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# *N*-acetylcysteine protects memory decline induced by streptozotocin in mice

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease characterized by cognitive impairment, associated with a reduced concentration of acetylcholine (ACh) in brain cortex and hippocampus. Recently we reported that the *N*-acetylcysteine (NAC) decreases brain acetylcholinesterase (AChE) activity *in vitro*. Thus, the aim of the current study was to investigate the effect of NAC against streptozotocin (STZ) induced AD in mice. Mice were divided into four groups: I) Sham, II) NAC, III) STZ and IV) NAC + STZ. Animals were daily treated with NAC (50 mg/kg/day, p.o.) for nine consecutive days and with STZ (2.5 mg/kg i.c.v.) at the first and third days. Step down passive avoidance (SDPA, days 7–8) and Morris water maze (MWM, days 6–9) task were assessed to evaluate learning and memory. On the tenth day animals were euthanized for AChE and butyrylcholinesterase (BChE) activities and ACh, energy-rich phosphate and brain glucose uptake levels evaluations. A learning and memory impairment was observed in SDPA and MWM in those animals that receive STZ. Nevertheless, the same was not observed in those animals that also received NAC. Brain cortex and hippocampus AChE and hippocampus BChE activities increase induced by STZ were also prevented by NAC treatment. The STZ induced a brain energy metabolism imbalance, decreasing adenosine triphosphate and increasing adenosine levels. The glucose uptake decrease in hippocampus was prevented by NAC. In conclusion, NAC treatment prevented the cognitive disturbance, by restoring the cholinergic system and brain energy metabolism disorders. NAC could modulate cholinergic imbalance without causing any changes *per se* in the same.



## 1. Introduction

Cognitive impairment is a broad term to describe any characteristic that acts as a barrier to the process of cognition [1]. Some of the causes of cognitive impairment in humans are neurodegenerative diseases such as Alzheimer's disease (AD), comprising 50 to 70% of all cases [2]. AD is characterized by a progressive loss of memory, thinking, learning capacity and judgment [3]. This neurodegenerative disease is coupled to mitochondrial dysfunction, which leads to an impaired energy metabolism and consequent activation of proapoptotic pathways signaling [4]. The death of neurons, particularly in the cerebral cortex and hippocampus, have also been related to AD [5]. Severe alterations in glucose metabolism are also reported in all forms of sporadic dementia resulting from AD, since the use of glucose and the levels of energy-rich phosphate compounds are reduced in brain tissue from AD patients. Disruption in the control of neuronal glucose metabolism, consequence of the failure in signal transduction triggered by insulin, is also observed in the early AD pathogenesis [6]. This neuronal glucose metabolism controls the release of neurotransmitters at synapses, activating pathways associated with learning and long-term memory [7].

Streptozotocin (STZ) is a naturally occurring nitrosamine, which has been widely used as diabetes inducing in experimental animals. However, an intracerebroventricular (i.c.v.) injection of STZ in a sub diabetogenic dose has been reported as a model of AD, leading to a memory deficit [8]. This model is associated with disorders in the glucose use and consequent changes in energy metabolism [9],

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leading to cognitive dysfunctions by reducing the synthesis of adenosine triphosphate (ATP) and acetyl coenzyme A (acetyl-CoA). These changes result in a cholinergic dysfunction characterized by the choline acetyltransferase (ChAT; EC 2.3.1.6) activity inhibition and the acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) activities increase in the nervous system [10,11].

AChE along with BChE belong to the hydrolases family. In the brain, AChE is primarily associated neurones [12], while BChE is mainly located in glial cells [13]. The main function of AChE is the acetylcholine (ACh) hydrolysis, this neurotransmitter is synthesized from choline and acetyl-CoA in the presynaptic neuron and released into the synaptic space where it will bind to the pre and post-synaptic muscarinic and nicotinic receptors, stimulating both excitatory and inhibitory transmission [14]. BChE is less substrate-specific [15]. Nevertheless, both enzymes are able to hydrolyze ACh at a rate of >10000 molecules per second [16]. Thus, these enzymes are strongly involved in cognitive learning and memory function [17,18]. In regard of the AD cholinergic hypothesis, which is based on the decline in cognitive function due to loss of cholinergic neurotransmission mainly in brain cortex and hippocampus, along the years the focus of drug development for AD has been on strategies that enhance central cholinergic function. Amongst them, the use of AChE inhibitors seems to be the one with best results [14].

*N*-acetylcysteine (NAC) is a derivative of the amino acid cysteine molecule clinically used in respiratory diseases, acetaminophen poisoning and renal disease treatments [19,20]. This molecule also have anti-inflammatory, anti-apoptotic and pro-neurogenic properties [21]. Knowing this, the use of NAC as neuroprotective in psychiatric and neuroprogressive disorders [22] and in cognitively related treatments [23], have been propose. Moreover, the neuroprotective effect of NAC against STZ-induced memory dysfunction in rats after three weeks treatment has been reported [24]. Based on the above considerations, the purpose of the present study was to investigate the potential neuroprotective effect of NAC on the cognitive deficit induced by i.c.v. STZ in mice.

