

# Angiotensin IV improves spatial memory in streptozotocin-induced diabetic rats by reducing oxidative stress and altering BDNF levels

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In this study, we investigated the protective effects of angiotensin IV (Ang IV) on cognitive function in streptozotocin (STZ)-induced diabetic rats. Male Wistar albino rats, were randomly divided into four groups; control (C), diabetes (Dia, 60 mg/kg, STZ, i.p.), Ang IV (5 µg/kg, s.c.) and Dia+Ang IV. The passive avoidance and Morris water maze (MWM) tests were used to evaluate learning and memory performance. Behavioral tests were carried out between 21 and 30 days after the initial Ang IV injection. Hippocampi were dissected and retained for biochemical and Western blot analysis. The Dia group exhibited the poorest behavioral results, while the Dia+Ang IV group performed highest on the MWM task. Superoxide dismutase, glutathione peroxidase, and malondialdehyde levels increased significantly in the Dia group compared to Dia+Ang IV. Brain-derived neurotrophic factor (BDNF) and N-methyl-D-aspartate levels were significantly elevated, while levels of GABA<sub>A</sub> significantly decreased, in the Dia+Ang IV group compared to the Dia group. These findings suggest that peripheral administration of Ang IV ameliorated spatial memory in diabetic rats by decreasing hippocampal oxidative stress and BDNF levels.

**Key words:** angiotensin IV, cognitive functions, diabetes mellitus, hippocampus, oxidative stress

## INTRODUCTION

Diabetes mellitus (DM), which has been rapidly increasing in prevalence worldwide, causes many complications in the body, particularly structural and functional disorders in peripheral and central nervous system (Charnogursky et al., 2014). Hyperglycemia is known to cause cognitive dysfunction in individuals with DM (Saedi et al., 2016). Although the underlying pathophysiology of cognitive dysfunction in diabetic patients has not been fully elucidated, cerebrovascular and neurodegenerative alterations are thought to play a role in the cognitive impairments. It has been

suggested that disturbances in receptor levels and neurotransmitter release may lead to apoptosis in neurons which might impair cognitive function in individuals with DM (Moheet et al., 2015; Sadeghi et al., 2016). DM is also defined as a chronic metabolic disorder as it is characterized by increased oxidative stress. It has been reported that oxidative stress contributes greatly to the development of diabetes-related complications (Asmat et al., 2016).

Since the discovery of renin in 1898, many studies have investigated the renin angiotensin system (RAS) (Tigerstedt and Bergman, 1898). This complex system has important physiological functions in regulating

water and electrolyte balance, systemic vascular resistance, blood pressure and cardiovascular homeostasis. However, chronic activation of the classical RAS may cause oxidative stress, endothelial dysfunction and inflammation, which leads to many pathological conditions ranging from hypertension and kidney disease to heart failure (Mentz et al., 2013). Furthermore, recent data suggests that inhibition of this system may be useful in reducing cognitive impairment, which is observed during aging, Alzheimer's disease, Parkinson's disease and post-stroke cognitive impairment (Fouda et al., 2016; Hamel et al., 2016).

Angiotensin IV (Ang IV), which is formed from angiotensin II (Ang II) by aminopeptidases, is an endogenous peptide and a primary molecule involved in the RAS. Ang IV and its analogs do not activate any of type 1 or type 2 Ang II receptors, unlike Ang II, because the effects of Ang IV are mediated by a different receptor called angiotensin type 4 receptor (AT4R) (Royea et al., 2017). AT4R activation is thought to enhance cognition, cell signaling and synaptic conduction, and has antioxidant and anti-inflammatory properties (Albiston et al., 2003). The AT4R signaling mechanism in the hippocampus of rodents has been shown to activate acetylcholine and glutamate release and induce long-term potentiation (LTP) (Jackson et al., 2018). In addition, inadequate AT4R signaling may lead to impaired neurotransmitter release and synaptic dysfunction, which may lead to impaired cognitive function.

Previous studies on the positive effects of Ang IV on learning and memory have led to the question of whether it might play a curative role in cognitive disorders that develop in DM. Therefore, in this study, we aimed to investigate the effect of Ang IV on hippocampal oxidative stress and diabetes-induced impairments in cognitive function in streptozotocin (STZ)-induced diabetic rats. The possible mechanisms underlying its effect on cognitive functions were investigated by measuring levels of N-methyl-D-aspartate (NMDA) and gamma-aminobutyric acid A (GABA<sub>A</sub>) receptors, as well as brain-derived neurotrophic factor (BDNF), as molecular markers of hippocampus-dependent mechanisms of learning and memory.

## METHODS

### Ethics and animals

Thirty-two male Wistar albino rats, weighing between 350–400 g were used. The animals were obtained from the Bezmialem Vakif University Experimental Animal Centre and housed under standard laboratory

conditions (12 h light/dark cycles, 22°C and 60% humidity), with *ad libitum* food and water, and received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institutes of Health. Ethical regulations were followed in accordance with The National and Institutional guidelines for the protection of animal welfare during all experiments. All animal procedures were approved by the Laboratory Animals Ethical Committee of Bezmialem Vakif University (2018/150).

