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An inhibitor of soluble epoxide hydrolase ameliorates diabetes-induced learning and memory impairment in rats

Nathani Minaz[a], Rema Razdan[a], Bruce D. Hammock[b], Sumanta Kumar Goswami[c,*]

[a] Department of Pharmacology, Al-Ameen College of Pharmacy, Bangalore, Karnataka, India
[b] Department of Entomology and Nematology, and Comprehensive Cancer Center, University of California, Davis, CA, USA
[c] Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA, USA

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ABSTRACT

Background: Pharmacological inhibition of soluble epoxide hydrolase (sEH) enhances the synaptic function in the CNS and has a protective role in cognitive decline. We hypothesized that the sEH inhibitor TPPU might prevent the diabetes-induced decline in learning and memory which is associated with an alteration in the level of neurotransmitters and oxidative stress.

Methods: Type 1 diabetes was induced in rats and the animals were treated with TPPU for 8 weeks. The learning and memory functions were assessed by the Barnes maze and a step-down test. Indicators of oxidative stress, levels of neurotransmitters, and activity of acetylcholinesterase were measured in the discrete regions of the brain.

Results: Our results revealed that treatment with TPPU significantly improves learning and memory performance in diabetic rats along with decreasing the level of blood sugar. Moreover, treatment with TPPU significantly prevented the diabetes-induced alteration in levels of neurotransmitters, the activity of acetylcholinesterase and preserved anti-oxidant defence system.

Conclusion: Inhibition of the sEH alleviates diabetes-induced decline in learning and memory.

1. Introduction

Diabetes mellitus (DM) is one of the most common metabolic diseases whose occurrence and prevalence is increasing. It is among the diseases with high rates of complications which significantly lower the quality of life in patients. Type 1 diabetic patients have deficits in executive function, general intelligence, attention, and memory [1].

The hippocampus, a crucial part of the brain for learning and memory, is highly sensitive to uncontrolled peripheral hyperglycemia. A decrease in general cognitive performance, memory impairment, and atrophy are observed in diabetes [2]. The uncontrolled hyperglycaemia endorses the production of advanced glycation end products (AGEs), a critical mediator in the pathogenesis of diabetic complications [3]. AGEs by binding to their membrane receptors provoke the pro-oxidant and pro-inflammatory environment [4], which imparts neuronal degeneration [5]. Further, diabetes-induced neuronal loss and the allied neurotransmitter alterations are crucial steps leading to impaired cognitive functions. In addition to this risk, the high blood pressure associated with diabetes also leads to shrinkage of the brain and cognitive decline [6]. Pharmacological interventions including reduction of oxidative stress and protecting neurons have shown beneficial effects in diabetes-induced cognitive impairment [7,8,9].

Epoxyeicosatrienoic acids (EETs) and other epoxy fatty acids are signaling molecules formed by cytochrome P450 epoxidation of arachidonic acid. Many studies suggest that EETs are beneficial in diabetes, hypertension and in inflammatory diseases [10,11]. These EETs are generally short-lived because these lipid mediators are easily converted into inactive diols by the enzyme soluble epoxide hydrolase (sEH). Inhibition of this enzyme enhances therapeutic actions of EETs. Selective sEH inhibitors possess multiple pharmacological activities including antihypertensive, analgesic, anti-inflammatory and other effects which protect the brain, heart and kidney from pathological insult [12–16]. Effects of the sEH inhibitors in diabetic cognitive impairment are still unknown. So, in this study, we evaluated the protective activity of the sEH inhibitor, TPPU in streptozotocin (STZ)-induced cognitive impairment in rats. Further, we have quantified the levels of key neurotransmitters including γ-Aminobutyric acid (GABA), dopamine (DA) and norepinephrine (NE/NA, also called noradrenaline), and activity of acetylcholine esterase (AChE) which regulates level of neurotransmitter acetylcholine (ACh).

* Corresponding author.
E-mail address: skg74@drexel.edu (S.K. Goswami).

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2. Materials and methods

2.1. Chemicals

The soluble epoxide hydrolase inhibitors were synthesized as described previously [17]. STZ (MP Biomed, India) and glucose kit (Span diagnostic, India) were purchased. All other chemicals and reagents used were of analytical grade.

2.2. Animals and vivarium condition

Male Wistar albino rats aged 3 months were procured from the in-house animal facility of Al-Ameen College of Pharmacy, Bangalore, India. The animals were housed under general laboratory conditions of temperature (25 ± 1 °C) and relative humidity (55–75%) with a 12: 12 h light–dark cycle. The animals were fed with standard pellet diet and water ad libitum. All the experiments were performed with a prior approval from the Institutional Animal Ethics committee and study protocol complied with Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) guidelines for the care and use of animals. A complete effort was made to alleviate the stress to the animals.

