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Research report

Candesartan improves memory decline in mice: Involvement of AT1 receptors in memory deficit induced by intracerebral streptozotocin

Santoshkumar Tota, Pradeep K. Kamat, Himani Awasthi, Nilendra Singh, Ram Raghubir, Chandishwar Nath, Kashif Hanif*

Division of Pharmacology, Central Drug Research Institute (CDRI), P.O. Box 173, Lucknow, U.P. 226001, India

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ABSTRACT

The Renin-angiotensin system, besides blood pressure regulation, affects learning and memory as evidenced by improvement of cognition in hypertensive patients being treated with AT1 receptor blockers like candesartan. The present study examined the influence of candesartan on memory impairment induced by intracerebral streptozotocin (IC STZ 0.5 mg/kg) in mice. Candesartan (0.05 mg/kg and 0.1 mg/kg, i.p.) was given for 14 days following IC STZ administration. The dose of 0.1 mg/kg significantly improved latency period in passive avoidance test. Further, treatment with 0.1 mg/kg candesartan for 14 days significantly improved spatial memory in mice in water maze test also. In another group, after memory impairment in mice following IC STZ administration, memory improving effect of a 7 days treatment with 0.1 mg/kg candesartan lasted only for 3 subsequent days in water maze task. IC STZ increased oxidative stress but pretreatment with 0.1 mg/kg candesartan decreased oxidative stress as indicated by a decrease in MDA and increase in GSH. Further, candesartan decreased free radicals as evidenced by flow cytometry. IC STZ affected cholinergic system also by increasing acetylcholine esterase activity that was restored by pretreatment with 0.1 mg/kg candesartan. Locomotor activity and serum glucose level remained unaffected by candesartan treatment. These results suggest that AT1 receptors play a facilitatory role in STZ induced memory deficit and corroborate number of human studies that AT1 receptor blockers can be used therapeutically against cognitive decline in hypertensive patients.

1. Introduction

The Renin-angiotensin system (RAS) plays a pivotal role in the homeostasis of blood pressure regulation in peripheral vasculature [1]. However, besides vasculature, presence of the RAS has also been established in CNS [2,3] where it regulates several homeostatic/physiological and behavioral processes such as thirst, temperature, sexual behavior [3,4] and cognitive functions [5,6]. However, the exact role played by Ang II, one of the main peptide of RAS, and its related metabolites in learning and memory is not yet clear [5]. In experimental models of cognitive functions, brain RAS and its effector peptide i.e. angiotensin II (Ang II) and its different metabolites like Ang IV and Ang III [6–8] exert modulatory effects on learning and memory by influencing the activity of neurotransmitters such as acetylcholine [9]. Braszko's group demonstrated enhancement of learning by Ang II administration, in a number of tasks including passive avoidance and object recognition, [7,8,10] was mediated by AT1 receptors [11,12]. Opposing effects have also been reported; when infused into hippocampus after training, Ang II produced a dose-dependent amnesic effect in the inhibitory avoidance task in rats, which was blocked by the AT2 antagonist, PD123319, but not by the AT1 antagonist, losartan [13]. Another study showed that intrahippocampal administration of Ang II disrupted retrieval of aversive memory in the inhibitory avoidance task, an effect that was mediated via the AT2 receptor [14].

Further, clinical studies also prove that the RAS and cognition are intricately intertwined. Antihypertensive drugs interacting with RAS i.e. ACE inhibitors and AT1 receptor blockers (ARBs) prevent cognitive decline and even improve cognitive functions in hypertensive patients [15–18] and animals [19–21]. The ARBs as compared to ACE inhibitors effectively improve some of the components of cognitive functions, particularly episodic memory [17]. Involvement of the RAS and AT1 receptors in memory got further strengthened when it was shown that valsartan, an ARB, attenuated oligomerization of Aβ peptides in *in vitro* and *in vivo* conditions and the development of Aβ-mediated cognitive deterioration at non-hypotensive dose in AD mouse model [22].

The present study was designed to find out the role of AT1 receptors and assess the effect of administration of candesartan, an AT1 receptor blocker, in model of STZ induced memory deficit. Alzheimer's disease (AD) and related dementia, characterized by progressive memory loss and diminished cognitive ability [23],

* Corresponding author. Tel.: +91 522 2625036; fax: +91 522 2623405.
E-mail address: k_hanif@cdri.res.in (K. Hanif).