### Experimental groups and chemicals

At the beginning of the experiment, animals were divided randomly into four groups; control (C, n=8), angiotensin IV (Ang IV, n=8), streptozotocin (STZ)-induced diabetes mellitus (Dia, n=8) and STZ-induced diabetes mellitus and angiotensin IV (Dia+Ang IV, n=8). Diabetes was induced by an intraperitoneal (i.p.) injection of 60 mg/kg STZ (Sigma-Aldrich, Munich, Germany) dissolved in citrate buffer (pH: 4.5). An equal volume (1 ml) of citrate buffer was injected into the control group. Before STZ/citrate buffer injection, initial blood glucose levels were measured from blood obtained from the tip of the rat tails with a glucometer (AccuCheck Nano, Roche, Switzerland). Three days after STZ injection, blood glucose levels were re-measured, and rats with blood glucose values over than 200 mg/dl were accepted as diabetic, which has also been accepted by other research studies (Katsumata and Katsumata, 1992; Patel et al., 2006). Ang IV (Sigma-Aldrich, Munich, Germany) was dissolved in 1 ml saline (5 µg/kg) (Gard et al., 2012; Fidalgo et al., 2017) and administered subcutaneously to the rats in the Ang IV and Dia+Ang IV groups for 21 days from the day the rats were accepted as diabetic (Day 1 of the experiment). Equal volumes of saline were injected subcutaneously into the rats in the C and the Dia groups.

Learning and memory was evaluated between the Day 21 and Day 30. All animals were weighed and their blood glucose levels were measured at Day -3, Day 1, Day 14, Day 21 and Day 30 of the experiment (Fig. 1).

### Behavioral tests

#### Passive avoidance task

The passive avoidance task was performed on Day 21 of the experiment. A shuttle box consisted of two parts, a light compartment and a dark compartment

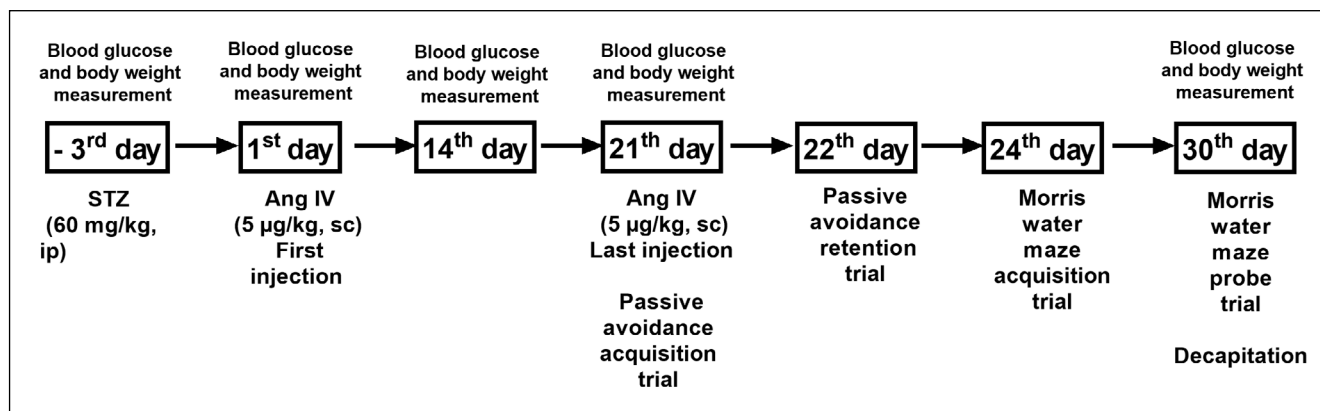


Fig. 1. Experimental timeline.

separated by a guillotine door (MAY-APAV-214 ELS, MAY-PA1014, Commat Ltd., Cankaya, Ankara). The test consisted of two stages: an acquisition and retention session. During the acquisition period (Day 21), all experimental animals were first habituated to the equipment. The animal was placed in the light chamber and 20 s later, the guillotine door was opened. After the rat entered into the dark compartment, a foot shock (1 mA) was delivered for 2 s. At the end of the acquisition trial the rat was removed from the apparatus and placed in its home cage. The retention tests were performed 24 hours after training to evaluate memory function (Day 22). For the retention test, each animal was placed in the light compartment, and the session ended when the animal entered the dark compartment or if it remained in the light compartment for 300 s. In these sessions, no electric shock was given to the animals. Time spent to enter the dark compartment was measured.

#### Morris water maze

The Morris water maze (MWM) test was used to evaluate hippocampal spatial learning and memory. A circle pool (150 cm in diameter and 51 cm high) was filled with opaque-colored (non-toxic dye) water ( $23 \pm 1^\circ\text{C}$ ) to a depth of 45 cm and divided into four quadrants (north, south, east and west). A computerized video tracking system (Ethovision XT Noldus Information Technology, The Netherlands) was used to track the animal in the pool and record the data. A movable escape platform (11 cm x 11 cm) was placed in the west quadrant (target quadrant) of the pool, hidden 2 cm below the water surface. Geometric visual cues were located on the walls of experimental room. The test included an acquisition period (Day 24–Day 29) and probe trial (Day 30). During the acquisition period, each rat performed 4 trials per day for 5 days. Rats were randomly

introduced into the pool from different quadrants, facing the wall, and allowed 60 s to locate the escape platform. If the rats did not find the platform within 60 s in the first trial, they were gently guided to the platform and allowed to remain for 30 s. The rat was then returned to its home-cage for a 5 min inter-trial interval. Swim latency, swim velocity and swim distance to find the escape platform during training were automatically recorded using the video tracking system. On the sixth day of the test, during the probe trial (Day 30), rats were assessed in the pool and allowed to swim for 60 s with no platform. At the end of the probe trial, the percentage of time spent in the platform quadrant was recorded for each animal.

