Original Article

Pelargonidin improves amyloid β-induced deficits in the long-term potentiation in hippocampus of male rats

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Abstract

Introduction: Given that pelargonidin is an anthocyanin that exhibits neuroprotective effects and Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by the accumulation of amyloid-beta (Aβ) and cognitive dysfunction subsequently, herein, we examined the effects of pelargonidin on Aβ-induced long-term potentiation deficits in rats.

Methods: AD was induced using intrahippocampal injections of the Aβ in the adult Wistar male rats. The rats received single intraperitoneal injections of pelargonidin (3mg/kg). Long-term potentiation in the perforant path- dentate gyrus synapses was assessed electrophysiologically by measuring the field excitatory post-synaptic potential (fEPSP) slope and population spike (PS) amplitude.

Results: Our results showed that Aβ significantly decreased fEPSP slope and SP amplitude in comparison with the control and sham groups, whereas pelargonidin increased these parameters in comparison to the Aβ group.

Conclusion: It is probably that pelargonidin could improve Aβ-induced cognition deficit in rats.

Introduction

Alzheimer's disease (AD) as the most common cause of dementia is characterized by the accumulation of amyloid-beta (Aβ), neurofibrillary tangles (tau) and synaptic loss in the brain (Cvetković-Dožić et al., 2001; Ghavami et al., 2014). The hippocampus is one of the most important areas of the brain affected by AD. Following deposition of Aβ and tau in the hippocampus, the long-term potentiation (LTP) and synaptic plasticity were disrupted. It has been suggested that Aβ induces the lipid and protein oxidation, damages the mitochondrial membrane, increases intracellular H_2O_2 and subsequently results in neuronal death in the hippocampus (Mark et al., 1997). Aβ also destabilizes cellular homeostasis of Ca^{2+} and consequently inhibits LTP in the hippocampus and disrupts neuronal plasticity (Shankar et al., 2008). LTP is a manifestation of synaptic plasticity characterized by long-lasting enhancement of synaptic strength induced by high-

Days 0 — 14 — 28

Injection of Aβ

Injection of Pelargonidin

Electrophysiological recording
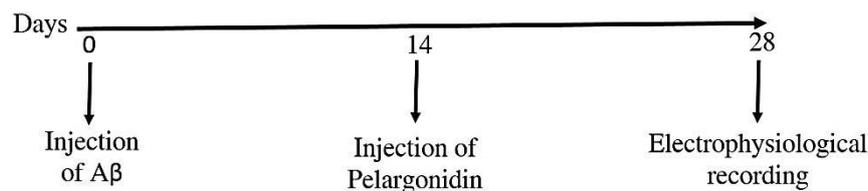
Fig.1. A schematic representation of the experimental procedures.

frequency activities of the synaptic input. It occurs during learning and is considered to be a potential neuronal mechanism for the formation of the associative memory (Whitlock et al., 2006). Hence, the alteration in LTP is accompanied by deficits in learning and memory (Hölscher, 1999). It has been reported that Aβ-induced impairment in LTP is accompanied by enhanced activity of stress oxidative and there is a negative relationship between oxidative stress and nervous system ability to detoxify the reactive oxygen species (Rothman and Mattson, 2010).

Administration of antioxidants is a therapeutic approach to alleviate Aβ-induced LTP deficit (Haque et al., 2008). Anthocyanin, as a type of flavonoid, is a bioactive compound isolated from wide range plants that exhibit antioxidant activity and subsequently neuroprotection (Gutierres et al., 2014). Pelargonidin is an anthocyanin that exhibits neuroprotective effects in diabetic hyperalgesia and 6-hydroxydopamine-induced hemi- Parkinsonism in rats (Roy et al., 2008; Roghani et al., 2010). It has been reported that administration of pelargonidin caused an increase in catalase and serum superoxide dismutase (free radical scavenging enzymes) in diabetic rats and a reduction in serum MDA levels as an indicator of stress oxidative (Roy et al., 2008). Because Aβ administration cause to stress oxidative and resulting to cognition deficit and due to antioxidant capacity of pelargonidin, in this study, therefore, we investigated the effects of pelargonidin on the Aβ-induced LTP deficit.

