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BRAIN RESEARCH

Research Report

Estrogen receptor beta agonist diarylpropiolnitrile (DPN) does not mediate neuroprotection in a rat model of permanent focal ischemia

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ARTICLE INFO

Article history:
Accepted 5 September 2007
Available online 16 September 2007

Keywords:
Estrogen receptor beta
DPN
Stroke
Neuroprotection
Behavior

ABSTRACT

Selective estrogen receptor (ER) agonists can indicate which receptor subtypes are implicated in neuroprotection. This study investigated the contribution of ERβ, using the selective agonist diarylpropiolnitrile (DPN), in a rat model of stroke. Lister Hooded rats were ovariectomized and implanted with mini-pumps containing either DPN (8 mg kg^{-1} day^{-1}) ($n=7$) or vehicle ($n=5$). Sensorimotor function was assessed using a neurological score and the spontaneous forelimb use asymmetry (cylinder) test. One week later the animals received a middle cerebral artery occlusion (MCAO), and T_2-weighted MRI at 48 h post-MCAO quantified ischemic damage. Functional recovery was tested for 7 days post-MCAO and brains processed for histological verification of infarct size. The MRI images revealed no significant differences in hemispheric lesion volumes between vehicle- and DPN-treated groups (35.6±3.5% and 30.8±1.7%, respectively [mean±SEM]; Student's unpaired *t*-test $df=10$, $t=-1.357$, $p=0.453$); this was confirmed histologically at 7 days. MCAO induced significant decline in neurological score performance (from 22 to 11 at 2 h post-MCAO) in the vehicle-treated animals, which was not significantly influenced by DPN. MCAO also induced significant changes in forelimb use in the cylinder test (10% reduction in contralateral, 20% reduction in both, and 30% increase in ipsilateral forelimb use) but this response was not significantly different between groups [$F(1,1)=2.929$, $p=0.118$, repeated-measures ANOVA]. In conclusion, pretreatment with the ERβ agonist DPN did not influence infarct size or sensorimotor function in rats exposed to MCAO.

1. Introduction

The influence of estrogen in animal models of cerebral ischemic injury has been examined repeatedly and the majority of reports indicate that estrogens exert neuroprotective effects, reviewed in Gibson et al. (2006), Hurn and Macrae (2000), Murphy et al. (2004). However, recent reports also reveal that estrogen has the capacity to exacerbate ischemic damage (Bingham et al., 2005; Carswell et al., 2004a; Gordon et al., 2005; Harukuni et al., 2001; Theodorsson and Theodorsson, 2005). These results emphasize the need for more studies that examine the mechanisms of estrogen actions during experi-

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doi:10.1016/j.brainres.2007.09.009

mental stroke in order to gain an improved understanding of the hormone's neuroprotective potential. There are two subtypes of estrogen receptors (ER): ERα and the more recently discovered ERβ (Kuiper et al., 1996). While both subtypes retain considerable homology, there are differences in their brain distribution (Shughrue et al., 1997), the structure of the ligand binding domains (Harrington et al., 2003), and gene activation abilities (Curtis Hewitt et al., 2000; van de Stolpe et al., 2004). Therefore, it is likely that each subtype is capable of mediating different effects.

One approach in which to examine this issue is to use selective ER agonists and antagonists. Selective ER α and β agonists have been reported to protect neuronal cultures from β-amyloid (Cordey and Pike, 2005) and glutamate toxicity (Zhao et al., 2004). An in vivo study reported that the non-selective ER antagonist, ICI 182,780, increased striatal infarct volume in female mice exposed to transient middle cerebral artery occlusion (MCAO) (Sawada et al., 2000). These studies confirm the involvement of receptor-dependent pathways, but not which subtype is primarily implicated. Very few studies have examined the effects of ER subtype selective agonists in vivo, and those that do exist were conducted in global ischemia models. The first report indicated that both a selective ERα agonist, propyl pyrazole triol (PPT), and a selective ERβ agonist, WAY 200070-3, afforded protection for hippocampal neurons of ovariectomized female rats (Miller et al., 2005). Another study, from our group, found that the selective ERβ agonist diarylpropiolnitrile (DPN) was able to mediate neuroprotection in ovariectomized mice (Carswell et al., 2004b) however, a recent study revealed this may not be the case for male rats, in which no observable effect of DPN was detected (Dai et al., 2007).

To our knowledge, there are no studies that have examined the effects of selective ER agonists in rodent models of focal ischemia. The purpose of the present study was to continue the ongoing work in our laboratory concerning the role of ERβ and test the neuroprotective potential of DPN in a focal ischemia model.