#### Biochemical analysis

Upon the completion of behavioral testing (Day 30), rats were anesthetized, decapitated, and their brains were removed and the hippocampi were dissected for molecular and biochemical analysis. The hippocampus was frozen on ice and stored at  $80^\circ\text{C}$ . The tissue samples were homogenized with PBS (phosphate buffered saline, pH: 7.4) using a steel bead homogenizer (Retsch MM400, Haan, Germany) in 2 ml microtubes. The homogenates were then taken into microtubes and centrifuged at  $15,000 \times g$  for 20 min at  $4^\circ\text{C}$ . First, the total protein content of supernatants was determined by the Bradford method (Kruger, 2009). The tissue homogenates' malondialdehyde (MDA) (SinoGeneClon Biotech, HangZhou, China) glutathione peroxidase (GSH-Px) (SinoGeneClon Biotech, HangZhou, China), superoxide dismutase (SOD) (Y&L Biotech Co, Shanghai, China), and BDNF (SinoGeneClon Biotech, HangZhou, China) levels were determined by ELISA using corresponding kits according to manufacturers' instructions.

## Western blotting assay

The hippocampi were dissected and homogenized in lysis buffer containing protease inhibitor cocktails (MP FastPrep-24, USA). The homogenates were centrifuged at 14,000 x rpm (Beckman Coulter, Krefeld, Germany) for 10 min at 4°C, and the final supernatant was used as the cytosolic fraction. Total protein concentrations of supernatants were measured using the Bradford method at a 595 nm wavelength (Thermo Scientific Multiskan FC, 2011-06, USA). The supernatants were used for sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After the proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane, the membranes were incubated in blocking solution (5% skimmed milk) and immersed into primary antibody and horseradish peroxidase (HRP)- conjugated secondary antibody solutions (Cell Signaling Technology). Protein bands for anti-GABA<sub>A</sub> receptor alpha (Novusbio, Centennial, USA), ACE2 (GeneTex, Irvine, California, USA) and NMDAR2A antibody (Novusbio, Centennial, Colorado, USA) were visualized with chemiluminescence (ECL) Western blot substrate (Pierce, Thermo Scientific, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Cell signal, USA) was used as a housekeeping reference antibody.

## Statistical analysis

Group means  $\pm$  standard error of mean (SEM) were calculated for all values. The Shapiro-Wilk test was used to test the normality of the data. The biochemical and western blotting assay results for tissue samples in different groups were analyzed by one-way ANOVA, and the behavioral tests were compared using two-way ANOVA, one-way ANOVA and Kruskal Wallis tests. *Post-hoc* comparisons between the groups were performed with Bonferroni and Dunn tests using GraphPad Prism software (GraphPad Prism Version 6 Software Program San Diego, CA). A value of  $p < 0.05$  was considered statistically significant.

## RESULTS

### Changes in body weights and blood glucose levels

On the Day 14 of the experiment, significant decreases were observed for body weight in the Dia and the Dia+Ang IV groups compared to control ( $p < 0.01$  and  $p < 0.001$ , respectively), which were maintained at Day 21 and Day 30 ( $p < 0.001$ ) (Fig. 2). There was no significant difference in the initial blood glucose levels among groups (Day 3). Both diabetic groups (Dia and Dia+Ang IV) showed significantly ( $p < 0.001$ ) higher blood glucose levels than the control group on the Days 1, 14, 21 and 30 (Table I).

### Behavioral tests

The mean swim latency and the mean swim distance for all groups decreased from the 1<sup>st</sup> training day to the 5<sup>th</sup> training day ( $p < 0.001$ ) However, there were no significant differences between groups for swim latency

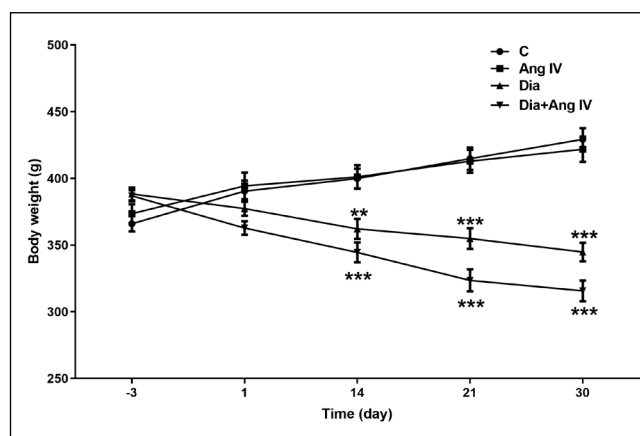


Fig. 2. Changes in body weight of animals (n=8) during the experiment. Data is presented as mean  $\pm$  SEM. \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ , represent statistical significance determined using one-way ANOVA followed by Bonferroni *post-hoc* test.

Table I. Changes in animal blood glucose levels (n=8) during the experiment.