Materials and methods

Experimental design and animals' classification

All experiments were approved by the Ethics Committee of Iran University of Medical Science (No; 93/105/610). Adult male Wistar rats (250-300g) were obtained from the animal facility of the Hamadan University of Medical Sciences and maintained under standard laboratory condition (12h light/dark cycle, 20±2°C and 50% relative humidity) with free access to food and water.

Animals were randomly allocated into 4 experimental groups (n=7 per group) as follows: 1-control group that was left undisrupted; 2- sham operated group went under stereotaxic surgery without any treatment; 3- Aβ–injected group which received intrahippocampal injection of Aβ and 4- pelargonidin-treated Aβ groups that received single intraperitoneal (IP) injection of 3mg/kg pelargonidin two weeks after Aβ administration (Fig. 1).

Intrahippocampal injection of Aβ

Injection of Aβ (25-35) was performed as previously described method (Zargooshnia et al., 2015). In brief, under the ketamine (100mg/kg) and xylazine (10mg/kg) anesthesia and stereotaxic apparatus the Aβ (5μg) was bilaterally injected into the hippocampi over 1μl/2min at the coordinates for the dentate gyrus (relative to bregma, AP: -3.6mm, ML: ±2.3mm and DV: 3mm) (Paxinos et al., 1985). Pelargonidin was dissolved in normal saline and two weeks after Aβ injection, pelargonidin group received a single IP injection of 3mg/kg pelargonidin (Roy et al., 2008).

Electrophysiological techniques

The electrophysiological procedure to record field potentiation was carried out as described previously (Tahmasebi et al., 2015). Briefly, rats were anesthetized with urethane (1.5g/kg) and placed in a stereotaxic frame for the insertion of electrodes. As shown in Figure 1, a bipolar electrode was placed in the perforant pathway (AP: 7.8-8.1mm from bregma, ML: +4.3mm from midline and DV: 2.7mm from skull surface) and the recording electrode was positioned in the granular cell layer of the (AP: 3.6mm from bregma; ML: +2.3mm from midline and DV: 3mm from skull surface, Fig. 2) according to Paxinos and Watson's atlas (Paxinos et al., 1985). Base line recording was taken at least 40min prior to each
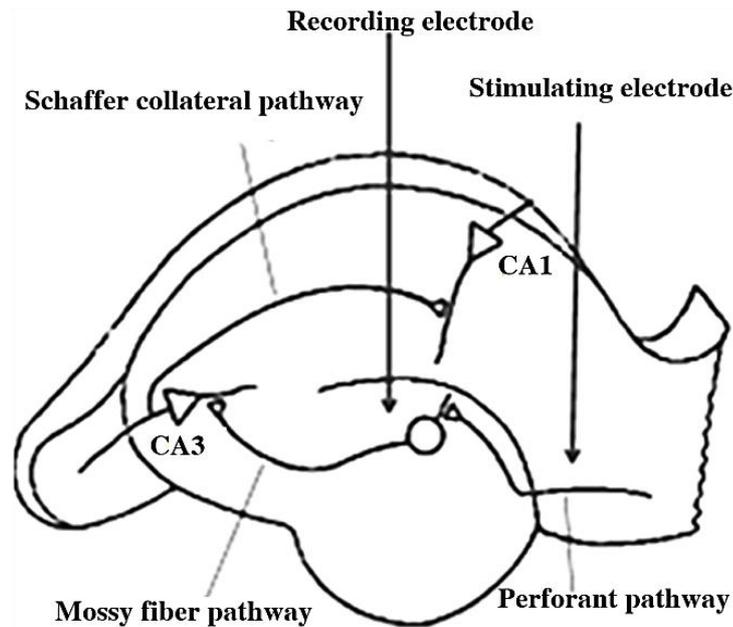
Fig.2. Schematic graph of hippocampus that illustrates the stimulating (perforant pathway) and recording (dentate gyrus) electrodes sites. Sites important of illustration of performed experiments are indicated by arrows (Karimi et al., 2013).

experiment. LTP was induced using high frequency stimuli (HFS) protocols of 400Hz (10 bursts of 20 stimuli, 0.2ms stimulus duration and 10s interburst interval) at a stimulus intensity that evoked a population spike (PS) amplitude and field excitatory postsynaptic potential (fEPSP) slope of approximately 80% of the maximum response. Both fEPSP and PS were recorded for the period of 5, 30, and 60min after HFS in order to determine any changes in the synaptic response of DG neurons. The parameters of the stimuli were amplified (100x) and filtered (1Hz to 3kHz band pass) using an A365 differential amplifier (WPI, USA). Signals were passed through an analogue to digital interface to a computer and data were analyzed using Biochart software.

