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Angiotensin-(1-7) improves cognitive function in rats with chronic cerebral hypoperfusion

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ABSTRACT

Chronic cerebral hypoperfusion (CCH) is associated with cognitive decline in aging, vascular dementia and Alzheimer's disease. Recently, angiotensin-(1-7) (Ang-(1-7)), one of the physiological constituents of the brain, was found to protect against cognitive dysfunction and brain ischemia. However, the effects of Ang-(1-7) on CCH-induced cognitive deficits remained unknown. In the present study, Ang-(1-7) significantly alleviated CCH-induced cognitive deficits in rats subjected to permanent bilateral occlusion of the common carotid arteries (a model of CCH). This neuroprotective effect was associated with increased nitric oxide generation, attenuated neuronal loss and suppressed astrocyte proliferation in the hippocampus. These findings demonstrate that Ang-(1-7) is a promising therapeutic agent for CCH-induced cognitive deficits.

1. Introduction

Chronic cerebral hypoperfusion (CCH) is an important factor underlying memory and learning impairment, and is associated with various neurological and psychiatric diseases, including Alzheimer's disease and vascular dementia(Austin et al., 2011). Cerebral hypoperfusion results from chronic disruption of cerebral blood flow, and the renin–angiotensin system (RAS), one of the most important systems regulating vascular homeostasis, is linked to cognitive function(Ciobica et al., 2009). Indeed, drugs that target the RAS seem to have great potential for the prevention and treatment of dementia (Ciobica et al., 2009).

Angiotensin-(1-7), (Ang-(1-7)), the main vasodilator in the RAS, has received considerable attention recently. Its effects through the receptor MAS have been well characterized in numerous tissues, including the brain (Passos-Silva et al., 2013). Central administration of Ang-(1-7) into a rat model of ischemic stroke is accompanied by reduced infarct size and an improvement of neurological deficits. The anti-ischemic effects are linked to increased brain blood supply, reduced oxidative stress, improved brain energy, stimulated

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neuroprotective factor production, and blunted inflammatory cascades (Lu et al., 2013; Mecca et al., 2011; Regenhardt et al., 2013; Zhang et al., 2008). Nitric oxide (NO) is a major mediator of the anti-ischemic effects and the role of MAS in these effects is confirmed by the receptor antagonist A779(Jiang et al., 2013). Moreover, brain Ang-(1-7) is critical for cognitive processes. In *in vitro* electrophysiological studies, Ang-(1-7) enhances long-term potentiation (LTP) in the CA1 region of the hippocampus, and genetic deletion of its receptor MAS abolishes this enhancement(Hellner et al., 2005). This LTP-enhancing effect of Ang-(1-7) is also observed in the lateral amygdala in mice, and is mediated by the various isoforms of NO synthase (NOS) (Albrecht, 2007; Staschewski et al., 2011). In a more recent study, the Ang-(1-7)/MAS pathway was found to be essential for normal processing of object recognition memory (Lazaroni et al., 2012), suggesting that it is an important modulator of learning and memory. Indeed, risk factors for cognitive impairment, such as hypertension, obesity, insulin resistance and dyslipidemia, can be controlled by Ang-(1-7) (Fraga-Silva et al., 2013; Oh et al., 2012; Santos et al., 2013a, 2013b), implicating the peptide in cognitive protection.

In the present study, we aimed to investigate whether Ang-(1-7) protects against cognitive dysfunction in rats subjected to CCH. CCH was induced by permanent bilateral common carotid artery occlusion (the 2-vessel occlusion (2VO) model), which is well established and extensively used for examining hypoperfusion-mediated neuronal damage as well as the effects of neuroprotective drug candidates (Farkas et al., 2007).

2. Results

2.1. Central Ang-(1-7) administration attenuates the 2VO-induced increase in systolic blood pressure (SBP)

SBP was measured with the tail cuff method before surgery (2 h before model induction), after surgery (7 days after model induction), and after treatment (7 days after Ang-(1-7) infusion). As shown in Table 1, the baseline SBPs are approximately 125 mmHg. SBPs were increased to approximately 155 mmHg in 2VO rats ($p<0.01$). The 2VO-induced increases in SBPs were not significantly changed by intracerebroventricular (ICV) infusion of low-dose Ang-(1-7), although there were slight decreases ($p>0.05$). In comparison, SBPs were reduced to 136 mmHg by high-dose Ang-(1-7) treatment ($p<0.01$), and these reductions were blocked by A779, an antagonist of the Ang-(1-7) receptor MAS.

2.2. Central Ang-(1-7) administration alleviates 2VO-induced cognitive deficits in the Morris water maze test

On day 1 of the test, cognitive performance was significantly impaired in 2VO rats (Fig. 1A). This learning deficit was alleviated by high-dose Ang-(1-7) ($p<0.05$), though not by low-dose Ang-(1-7) ($p>0.05$). This effect of high-dose Ang-(1-7) was blocked by A779. On days 2 and 3, 2VO-induced learning deficits were alleviated by both low-dose and high-dose Ang-(1-7) (all $p<0.01$), and these effects were blocked by A779 (Fig. 1A). On day 4, the number of correct crossings and the time spent in the target quadrant were remarkably reduced in 2VO rats (all $p<0.01$, Fig. 1B and C), suggesting that memory performance was impaired by 2VO. This impairment was attenuated by Ang-(1-7) treatment ($p<0.01$). The beneficial effects were prevented by A779, implicating MAS in the Ang-(1-7) signaling pathway. The swimming speed was not altered by the 2VO model or by ICV infusion (all $p>0.05$, Fig. 1D), suggesting that locomotor activity was not impaired by these procedures.

2.3. Central Ang-(1-7) administration has different effects on hippocampal MAS and NOS expression

The levels of the receptor MAS, and the three isoforms of NOS, including eNOS, iNOS and nNOS, were assessed in rat hippocampus extracts. As shown in Fig. 2A and B, the expression of MAS is significantly suppressed in 2VO rats ($p<0.01$), but is promoted by high-dose Ang-(1-7) treatment ($p<0.01$). Similar results were obtained for eNOS ($p<0.01$). These effects of high-dose Ang-(1-7) were prevented by A779, suggesting that these effects were dependent on the receptor MAS. In comparison, the levels of iNOS and nNOS were not altered by the 2VO model or by ICV infusion (all $p>0.05$, Fig. 2C and D).

2.4. Central Ang-(1-7) administration increases hippocampal NO production

The levels of NO were measured in extracts of brain tissues subjected to ischemic injury. As shown in Fig. 3, after 2

Table 1 – Central Ang-(1-7) administration reduces the 2VO-induced increase in systolic blood pressure (mmHg).

Treatment	Before surgery	After surgery	After treatment
Sham+aCSF	128.6±7.6	124.8±6.4**	126.0±6.5**
2VO+aCSF	125.5±5.01;##	155.4±8.4	155.5±11.1
2VO+L-A7	126.3±7.31;##	153.1±9.5	145.1±10.0
2VO+H-A7	126.0±5.91;##	153.9±9.6	135.6±10.9**1;##
2VO+H-A7+A779	125.0±6.21;##	152.5±9.8	158.4±8.0
2VO+A779	125.4±6.21;##	153.5±11.1	151.0±10.4

Results are presented as mean±SD. $n=10$ for each group.
L-A: low-dose Ang-(1-7); H-A: high-dose Ang-(1-7).
** $p<0.01$ *vs.* 2VO+aCSF.
$p<0.01$ *vs.* after surgery.