Groups	Day -3 (mg/dl)	Day 1 (mg/dl)	Day 14 (mg/dl)	Day 21 (mg/dl)	Day 30 (mg/dl)
C	110.875 $\pm$ 1.6	105.125 $\pm$ 2.5	107.375 $\pm$ 2.7	104.250 $\pm$ 2.8	104.250 $\pm$ 2.8
Ang IV	108.125 $\pm$ 2.5	115.750 $\pm$ 3.7	105.750 $\pm$ 1.8	104.500 $\pm$ 2.1	104.500 $\pm$ 2.1
Dia	101.125 $\pm$ 2.1	506.875 $\pm$ 21.8***	520.125 $\pm$ 22***	520.875 $\pm$ 23.1***	549.375 $\pm$ 22***
Dia+Ang IV	108.125 $\pm$ 2.8	413.875 $\pm$ 18.3***	525.125 $\pm$ 26.1***	495.875 $\pm$ 26.6***	513.250 $\pm$ 32***

Data were presented as mean  $\pm$  SEM. \*\*\* $p < 0.001$ , represents statistical significance compared to control group.

or swim distance on the first three training days. On Day 4 and 5, both swim latency and swim distance of the Dia group were significantly higher than in the control group ( $p$ 's<0.001), which indicates an impairment in learning performance (Fig. 3A and 3B). On the other hand, in the Dia+Ang IV group, both swim latency and swim distance were significantly lower than the Dia group on both Day 4 and 5 ( $p$ 's<0.001), indicating relatively better learning (Fig. 3A and 3B). There was a significant difference between the Dia+Ang IV group and the control group ( $p$ <0.001) for swim latency on Day 4, however, this difference was no longer detectable on Day 5 (Fig. 3A).

Furthermore, swim distance was significantly lower in the Ang IV group compared to the control group on the 4<sup>th</sup> day of MWM training ( $p$ <0.01). In addition, compared to the Dia group, swim distance was significantly lower in the Ang IV group on Day 4 and 5 ( $p$ 's<0.001). There were no significant differences for swimming velocity between groups for all 5 training days (Fig. 3C).

For the MWM probe trial, the Dia group spent less time in the platform area compared to the control group ( $p$ <0.05) (Fig. 4A). Time spent in the platform area was significantly higher in the Dia+Ang IV group compared to the Dia group, demonstrating enhanced learning performance ( $p$ <0.05) (Fig. 4A).

In the retention trial of the passive avoidance task, the Dia group performed worse than the control group ( $p$ <0.05) (Fig. 4B). The Dia+Ang IV group exhibited a slightly better performance on passive avoidance compared to the Dia group, however, it did not reach the accepted significance level (Fig. 4B).

## Biochemical results

The BDNF levels of hippocampus in the Dia group were significantly lower than in the control group ( $p$ <0.01). In the Dia+Ang IV ( $p$ <0.05) group, the levels of BDNF were significantly higher than the Dia group (Fig. 5A). The levels of SOD (Fig. 5B), GPx (Fig. 5C) and MDA (Fig. 5D) were significantly elevated in the Dia group compared to the C group ( $p$ <0.01). In addition, the levels of SOD, GPx and MDA in the Dia+Ang IV group were significantly lower ( $p$ <0.05,  $p$ <0.05,  $p$ <0.01, respectively) compared to the Dia group.

## Western blotting assay

The highest levels of NMDA receptor expression were detected in the Ang IV group, and this elevation was statistically significant compared to the control group ( $p$ <0.001) (Fig. 6A). NMDA receptor expression