Data analysis
Data are expressed as mean±SEM. Statistical analysis were performed using ANOVA with repeated measure (GraphPad Prism 6 software) and Tukey's post hoc test. Value of $P \leq 0.05$ was considered significant.

Results

Effect of pelargonidin on Aβ- induced PS deficiency in the granular cells of DG
A two-way analysis of variance of PS revealed significant effects of treatment [F(3, 41005)= 10.27,

P<0.001] and time points (5, 30 and 60min) [F(3, 7739)= 19.39, P<0.001). No significant interaction between treatment and times point was observed. As shown in Figure 3, intra-hippocampal injection of Aβ negatively affects LTP in the DG and produced a substantial decrease in the PS amplitude after HFS in compared with the intact group (P<0.05). The administration of pelargonidin significantly increased PS amplitude LTP in comparison with the Aβ- treated group (P<0.001, Fig. 3).

Effect of pelargonidin on Aβ- induced EPSP deficiency in the granular cells of DG
Consistent with the PS data, a significant effect of treatment [F(3, 1662)= 12.11, P<0.001] and time points [F(3, 6208)= 45.24, P<0.001] in slope of EPSP of the granular cells of DG was observed. There was a significant interaction between time points and treatment [F(9, 280.69)= 2.04, P<0.05]. Rats that received Aβ showed a depressed EPSP when compared with intact group (P<0.05). Aβ-treated rats that received pelargonidin showed a significant increase in slope of EPSP LTP as compared to the Aβ group (P<0.001, Fig. 4).

Discussion

The results of this study showed that intrahippocampal injection of Aβ (25-35) impaired the
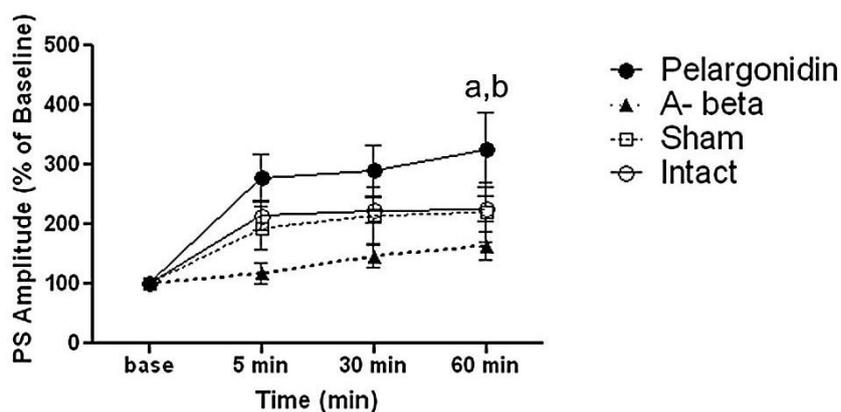
Fig.3. The effects of Aβ (25-35) and pelargonidin on the PS amplitude in DG granule cells following a 400Hz HFS applied to the perforant path (n=7). The PS amplitude was significantly reduced in Aβ- treated group (a, $P<0.05$ vs. intact group) and pelargonidin administration caused an increase in PS compared to Aβ group (b, $P<0.001$).
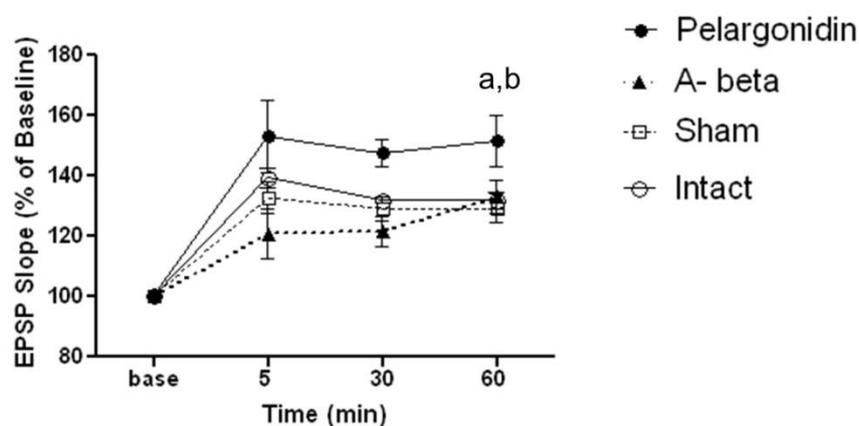

