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Research article

Enhancement of memory consolidation by the histone deacetylase inhibitor sodium butyrate in aged rats

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HIGHLIGHTS

- Sodium butyrate produced memory enhancement and persistence in aged rats.
- Memory formation in younger rats was not affected by sodium butyrate.
- Aged rats with normal memory might be particularly sensitive to sodium butyrate.

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ABSTRACT

Here we show that a systemic injection of the histone deacetylase inhibitor (HDACi) sodium butyrate (NaB) immediately after training in a step-down inhibitory avoidance task produced an enhancement of memory consolidation that persisted across consecutive retention tests during 14 days in aged rats, while it did not significantly affect memory in young adults. Control aged and young adult rats showed comparable basal levels of memory retention. Our results suggest that HDACis can display memory-enhancing effects specific for aged animals, even in the absence of age-related memory impairment.

1. Introduction

Over the last decade, several studies have demonstrated that transcriptional regulation involved in the formation of long-term memories (LTM) needs the synchronized interaction of several transcription factors and transcriptional co-activators in the chromatin structure [1,2]. It is well established that epigenetic mechanisms, such as histone acetylation, orchestrate molecular events during LTM formation by relaxing or condensing the chromatin structure altering gene transcription [3]. Proteins named histone acetyltransferases (HATs) add acetyl groups to lysine residues of histones and are responsible for the modulation of the histone-DNA interactions. The chromatin structure relaxation leads to enhanced transcription which is a reversible process by the action of histone deacetylases (HDACs). HDACs acts by removing the acetyl group from lysine residues of histones and non-histone proteins favoring the closed repressive state of chromatin [4].

Pharmacological treatment with histone deacetylase inhibitors (HDACis), such as trichostatin A and sodium butyrate (NaB), induce a histone hyperacetylated state regulating the accessibility of chromatin to the transcription machinery, affecting gene expression [5] and the essential mechanisms acting in neurological diseases as well as those underlying memory formation [6–8].

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HDACis were first designed as anticancer agents [5] and the treatment with HDACis have been shown to enhance memory and ameliorate deficits in aged rats and experimental models of memory dysfunction [8,9,10,11]. Recent evidence has demonstrated epigenetic alterations in specific brain areas of aged animals [9,12] that may play a crucial role in aging being correlated to diseases, such as diabetes, cancer, neurodegenerative and psychiatric disorders [13]. In the present study we sought to establish whether HDAC inhibition by an acute systemic treatment with NaB affects LTM formation for a one-trial inhibitory avoidance (IA) task in aged rats.

2. Materials and methods

Young adult (3 months) or aged (20–24 months) male Wistar rats were obtained from our institutional certified breeding colony (CREAL-UFRGS). Animals were housed three per cage in plastic cages with sawdust bedding and maintained on a 12 h light/dark cycle at a room temperature of 22 ± 1 °C. The rats were allowed *ad libitum* access food and water. Experiments using aged and young rats were carried out separately and took place between 8 AM and 4 PM. All experimental procedures were performed in accordance with the Brazilian Guideline for the Care and Use of Animals in Research and Teaching (DBCA, published by CONCEA, MCTI) and were approved by the institutional animal care committee under protocol number 12-0424.

Animals were allowed to acclimate to the laboratory for 2 h before any experimental manipulation. One week before experimental manipulation animals were handled once a day every 2 days during home-cage cleaning. We used the single-trial step-down IA conditioning as an established model of fear-motivated memory. In step-down IA training, animals learn to associate a location in the training apparatus (a grid floor) with an aversive stimulus (foot-shock). The general procedures for IA training and retention test were described in previous report [14]. On training trials, rats were gently placed on the platform facing the left rear corner of the apparatus box and their latency to step down on the grid with all four paws was measured. Immediately after stepping down on the grid, rats received a 0.4-mA, 3.0-s foot shock and were removed from the apparatus. Retention test trials took place at different intervals after training. No foot shock was presented during retention test trials. No cut-off value of step-down latencies for the training session was assigned. A ceiling of 300 s was imposed on retention test measures. Step-down latencies on the retention test trials were used as a measure of IA memory retention. At the 21 day, rats were given a mild reminder shock (0.3-mA, 3 s), followed by a retention test 24 h later. Immediately after training rats received a single intraperitoneal (i.p.) injection of saline (NaCl 0.9%) or NaB (1.2 g/kg; Sigma, St. Louis, MO, USA) dissolved in saline in a 1.0 ml/kg injection volume. The dose of NaB was chosen on the basis of previous studies [10,11,15]. Rats were tested for memory retention 1 day after training and subsequently they were submitted to test sessions daily until 7 days. Animals were tested again at 14 days and 21 days after training. Additional test and reminder shock session were performed at 23 and 24 days after training.