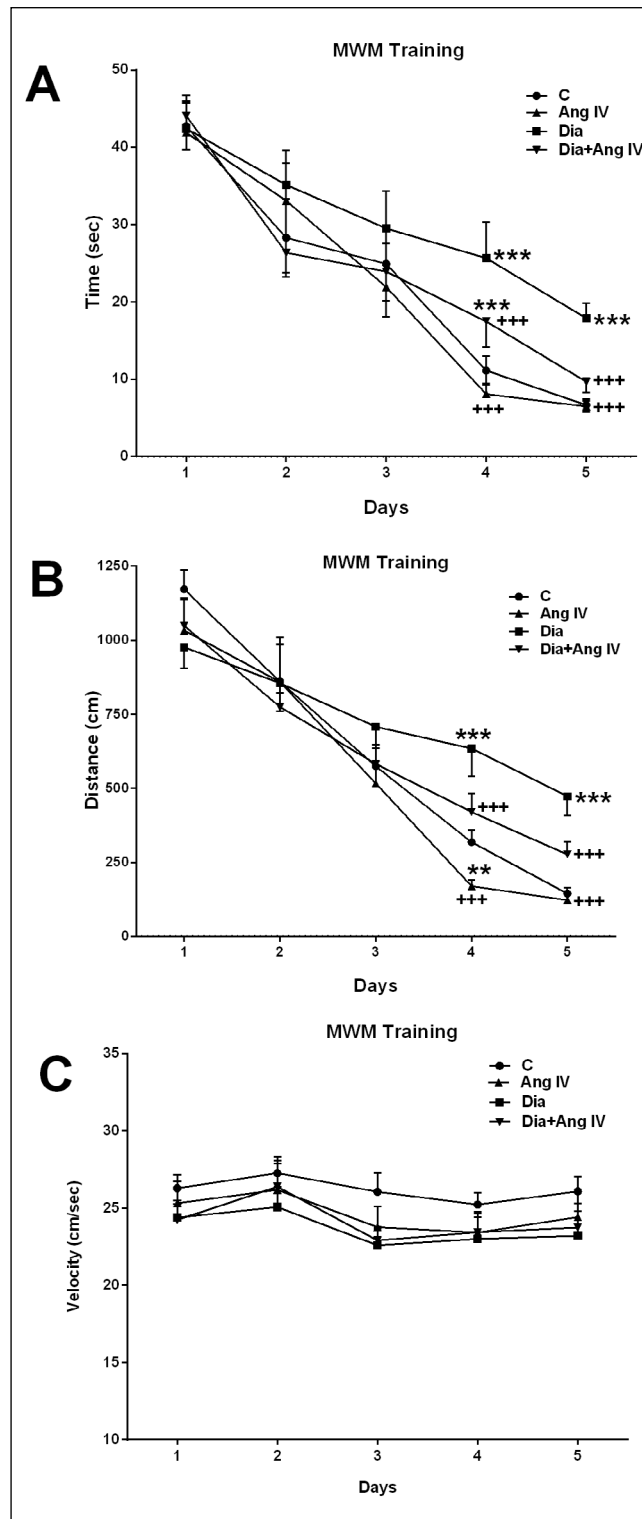


Fig. 3. Behavioral tests for all groups (n=8). Mean swim latency (A), mean swim distance (B) and velocity (C) analyzed with two-way ANOVA followed by Bonferroni *post-hoc* test for the first 5 days of Morris Water Maze (MWM) training. Data is presented as mean  $\pm$  SEM. \*\* $p$ <0.01, and \*\*\* $p$ <0.001, represent statistical significance compared to the C group; +++ $p$ <0.001, represents statistical significance compared to the Dia group.

in the Dia and the Dia+Ang IV groups was significantly lower than in the control group ( $p < 0.001$ ); however, it was significantly higher in Dia+Ang IV group compared to the Dia group ( $p < 0.05$ ) (Fig. 6A).

The highest levels of protein expression for GABA<sub>A</sub> receptor were observed in the Dia group ( $p < 0.01$ ) (Fig. 6B). GABA<sub>A</sub> receptor expression levels for the Dia+Ang IV group were significantly lower compared to the Dia group ( $p < 0.05$ ) (Fig. 6B).

Lastly, the highest ACE2 levels were found in the Ang IV group (Fig. 6C). The expression level of ACE2 was significantly lower in both the Dia and the

Dia+Ang IV groups compared to the control group ( $p < 0.05$ ). Surprisingly, ACE2 expression levels in the Dia group were significantly lower than the Dia+Ang IV group (Fig. 6C).

## DISCUSSION

Injection of STZ, which induces one of the most common experimental diabetes model in rats, results in a number of diabetes-specific symptoms such as decreased body weight, hyperglycemia and neuroendocrine disorders (Crawford, 2017). Blood glucose levels were found to be significantly elevated in both the Dia and the Dia+Ang IV groups and remained within normal limits in both the control and the Ang IV groups throughout our study. The body weights of the diabetic rats decreased significantly throughout the experiment, as shown in other studies (Pournaghi et al., 2012; Wang-Fischer and Garyantes, 2018). The body weights for both the control and the Ang IV groups increased slightly during the experiment. These results indicated that the dose of Ang IV used had no effect on blood glucose level and body weight in the STZ-induced diabetes model.

Type 1 (T1DM) and type 2 diabetes mellitus (T2DM) have been associated with reduced performance in multiple domains of cognitive function. Cognitive deficits can occur at the very earliest stages of diabetes and are further exacerbated by metabolic syndrome due to hyperglycemia (Low et al., 1997). The duration of diabetes and glycemic control may have an impact on the type and severity of cognitive impairment (Zilliox et al., 2016). Indeed, in a previous study cognitive impairment was even identified seven days after STZ administration to rats (Liu et al., 2016). In our study, both in the passive avoidance and MWM tasks, it was demonstrated that STZ injection disrupted both fear-conditioned memory and spatial learning and memory.

Several studies showed that intracerebroventricular (i.c.v.) injection of Ang IV or its analogues enhanced performance of rats in the MWM test (De Bundel et al., 2009; Lee et al., 2004; Wright and Harding, 2009; Wright et al., 1999). In addition, Wright and Harding (2009) suggested that Ang IV increased the escape latency in the passive avoidance task. There are very few studies on the peripheral administration of Ang IV. Although Ang IV is thought to have a low blood brain barrier affinity (Ho et al., 2018), due to its low weight compared to other RAS components and impaired blood brain barrier permeability in STZ-induced diabetes (Huber et al., 2006), the behavioral test finding suggests that a learning-memory mech-

