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Effects of rolipram and roflumilast, phosphodiesterase-4 inhibitors, on hypertension-induced defects in memory function in rats

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ABSTRACT

Hypertension (HT) is a prevailing risk factor for cognitive impairment, the most common cause of vascular dementia; yet, no possible mechanism underlying the cognitive impairment induced by hypertension has been identified so far. Inhibition of PDE-4 has been shown to increase phosphorylation of cAMP-response element binding protein in the hippocampus and enhance the memory performance. Here, we examined the effects of PDE-4 inhibitors, rolipram and roflumilast, on the impairment of learning and memory observed in hypertensive rats. We used 2k-1c hypertensive model to induce learning and memory defects. In addition, mRNA expression of PDE-4 sub-types A-D was also assessed in the hippocampus tissue. Systolic blood pressure (SBP) was measured by tail-cuff method was significantly increased in 2k-1c rats when compared to sham operated rats; this effect was reversed by clonidine, whereas, PDE-4 inhibitors did not. PDE-4 inhibitors significantly reversed time induced memory deficit in novel object recognition task (NORT). Further, the retention latency on the second day in the elevated plus maze model was significantly shortened after repeated administration of rolipram and roflumilast. Plasma and brain concentrations of rolipram, roflumilast and roflumilast N-oxide were also measured after the NORT and showed linear increase in plasma and brain concentrations. The PDE4B and PDE4D gene expression was significantly enhanced in hypertensive rats compared with sham operated however PDE4A and PDE4C remained unaltered. Repeated treatment with PDE-4 inhibitors caused down regulation of PDE4B and PDE4D in hypertensive rats. These results suggest that inhibition of PDE-4 ameliorates HT-induced impairment of learning and memory functions.

1. Introduction

Hypertension is a prevailing vascular risk factor for cognitive decline and dementia, leading to a distressing loss of independence of the individual (Duron and Hanon, 2008). Several studies have focused on the possible mechanisms underlying the cognitive impairment induced by hypertension but a pathophysiological mechanistic link is still to be ascertained (Carnevale et al., 2012). The existence of memory deficits correlates with the presence of hypertension, and the subsequent pathological changes are generally foci of ischemic damage in deep cerebral white matter (Fazekas et al., 1993; Vermeer et al., 2003a). White matter damage may contribute to cognitive impairment and its most serious manifestation being dementia (Pantoni and Garcia, 1995; O'Brien et al., 2003; Vermeer et al., 2003b). People with controlled hypertension seem to have a lesser prevalence of white matter lesions than people with uncontrolled hypertension (Liao et al., 1996; de Leeuw et al., 2002).

Several clinical studies suggest that high blood pressure contributes to cognitive deficits in aging individuals (Elias et al., 1997; Skoog, 1997). Numerous pre-clinical models have shown positive (Wyss et al., 1992; Meneses et al., 1996; Meneses and Hong, 1998; Hirawa et al., 1999; Wyss et al., 2000; Hacioglu et al., 2003) but also negative (Kadish et al., 2001) correlations between hypertension and cognitive impairment in rats.

Phosphodiesterase-4 (PDE-4) is a critical regulator of intracellular level of cAMP that is expressed throughout the brain (Perez-Torres et al., 2000). Activation of cyclic AMP (cAMP) signaling enhances memory function and synaptic plasticity (Wang et al., 2012). Therefore, targeting PDE-4 with a selective inhibitor may offer novel strategies in the treatment of memory impairment (Ghavami et al., 2006; Kodimuthali et al., 2008). A number of

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studies have identified that PDE-4 inhibition leads to elevation of cAMP levels (Barad et al., 1998), increased protein kinase A (PKA)/phosphorylation of cAMP response element binding protein (pCREB) in the hippocampus signaling. This signaling cascade is essential for mediating memory, in particular hippocampus dependent long-term memory (Monti et al., 2006; Nagakura et al., 2002; Bourtchouladze et al., 2003). Rolipram, a specific PDE-4 inhibitor, has been shown to improve the working memory deficits caused by administration of scopolamine and MK-801in preclinical models (Zhang and O'Donnell, 2000; Zhang et al., 2000). Also, rolipram treatment reverses cognitive impairment and neuroinflammation induced by β-amyloid in rats (Wang et al., 2012). Very recently, brain penetrant selective PDE-4 inhibitor-GSK356278 demonstrated anxiolytic and improvement of cognition effects in preclinical models (Rutter et al., 2014).

Roflumilast is a second generation, highly selective PDE-4 inhibitor, and has recently been approved in several countries for severe COPD (Rabe, 2011). However, the role of PDE-4 inhibitors in improving learning and memory dysfunction induced by hypertension has not been studied so far. To evaluate the possibility of PDE-4 inhibitor as a therapeutic agent for cognitive dysfunction by hypertension, we examined the effects of rolipram and roflumilast on impairment of learning and memory function in the hypertensive rats using a two-kidney one-clip (2k-1c) hypertensive model to induce deficits in learning and memory function.