Fig.4. The effects of Aβ (25-35) and pelargonidin on the EPSP slope in DG granule cells following a 400Hz HFS applied to the perforant path (n=7). There was a significant difference between Aβ-injected group, intact (a, $P<0.05$) and pelargonidin (b, $P<0.001$) groups.

PS amplitude and fEPSP slopes in DG hippocampal granule cells and administration of pelargonidin could attenuate Aβ-induced deficits in LTP. We used the Aβ (25–35) to induce Alzheimer's disease model because it is more soluble than (1–42) fragment and contains the active sequence of (32–35) beta-amyloid that displays neurotoxic effects (Pike et al., 1995). Consistent with the results of this study, Gault and Hölscher (2008) found an impaired LTP following injection of Aβ (25–35) in the brain of male Wistar rats. Furthermore, a previous study by Cooper and Bear (2012) has suggested a reduction in synapses prior to apoptosis and dementia in the AD patients. Therefore, from previous observations, It has been indicated that deposition of Aβ inhibits the reuptake of glutamate, decreases the cell surface expression of N-methyl-D- aspartate receptor (NMDAR) and disrupts cAMP-mediated form of LTP (Lacor et al.,

2007; Shankar et al., 2007). Furthermore, Aβ alters calcium dynamics and dendritic spine density and subsequently decrease neuronal synaptic plasticity (LTP) in the hippocampus (Gonzalo-Ruiz et al., 2019; Morley et al., 2019). In this context, NMDA receptor-mediated LTP in the hippocampus is considered as the most important form of synaptic plasticity (Yun et al., 2006) and have been shown that Aβ deposition induces massive oxidative challenge in the hippocampus through a mechanism involving impaired energy metabolism and stress-related signaling pathways in nerve cells (Matsuda et al., 2018). There are several therapeutic approaches (such as chemical and herbal medicine) to reduce Aβ-induced LTP deficit (Almasi et al., 2018; Komaki et al., 2019). Among the herbal medicine, pelargonidin is an anthocyanin that produces a characteristic orange- red color in the plants and

easily cross the brain blood barrier (Youdim et al., 2004). Consistent with others, the results of this study showed attenuation in the Aβ-induced neurotoxicity following administration of pelargonidin (Roghani et al., 2010). They reported that administration of pelargonidin dose-dependently attenuated the rotational behavior and thiobarbituric acid reactive substances formation and protected the neurons of substantia nigra pars compacta against 6-OHDA toxicity (Roghani et al., 2010). Ho et al. have reported that the administration of quercetin-3-O-glucuronide as a phenolic compound, improved AD-type deficits in hippocampal formation basal synaptic transmission and LTP. They concluded that quercetin-3-O-glucuronide may possibly improve Aβ-induced LTP deficit through mechanisms involving the activation of the c-Jun N-terminal kinases and the mitogen-activated protein kinase signaling pathways (Ho et al., 2013). In another study administration of abacopterin E (a flavonoid) reversed the d-Gal induced LTP suppression via inhibiting the generation of free radical and enhancing the activities of endogenous antioxidant enzymes (Lei et al., 2011). Moreover, administration of berberine in pelargonidin ameliorates lipopolysaccharide- induced a cognitive decline in the rats via suppression of stress oxidative, neuroinflammation and apoptotic cascades (Sadraie et al., 2019). Taken together, the protective effects of pelargonidin may be related to its ability in the control of essential intracellular signaling cascades. It seems that pelargonidin could improve LTP deficit through moderation of cholinergic dysfunction and oxidative stress that needs further studies.

Conclusion

Our results suggest that pelargonidin may be recommended as an adjunct medication to improve cognition deficit in neurodegenerative diseases that needs further studies.

Acknowledgments

Conflict of interest

None of the authors has a financial interest to be reported.

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