Western blot analysis was performed as previously described [11,16]. Histones were extracted from hippocampal brain region of aged rats that were systemically treated with SAL or NaB immediately after training and euthanized 1 h after injections. Rats were trained in the IA learning task (TRAIN group), exposed to the context alone (1 min habituation in task chamber, HAB group) or exposed to aversive stimulus alone (04-mA 3-s shock, SHOCK group). The tissue was stored at −80 °C. The samples were homogenized ($n = 4$ per group) in 400 μl of 50 mM Tris, pH 7.0, 1 mM EDTA, 100 mM NaF, 0.1 mM PMSF, 2 mM Na_3VO_4, 1% Triton X-100, 10% glycerol and protease inhibitor cocktail (P2714; Sigma–Aldrich). After 20 min on ice, samples were centrifuged at 12,000 rpm for 1 min. The supernatant was collected and the same volume of 0.2-N HCl was added. Acid extraction of histones was carried out over night at 4 °C then samples were centrifuged at 6500 g for 10 min at 4 °C. The supernatants were diluted 1/1 (v/v) in 100 mM Tris, pH 6.8, 4 mM EDTA, and 8% SDS and were then boiled for 5 min. The protein content was determined by the method of Lowry modified [17]. Thereafter, the loading buffer (40% glycerol, 100 mM Tris, bromopehnol blue, pH 6.8, 8% β-mercaptoethanol) was added to the sample. Twenty-five μg total protein was separated on a 10% SDS-polyacrylamide gel and transferred electrophoretically to a nitrocellulose membrane. Membranes were blocked with 5% non-fat dry milk in TBS containing 0.05% Tween 20 (TBS-T) and were incubated overnight with the following antibodies: β-actin at 1:3000, H3 at 1:3000, acetyl-H3K14 at 1:1000; acetyl-H3K9 at 1:500 (ab34731, ab1791, ab52946, ab10812; Abcam, San Francisco, CA, USA). Thereafter, the membranes were incubated with goat anti-rabbit (ab6721, HRP) radish-conjugated secondary antibodies and reactions were developed by chemiluminescent substrate (LumiGlo). All steps were followed by three washes with TBS-T. The bands were quantified using the Scion Image® software, which is a derivative of NIH Image (Frederick, MD, USA). Total protein levels in the blotting were normalized according to each sample's β-actin protein levels and the results were expressed as a ratio of acetylated H3 residues to total H3.

Data are expressed as mean ± SEM. Comparisons between groups were performed using a Kruskal–Wallis analysis of variance followed by Mann–Whitney *U*-tests. Comparisons between trials within the same group were performed by Wilcoxon signed-rank test. Western blotting data were analyzed using an ANOVA followed by a Tukey's multiple comparison test. In all comparisons, $P < 0.05$ was considered to indicate statistical significance.

3. Results

The effects of intraperitoneal administration of NaB immediately after training on the retention and persistence of IA memory for aged rats are shown in Fig. 1. There was no significant difference between rats given SAL and NaB in training performances ($P > 0.05$; U 33.000). All rats were tested for retention 1 (Test 1d), 2 (Test 2d), 3 (Test 3d), 4 (Test 4d), 5 (Test 5d), 6 (Test 6d), 7 (Test 7d), 14 (Test 14d), 21 (Test 21d) and 23 (Test 23d) days after training. Immediately after Test 23d, rats were given a reminder foot shock and tested again 1 day later. Statistical comparison using Wilcoxon signed-rank test showed that animals in both groups displayed significant memory retention on Test 1d compared to training ($P < 0.001$ for SAL group and $P < 0.01$ for NaB group). Further analysis with Mann–Whitney *U*-tests showed that there were significant differences between SAL-treated rats and rats given NaB in Test 1d ($P < 0.01$; U 103.000), Test 2d ($P < 0.05$; U 94.000), Test 3d ($P < 0.01$; U 101.000), Test 4d ($P < 0.05$; U 90.000), Test 5d ($P < 0.05$; U 95.000), Test 6d ($P < 0.05$; U 92.000), Test 7d ($P < 0.05$; U 96.000) and Test 14d ($P < 0.05$; U 94.500), but not in the other behavioral trials. Both groups demonstrated significant retention levels at Reminder test when compared to training by Wilcoxon signed-rank test ($P < 0.001$ for SAL and $P < 0.01$ for NaB), additionally the retention level of NaB-treated aged rats in the Reminder test was significantly greater then SAL-treated aged rats as showed by Mann–Whitney U test ($P < 0.01$; U 95.000). The results indicate that NaB administration in aged rats resulted in significant enhancement of IA memory retention that lasted for 14 days compared to SAL treated rats.

On the other hand, younger animals treated with NaB did not demonstrate enhancement of IA retention (Fig. 2). Young adult rats were treated with NaB intraperitoneally immediately after train-