2. Materials and methods

2.1. Animals

Adult male Wistar rats were obtained from Orchid Chemicals Pharmaceuticals Ltd, Chennai, India. Animals were housed in groups on soft bedding with food and water available ad libitum, in a temperature controlled environment with a light dark cycle of 12:12 h. All animals were allowed to habituate to the housing facilities for at least 1 week prior to surgery. Guidelines of "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academic Press 1996; NIH publication number #85-23, revised 1996) were strictly followed throughout the study. All experimental procedures were approved by the Institutional Animal Ethical Committee (IAEC), Sri Ramachandra University, constitute as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, (IAEC NO: IAEC/XXX111/SRU/258/2013).

2.2. Drugs

Rolipram and clonidine hydrochloride were purchased from Tokyo Chemical Industry Co., Ltd, Japan. Roflumilast was kindly gifted by Matrix Laboratories, Hyderabad, India.

2.3. Two-kidney one-clip induced hypertension in rats

Prior to surgical procedure, systolic blood pressure was measured by tail-cuff method (model MC 4000; Hatteras Instruments, Cary, NC, USA). Two-kidney one-clip induced Reno Vascular Hypertension (RVHT) was performed as per the method described previously with minor modifications (Zeng et al., 1998; Kalaivani et al., 2013). In brief, rats weighing 140–180 g at the time of surgery were anaesthetized by intra peritoneal injection of ketamine (75 mg/kg) and midazolam (1 mg/kg) mixture. A small incision was made and the left renal artery was exposed and cleared. Then a U-shaped silver clip with a gauge of 0.25 mm was placed around the renal artery and secured in place and the incision was sutured and the animals were returned to their cages. In sham animals, incision was made, to expose the left renal artery and incision was sutured. Post-operative care was given to the rats for one week. The rats were maintained on drinking water containing 0.9% NaCl except sham group. SBP was measured starting three weeks after the renal artery constriction for 11 weeks. Animals were selected based on the SBP (cut off score $\geq$ 170 mm Hg) at the 11th week post-surgery; selected animals were then stratified into groups based on their mean SBP, so that the mean baseline did not differ between the groups.

For testing the effects of repeated drug treatments on behavioral tasks such as novel object recognition task and transfer latency in elevated plus maze, vehicle (0.5% CMC) or clonidine (25 μg/kg., *p.o*), rolipram (0.03, 0.1, 0.3 mg/kg., *i.p*, dissolved in normal saline containing 5% DMSO), roflumilast (0.1, 0.3, 1 mg/kg., *p.o*) were given once per day for ten consecutive days. Here we selected clonidine, a α2 receptor agonist, centrally acting anti hypertensive drug to evaluate whether anti-hypertensive treatment would attenuate the cognitive impairments associated with HT in therapeutic model. For both behavioral experiments, separate set of animals were used including sham operated rats (68 for NORT and 66 for transfer latency; total no = 134). Both behavioral tests were performed 1 h following the treatment except rolipram group (45 min after dosing) as per the schedule (Fig. 1).

2.4. Novel object recognition task paradigm

The effects of repeated administration of PDE-4 inhibitors on recognition memory in hypertensive rats were assessed using NORT similar to that described previously (Prickaerts et al., 2002). Rats were acclimated to the open arena (40 × 60 × 60) without objects for 15 min. NORT consisted of two trial periods, acquisition trial (T1) and discrimination trial (T2) separated by a 24 h inter trial period. A rat was taken from its home cage and placed into the apparatus, central from the two objects, facing the wall in front of the observer, and the time spent actively exploring (exploration was defined as the animal sniffing, or touching it) the objects during a 3 min test period (T1) was recorded and returned to its home cage. The arena and test objects were cleaned with alcohol in order to remove any olfactory/taste cues. After 24 h, each rat was again placed in the test arena for recording T2 in the presence of one of the familiar object and a novel object, and the time spent exploring both objects were again recorded and discrimination index (DI) was calculated according to the following formula.

$$\text{DI} = \text{RI}/(\text{Time spent in exploring novel object} + \text{Time spent in exploring familiar object})$$

$$\text{Recognition Index(RI)} = \text{Time spent in exploring novel object} - \text{Time spent in exploring familiar object}$$

2.5. Transfer latency in elevated plus maze

The procedure and technique for testing learning and memory were followed as per the parameters described earlier (Reddy and Kulkarni, 1998; Hlinak and Krejci, 2002). The elevated plus maze for rats consisted of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 40 cm) extended from a central platform (10 cm × 10 cm) and the maze was elevated to a height of 50 cm from the floor. On the 10th day of treatment, each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time taken by the animal to move from the open arm into one of the closed arms with all its four legs was recorded. If the animal did not enter into one of the closed arm within 180 s, it was gently pushed into one of the two closed arms and TL was assigned as 180 s and rat was allowed to explore the maze for another 60 s. Then, the rat was