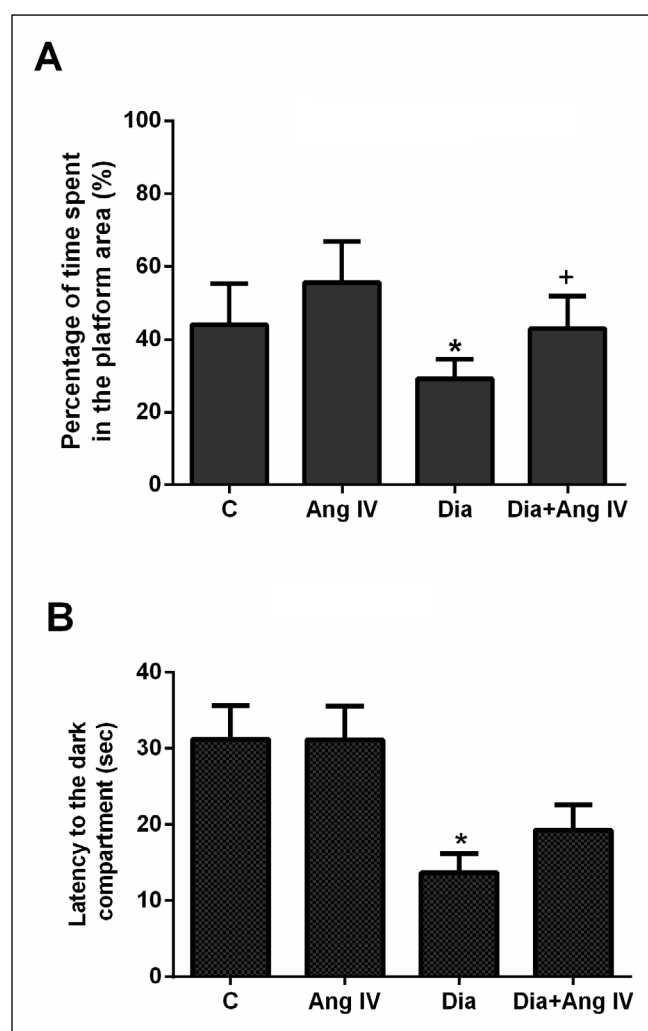


Fig. 4. Behavioral tests for all groups (n=8). Percentage time spent in the platform quadrant (A) on the probe trial of MWM analyzed by one-way ANOVA followed by Bonferroni *post-hoc* test. The time spent to enter the dark compartment in Passive Avoidance (B) analyzed by Kruskal-Wallis followed by Dunn's *post-hoc* test. Data is presented as mean  $\pm$  SEM. \* $p < 0.05$ , represents statistical significance compared to the C group; + $p < 0.05$ , represents statistical significance compared to the Dia group.

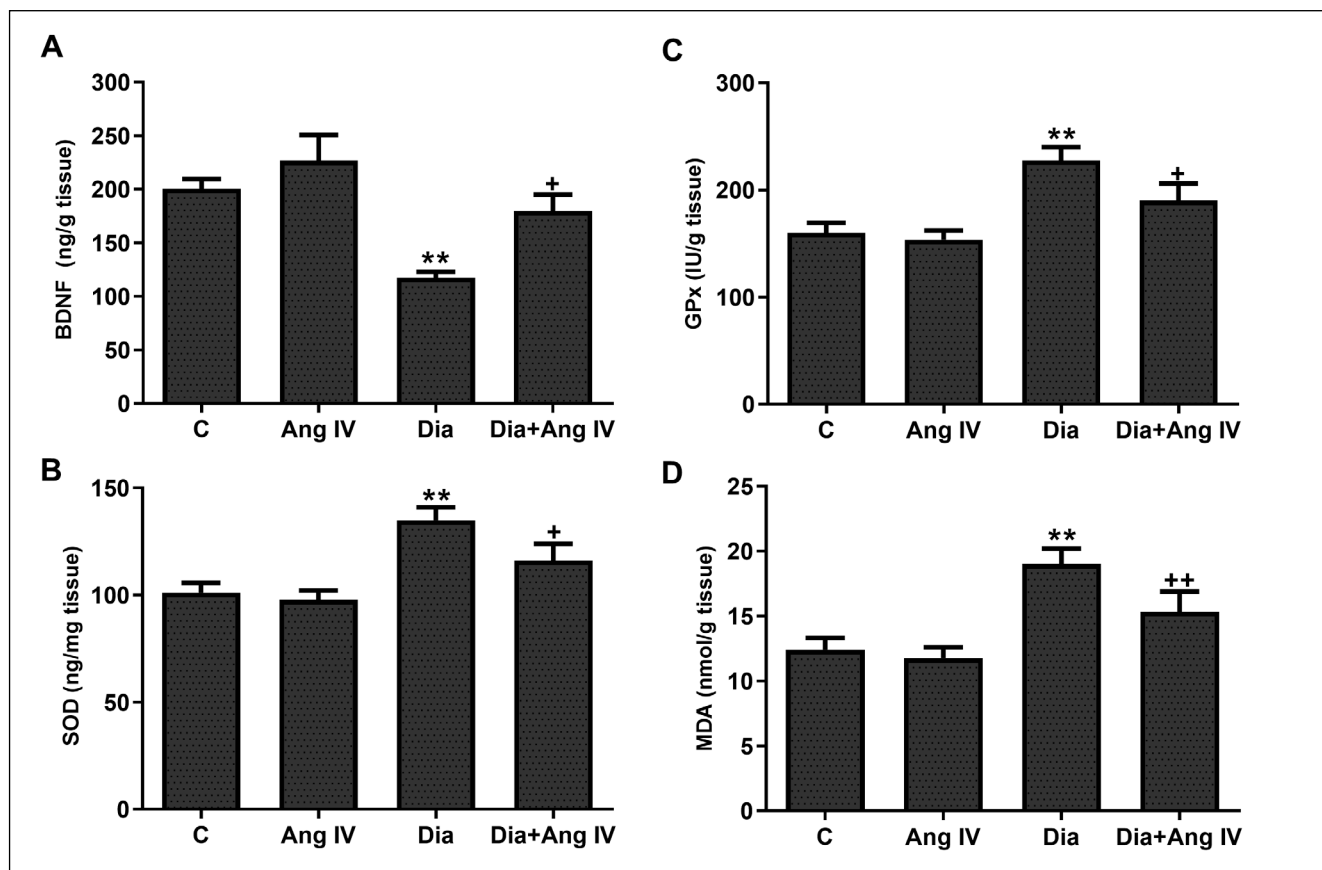


Fig. 5. The levels of hippocampal BDNF (A), SOD (B), GPx (C) and MDA (D) for each study group (n=8). BDNF; Brain derived neurotrophic factor, GPx; Glutathione peroxidase, SOD; Superoxide dismutase, MDA; Malondialdehyde. Data was analyzed using one-way ANOVA followed by *post-hoc* Bonferroni test and presented as mean  $\pm$  SEM. \*\* $p < 0.01$ , represents statistical significance compared to the C group; + $p < 0.05$ , and ++ $p < 0.01$ , represent statistical significance compared to the Dia group.

