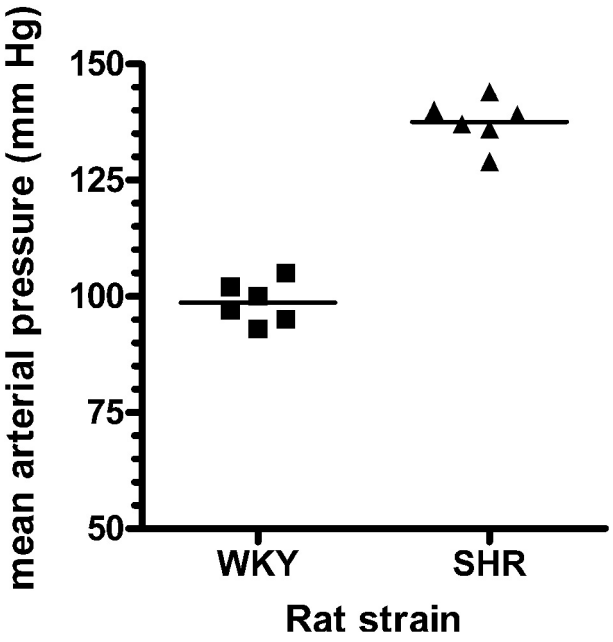

Fig. 1. Mean arterial blood pressure (MAP) in WKY and spontaneously hypertensive rats (SHR) rats. MAP of the 12-weeks-old WKY rats ($n = 6$) was 98.7 ± 4.5 mm Hg (mean ± SD) and 137.5 ± 5.0 mm Hg in the SHR rats ($n = 6$). In total 14 animals were used for this study of which one died and one was excluded because of bleeding. Differences between WKY and SHR found to be significant using the two-tailed student's *t*-test ($P = 0.0022$).

Ischemic stroke was induced as previously described (Van Hemelrijck et al., 2003; De Geyter et al., 2012) in animals weighing between 275 and 300 g. At that time the animals were between 12 and 14 weeks old. Animals were allocated to the placebo- or treatment-group using restricted randomization in a block experimental design and assessment of infarct volume, neurological deficit score (NDS), fluoro-Jade- GFAP- and Iba-1-labelling was blinded. To induce an ischemic stroke, 200 pmol Et-1 was injected into the piriform cortex, in the vicinity of the MCA (coordinates relative to Bregma: AP + 0.9, L.5.0, L + 2.8) (Paxinos and Watson, 2008). At 30 min after induction of ischemic stroke, rats received a subcutaneous injection of 17β-estradiol (1 mg/kg, E1024, Sigma, Schnelldorf, Germany), dissolved in corn oil (Sigma) containing 1% ethanol 30 min after the insult. Placebo-treated animals were injected with the same solvent. For the stroke experiments including shams, we used 35 WKY rats of which nine died during surgery or stroke and eight were excluded due to wrong placement of the probe or a bleeding (as observed by histological assessment). Of the 29 SHR rats four died and eight rats were excluded because of a bleeding or displacement of the probe.

Measurements of striatal blood flow using Doppler flowmetry

Blood flow measurements were performed using a Laser Flow Blood perfusion monitor probe (TSI, Inc., Shoreview,