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show reduced levels of glucose utilization, energy rich phosphates [24] and acetylcholine synthesis due to lower concentrations of acetyl-coenzyme A [25]. Oxidative stress increases resulting in oxidation of biomolecules [26–28]. These aspects of AD like disturbed glucose and energy metabolism and increased oxidative stress are closely mimicked after intracerebroventricular (ICV) injection of streptozotocin (STZ) in rats. STZ possibly desensitizes neuronal insulin receptor [29] and leads to progressive deterioration of memory, impairment of cerebral glucose and energy metabolism [24,26], oxidative stress [27] and reduced acetylcholine [30,31] due to decreased activity of glycolytic enzymes [32].

2. Materials and method

2.1. Animals

8 weeks old Swiss albino mice (25–30 g) were obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow. The animals were kept in polyacrylic cages (22.5 cm × 37.5 cm) with 5 mice per cage and maintained under standard housing conditions (room temperature 24–27 °C and humidity 60–65%) with a 12-h light and dark cycle. Food, in the form of dry pellets, and water were available ad libitum but food was not allowed from 1 h prior to till completion of the experiment (approximately 2–3 h). Experiments were performed according to international ethical standards and approved by the research ethics committee of Central Drug Research Institute and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

2.2. Drug administration

2.2.1. Intracerebral (i.c.) administration of streptozotocin (STZ)

The mice were anesthetized with chloral hydrate (300 mg/kg, i.p.). STZ (0.5 mg/kg, volume 10 μl) was dissolved in vehicle i.e. freshly prepared artificial CSF (aCSF) [27] and administered intracerebrally (IC) according to the method of Haley and McCormick [33]. The same dose of STZ was repeated 48 h after the first dose. Fourteen days after the first dose, the animals were subjected to testing of learning and memory functions in passive avoidance or Morris water maze test.

2.2.2. Administration of candesartan

Candesartan cilexetil was obtained from Ranbaxy. Non-hypotensive doses of 0.05 mg/kg and 0.1 mg/kg [34] were administered intraperitonealy (i.p.) daily for 14 days in pretreatment. In another set of experiment, 0.1 mg/kg candesartan was administered (i.p.) for 7 days after significant memory deficit following IC STZ. Candesartan was dissolved in a vehicle solution (1 mg in 950 μl of phosphate buffered saline and 50 μl 1 M Na_2CO_3) [35].

2.2.3. Experimental protocol

Animals were divided in seven groups and each group comprised of six animals.

Group 1: Control mice treated with vehicle of candesartan for 14 days.
Group 2: Mice were injected intracerebrally with artificial cerebrospinal fluid (aCSF) on day one and three and treated with vehicle for 14 days.
Group 3: Mice were injected with IC STZ on day one and three and treated with vehicle for 14 days.
Group 4: Mice were injected with IC STZ on day one and three and treated with 0.05 mg/kg candesartan starting from day one to 14 days.
Group 5: Mice were injected with IC STZ on day one and three and treated with 0.1 mg/kg candesartan starting from day one to 14 days.
Group 6: Mice were injected with IC STZ on day one and three and treated with vehicle for 14 days and subjected to water maze trial for 5 consecutive days followed by 1 week treatment with vehicle. On 26th day onwards, these animals were again subjected to water maze trial for 5 consecutive days.
Group 7: Mice were injected with IC STZ on day one and three and treated with vehicle for 14 days and subjected to water maze trial for 5 consecutive days followed by 1 week treatment with 0.1 mg/kg candesartan. On 26th day onwards, these animals were again subjected to water maze trial for 5 consecutive days.

Animals from Group 1–5 were sacrificed on day 21 and tissues were taken for biochemical and flow cytometry studies.

2.3. Assessment of learning and memory

Learning and memory were studied with the passive avoidance test and Morris Water Maze test in different groups of mice described in Section 2.2.

2.3.1. Passive avoidance test

The mice were subjected to the passive avoidance test by placing in a compartment with light at intensity of 8 [Scale from 0 to 10 (brightest)] in a computerized shuttle box with a software programme PACS 30 (Columbus Instruments, OH, USA). The light compartment was isolated from the dark compartment by an automated guillotine door. After an acclimatization period of 30 s, the guillotine door was opened and closed automatically after entry of the mouse into the dark compartment. The subject received a low-intensity foot shock (0.5 mA; 10 s) in the dark compartment. Infrared sensors monitored the transfer of the animal from one compartment to another which was recorded as transfer latency time (TLT) in seconds. The 1st trial was for acquisition and retention was tested in a 2nd trial (1st retention) given 24 h after the 1st trial. The duration of a trial was 270 s. Further, 2nd, 3rd and 4th retention trials on alternate days were given to measure retention in the IC STZ treated mice [36]. The shock was not delivered in the retention trials to avoid reacquisition. The criterion for learning was taken as an increase in the TLT on retention (2nd or subsequent) trials as compared to acquisition (1st) trial.