anism is activated by reaching the hippocampus. In support, several studies, using different behavioral tests, demonstrated healing effects of subcutaneously administered Ang IV on memory (Fidalgo et al., 2017; Gard et al., 2012; Golding et al., 2010). In accordance with these studies, we showed that subcutaneous Ang IV injection ameliorated cognitive function. However, the ameliorative effect of Ang IV on spatial memory was more drastic than its effect on fear conditioning. The poor performance of rats in the passive avoidance task may be marked by increasing the dose of Ang IV.

Effects of diabetes on the brain can include a reductions in antioxidant defense and a simultaneous increase in free radicals (Manschot et al., 2006). It is also known that hyperglycemia reduces antioxidant levels and concomitantly increases the production of free radicals (Muriach et al., 2014). The brain is considered to be particularly vulnerable to oxidative damage due to its high oxygen consumption rate, high lipid content and relatively smaller amounts of antioxidant enzymes compared to other tissues. The findings of

previous studies on the effects of STZ-induced DM on the activity of antioxidant enzymes such as SOD and GPx in tissues are inconsistent. It has been suggested that SOD and GPx levels in DM rat brain increased in the early stages (8<sup>th</sup> week) of STZ injection, but their levels decreased due to severity of dyslipidemia in brain tissue in subsequent stages after DM formation (Yang et al., 2013). In addition, it was also reported that oxidative stress markers in brain tissue were increased by STZ injection (Bathina et al., 2017). Similarly, in our study, it was determined that STZ-induced diabetes caused a significant increase in the levels of MDA, which are indicative of lipid peroxidation, and endogenous antioxidants SOD and GPx in the rat hippocampal tissue.

Oxidative stress occurs in pathological conditions such as DM, when there is an imbalance between free radical activity and antioxidant activity (Birben et al., 2012). Therefore, the reason for the lack of the oxidant markers and antioxidant markers in the Ang IV group may be related to the absence of oxidative dam-