## 2. Materials and methods

### 2.1. Animals

Experiments were conducted using male *Swiss* mice (25–30 g) about two months old. Animals were maintained at 22 ± 2 °C with free access to water and food under a 12 h: 12 h light/dark cycle with lights on at 07:00. All manipulations were carried out between 08:00 and 16:00. Animals were used according to the guidance of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil (Process Nº 088/2015) and the ARRIVE guidelines. All efforts were made to minimize animals' suffering and to reduce the number of animals used in the experiments.

### 2.2. Chemicals

NAC, STZ, adenosine (ADO), adenosine 5-monophosphate (AMP), adenosine 5-diphosphate (ADP), adenosine 5-triphosphate (ATP), acetylcholine (ACh), acetylthiocholine (ATCh), butyrylthiocholine (BTCh), acetylcholinesterase (AChE), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), choline oxidase (ChOx), ethopropazine, ethylenediaminetetraacetic acid (EDTA) and azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) were obtained from Sigma Chemical (St. Louis, MO, USA). All other chemicals were obtained in an analytical grade or from standard commercial suppliers.

### 2.3. Experimental procedure

#### 2.3.1. Memory impairment induction

Animals were divided into four groups (8 animals per group): I) Sham; II) NAC, III) STZ and IV) NAC + STZ. For treatment one: animals belonging to groups II and IV were treated p.o., through oral gavage, with a single NAC dose of 50 mg/kg body weight dissolved in distilled water each day, during nine consecutive days. NAC dose which showed no toxicity to animals was chosen based on a curve dose ranging from 0 to 150 mg/kg (data not shown). Animals belonging to groups I and III received distilled water instead. For treatment two: animals belonging to groups III and IV received an intracerebroventricular (i.c.v.) injection of STZ 2.5 mg/kg body weight dissolved in artificial cerebral spinal fluid [aCSF (mmol/L): 147.0 NaCl; 2.9 KCl; 1.6 $MgCl_2$, 1.7 $CaCl_2$ and 2.2 $C_6H_{12}O_6$], pH = 7.4 in a fixed volume of 5 μL at the first and third days. Animals from groups I and II received only aCSF (5 μl/site) [25]. The i.c.v. administration of STZ (or aCSF) was performed by a “free hand” protocol with the bregma fissure as a reference point based on the previous published method [26,27]. Briefly, the animals were anesthetized with isoflurane and grasped firmly by the loose skin behind the head, the skin was pulled tight and a hypodermic needle (27 gauge) attached to a 25 μl Hamilton microsyringe was inserted perpendicularly through the skull into the brain. The site of injection was 2 mm either side of the midline on a line drawn through the anterior base of the ears; the depth of insertion of the needle was about 3 mm. To find out if the STZ was administered exactly into the cerebral ventricle, mice were killed by cervical dislocation and the brains were dissected and macroscopically examined.

### 2.4. Behavioral tests

#### 2.4.1. Open field test

Spontaneous locomotor activity was measured in the open field (OF) test [28] at the day 6, previously to the memory task. The floor of the open field was divided into nine squares. Each animal was placed individually in the center of the arena, and the number of segments crossed (four-paw criterion) and rearings were recorded in a 5 min session.

#### 2.4.2. Step-down passive avoidance task

The step-down passive avoidance (SDPA) task has been used to study nonspatial long-term memory [29]. The apparatus consisted of a single box where the floor was made of a metal grid connected to a shock scrambler. A safe platform was also placed in the box. During the training session (acquisition trial, day 7), the mouse was placed on the platform, when the animal stepped down and placed their four paws on the grid floor, an electric shock (0.5 mA) was delivered for 2 sec. Some sec later (~5), the mouse was removed from the step-down passive avoidance apparatus and returned to its home cage. The retention trial was performed 24 h after training (day 8). Each mouse was placed again on the platform, and the transfer latency time (i.e., time took to step down from the platform) was measured in the same way as in the acquisition trial, nevertheless, shock was not delivered and the transfer latency time was recorded to a maximum of 5 min. The criterion for learning was taken as an increase in the transfer latency time on retention (day 8) trial as compared to the acquisition (day 7) trial. Therefore, short transfer latencies indicate poor retention.

#### 2.4.3. Morris water maze task

Spatial learning and memory were assessed using the Morris water maze (MWM) task, according to the method of Morris [30]. The maze consisted of a basin (diameter: 180 cm, wall height: 40 cm) made of black plastic and filled with water (25 ± 2 °C) at a