2.3.2. Morris water maze

The Morris water maze consisted of a large circular black pool of 120 cm diameter, 50 cm height, filled to a depth of 30 cm with water at 26 ± 2 °C. Four equally spaced points around the edge of the pool were designated as N (North), E (East), S (South) and W (West). A black colored round platform of 8 cm diameter was placed 1 cm below the surface of water in a constant position in the middle of the NE quadrant in the pool; the starting point was in the SW quadrant in all the trials. The water was colored with non-toxic black dye to hide the location of the submerged platform. The mice could climb on the platform to escape from the necessity of swimming. Trials were given for 5 consecutive days in order to train the mice in the Morris water maze. The mice were given a maximum time of 60 s (cut-off time) to find the hidden platform and were allowed to stay on it for 30 s [37]. The experimenter himself put the mice that failed to locate the platform onto it. The animals were given a daily session of 3 trials per day. Latency time to reach the platform was recorded in each trial. Mean latency time of all three trials was shown in the results. A significant decrease in latency time from that of 1st session was considered as successful learning [36].

2.4. Spontaneous locomotor activity

Each animal was observed for 10 min after a period of 30 min for acclimatization in Optovarimex activity meter (Columbus Inc., USA).

2.5. Estimation of biochemical parameters

Acetylcholine esterase (AChE) and biochemical parameters of oxidative stress, MDA and GSH, were measured in the brain on the 21st day after streptozotocin injection.

2.5.1. Brain tissue preparation

The mice were decapitated under ether anesthesia. The skull was cut open and the brain was exposed from its dorsal side. The whole brain was quickly removed and cleaned with chilled normal saline on the ice. A 10% (w/v) homogenate of brain samples in 0.03 M sodium phosphate buffer (pH 7.4) was prepared by using an Ultra-Turrax T25 (USA) homogenizer at a speed of 9500 rpm.

2.5.2. Protein estimation

Protein was measured in all brain samples for GSH and MDA activity by the method of Lowry et al. [38] and for acetylcholine esterase activity by the method of Wang and Smith [39]. Bovine serum albumin (BSA) (1 mg/ml) was used as standard and measured in the range of 0.01–0.1 mg/ml.

2.5.3. Acetylcholine esterase assay

The brain homogenate in volume of 500 μl was mixed with 1% Triton X-100 (1%, w/v in 0.03 M sodium phosphate buffer, pH 7) and samples were centrifuged at 100,000 × *g* at 4 °C in a Beckman Ultracentrifuge (LE 80, USA), using a fixed angle rotor (80 ti) for 60 min. Supernatant was collected and stored at 4 °C for acetylcholine esterase estimation [40]. The kinetic profile of enzyme activity was measured spectrophotometrically (Shimadzu, USA) at 412 nm with an interval of 15 s. One unit of acetylcholine esterase activity was defined as the number of μmol of acetylthiocholine iodide hydrolyzed per minute per milligram of protein. The specific activity of acetylcholine esterase is expressed in μmol/min/mg of protein.

2.5.4. Measurement of MDA

MDA, a product of lipid peroxidation, was measured spectrophotometrically [41], using 1,1,3,3-tetraethoxypropane as standard. MDA is expressed as nanomoles/mg of protein. To 500 μl of tissue homogenate in phosphate buffer (pH 7.4), 300 μl of 30% trichloroacetic acid (TCA), 150 μl of 5N HCl and 300 μl of 2%, w/v 2-thiobarbituric acid (TBA) were added and then the mixture was heated for 15 min at 90 °C. The mixture was centrifuged at 12,000 × *g* for 10 min. Pink colored supernatant was obtained which was measured spectrophotometrically at 532 nm.

2.5.5. Measurement of GSH

GSH (μg/mg of protein) was determined by its reaction with 5,5′-dithiobis (2-nitrobenzoic acid) (Ellman's reagent) to yield a yellow chromophore which was measured spectrophotometrically [42]. The brain homogenate was mixed with an equal amount of 10% trichloroacetic acid (TCA) and centrifuged (Remi cold centrifuge) at 2000 × *g* for 10 min at 4 °C. The supernatant was used for GSH estimation.