2.3. Induction of experimental diabetes and treatment

Type 1 diabetes was induced by injecting a single dose of STZ (dissolved in cold citrate buffer, pH 4.5, 52 mg/kg, i.p.) to rats fasted overnight [18,19]. We standardized the dose and effect of food on diabetogenic potential of STZ on rats before start of the study. Administration of the moderate dose of STZ (52 mg/kg) to fasting animals ensured robust induction of diabetes without mortality. The development of diabetes was confirmed by assessing fasting serum glucose post 72 h injection of STZ using a glucose assay kit (Span Diagnostics Ltd., India) [20]. The rats with glucose levels of 250–350 mg/dl were considered as diabetic and included in the study. This day was considered as day 1. The rats were divided into four groups with eight animals in each group. Group 1 and 2 animals received the vehicle and served as normal control and diabetic control, respectively. The group 3 and 4 animals received TPPU dose of 0.1 and 0.3 mg/kg, respectively for 8 weeks. The doses of TPPU were chosen based on its efficacy to reduce inflammation in our previous studies [15,16]. Insulin (3 IU/kg, s.c.) was administered twice in a week to diabetic rats to prevent mortality throughout the study. Body weight, blood glucose, and behavioural evaluations were carried out in all animals

2.4. Behaviour test

All behaviour tests were carried out in all animals (n = 8, each group) in a sound proof psychopharmacology laboratory between 10 a.m. to 12 p.m. The animals were brought to the lab 2 h before the start of experiment to avoid anxiety in animals. The behavioural studies were performed by another experimenter who was blinded about the grouping and treatment.

2.4.1. Barnes maze test

Barnes maze test is used to assess spatial memory, has similarities to Morris Water Maze and to radial arm maze task, but no strong aversive stimuli are applied during the test [21]. Strong aversive stimuli like water or shock are likely to produce stress in the animal, influencing the performance of the task. With the Barnes maze, animals receive reinforcement to escape from the open platform surface to a small dark recessed chamber located under the platform called an “escape box”. The method was performed according to the method of Rosenfeld et al. [22].

2.4.2. Step-down test

The passive avoidance response was assessed in an apparatus with the dimension of 34 cm × 34 cm × 20 cm. It consisted of grid floor through which a 20 mV electric shock was delivered. In the centre of the chamber, a shock-free zone (SFZ) was placed. After the 8 weeks of treatment, rats were exposed to a 5-min learning course, during which they were permitted to move freely throughout the chamber before being placed on the platform. If the animals stepped down from the platform, they were exposed to an electric foot shock (36 V, AC). After 24 h of trial, test was evaluated to measure step down latency [23].

2.5. Biochemical parameters

After the behaviour test, fasting blood glucose level was measured in all animals. Then animals were further subdivided into 2 subsets (n = 4) for measuring (i) activity of AChE and the level of neurotransmitters in the discrete regions of brain and (ii) oxidative stress in whole brain. Memory is dependent on the optimum levels of neurotransmitters in the hippocampus and cerebellum of brain, and the levels of neurotransmitters are altered in memory dysfunction including diabetes-induced memory dysfunction. Whole brain of the diabetic is subjected to oxidative stress. Therefore, we measured the levels of neurotransmitters in the discrete regions of the brain whereas the oxidative parameters were studied in the whole brain.

2.5.1. Plasma glucose levels

Blood was collected from the tail vein in microcentrifuge tubes containing heparin. Plasma was separated and blood glucose was estimated by GOD–POD kit as per the manufacturer’s instructions.

2.5.2. Quantification of activity of acetylcholinesterase (AChE) and level of neurotransmitters

After the completion of behavioural test, the animals were sacrificed, brains (n = 4) were isolated and different parts of brains were dissected out immediately and stored at −80 °C.

The hippocampus were homogenized separately in 0.1 M phosphate buffer and used for quantification of activity of AChE and the level of GABA. The activity of AChE was measured according to a published method [24]. The levels of GABA in homogenates were measured by its reaction with ninhydrin in the presence of glutamate to form a fluorescent product which was measured by a spectrofluorometric method [25].

The cerebellums were homogenized separately in 25 mL of HCl–butanol (0.85 mL of concentrated HCl was added to 1 L of N-butanol) for about 1 min, then centrifuged for 10 min at 2000 rpm. The aqueous phase was used for noradrenaline (NA) and dopamine (DA) quantification by a spectrofluorometric method [26].

2.5.3. Measurement of oxidative stress

Brains of the remaining animals (n = 4) were dissected out, washed with saline, chopped over ice and homogenates (10%, w/v) were prepared with 0.1 M (pH 6.8) phosphate buffer. The homogenates were centrifuged at 3000*g* for 10 min at 4 °C using Sorvall^{TM} refrigerated centrifuge and the supernatants were used for estimation of oxidative stress. Lipid peroxidation or formation of malondialdehyde (MDA) was quantified by the method of Slater and Sawyer [27]. Glutathione (GSH) was quantified by the method of Moron et al [28].

2.6. Statistical analysis

Results are expressed as mean ± standard error of mean (S.E.M) of 4–8 observations using 4–8 mice. Statistical significance with respect to diabetic control was evaluated using one way ANOVA followed by Dunnett’s test using GraphPad Prism 5 (Version 5.0, GraphPad Software Inc., San Diego, CA).