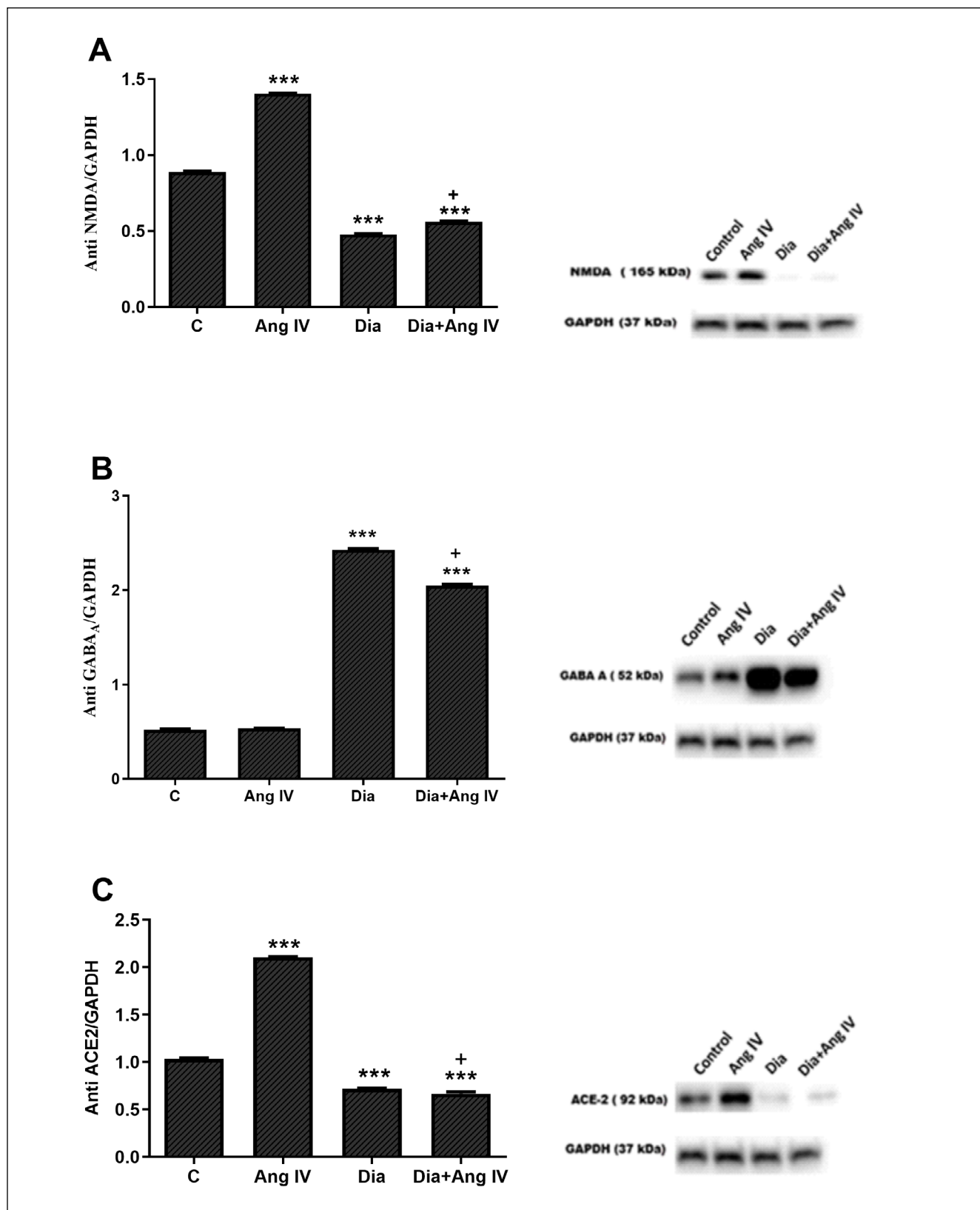


Fig. 6. Western blotting assay results from hippocampi for each group (n=8). (A) NMDA; N-methyl-d-aspartate, (B) GABA<sub>A</sub>; Gamma-aminobutyric acid-A, (C) ACE2; Angiotensin converting enzyme 2. Data was analyzed using one-way ANOVA followed by *post-hoc* Bonferroni test and presented as mean ± SEM. \*\*\*p<0.001, represents statistical significance compared to the C group; \*p<0.05, represents statistical significance compared to the Dia group.



age (Muriach et al., 2014). Royea et al. (2020) indicated that Ang IV reduced hippocampal oxidative stress. In support of this finding, the levels of MDA, SOD and GPx were significantly lower in the Dia+Ang IV group compared to the Dia group in our study. This suggests that Ang IV may act as an exogenous antioxidant to protect diabetic brain against oxidative damage-induced neuropathology.

While BDNF levels decreased in the Dia group, they increased in the Dia+Ang IV group. It is thought that low BDNF concentrations may contribute to cognitive impairment in diabetes (Zhong et al., 2019). Bathina et al. (2017) suggested that brain BDNF production is suppressed in STZ-induced diabetic rats resulting in cognitive dysfunction and impaired learning and memory in DM. Furthermore, it was also suggested that the increment of BDNF in the hippocampus correlates with increasing antioxidant levels and, accordingly, there is an improvement in cognitive function (Jain et al., 2013).

BDNF also promotes upregulation of NMDA receptors in the hippocampus (Caldeira et al., 2007). It is widely accepted that impaired synaptic plasticity or reduced activation of NMDA receptors will eventually result in spatial memory and learning disorders. In contrast, high GABA and GABA<sub>A</sub> receptor concentrations have been suggested to be associated with impaired spatial memory and learning (Kumar Datusalia and Sunder Sharma, 2016; Van Bussel et al., 2016). It has been shown that hyperglycemia is closely related to reduction of NMDA receptor levels (Xu et al., 2006). As in our study, previous studies with STZ-induced diabetic rats showed a significant reduction in NMDA receptor subtypes (Bean et al., 2006; Kamal et al., 2000). On the other hand, Ang IV treatment increased NMDA receptor levels and decreased GABA<sub>A</sub> receptor levels after STZ-induced DM. Ang IV may have increased BDNF levels and altered the NMDA and GABA<sub>A</sub> receptor levels due to its antioxidant effect.

Ang (1-7), another RAS component that is suggested to play a role in cognitive function, is formed by ACE2 from Ang II. It is an endogenous peptide that acts primarily by binding to a G-protein-coupled receptor called the MAS receptor (MasR) (Jiang et al., 2014). Chen et al. (2017) found that brain ACE2 levels significantly decreased in diabetic rats, as observed in our Dia group. Surprisingly, in the present study, ACE2 levels significantly increased in the Ang IV group whereas they decreased in the Dia+Ang IV group. It has been shown that the conversion of Ang (1-7) by ACE2 may cause an increase in BDNF expression via the MasR (Kamel et al., 2018). In addition, another study suggested that ACE2 deficiency impaired cognitive function, which was associated with increased ox-

idative stress and consequently decreased BDNF levels (Wang et al., 2016). However, according to our results, the lowest ACE2 levels, in the Dia+Ang IV group, may be independent of BDNF level. This could be due to Ang (1-7) formation increasing with Ang IV administration, which may result in a healing effect on cognitive function. According to these results, it was concluded that Ang IV might contribute to the ACE2/Ang (1-7)/MasR axis, however the findings relating to this pathway are inadequate in this study, and further investigation is required.

## CONCLUSION

In conclusion, as suggested by other studies, cognitive dysfunction due to hyperglycemia causes oxidative damage and our study suggests that systemic administration of Ang IV can significantly improve learning and memory performance even at the cellular level, as demonstrated by increased NMDA receptor expression and BDNF levels and decreased GABA<sub>A</sub> receptor expression in hippocampus of rats. Additionally, these alterations may be related to a partially curative effect of Ang IV on oxidative damage caused by diabetes. These findings also highlight the therapeutic potential of the peripheral administration of Ang IV.

## LIMITATIONS

This study had a technical limitation. Due to technical problems with equipment we could not measure blood pressure, which we had planned to perform with the non-invasive tail-cuff method.

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