2. Results

Overall mortality for the entire study was 7%: two animals from the DPN-treated group died during MRI acquisition due to malfunction of anesthetic equipment. An additional two animals from the vehicle-treated group were excluded due to signs of hemorrhage on the MRI images. Two animals (one from each group) were euthanized prematurely following the

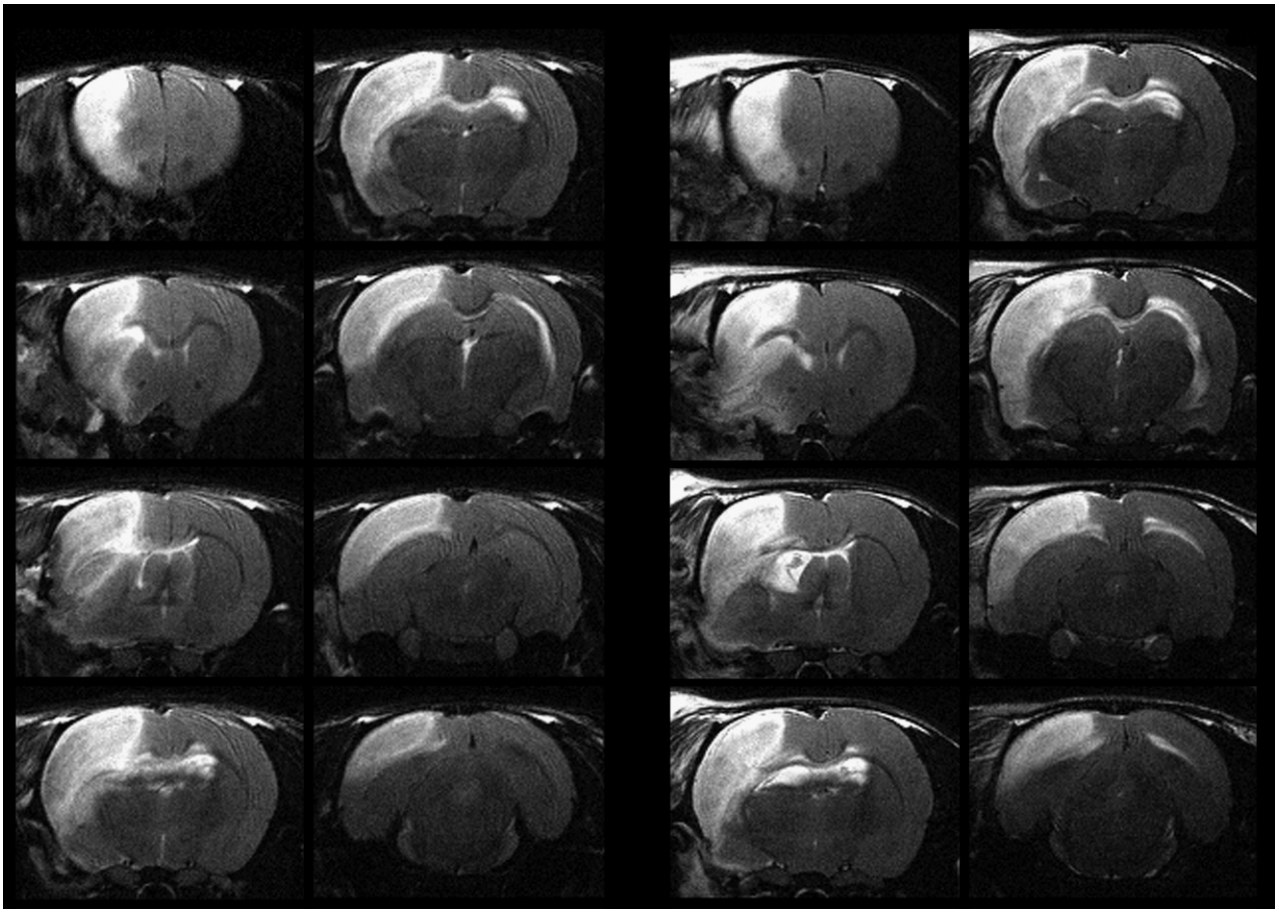

Fig. 1 – Assessment of infarct distribution. T_2-weighted images at 48 h post-MCAO: eight coronal slices, corresponding to approximately 3.2, 1.2, –0.26, –0.92, –1.8, –3.8, –5.3, and –6.3 mm from bregma from the median vehicle- (left) and DPN- (right) treated animal.