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Chronic difluoromethylornithine treatment impairs spatial learning and memory in rats

Neeraj Gupta [a,c], Hu Zhang [b,c], Ping Liu [a,c,*]

[a] Department of Anatomy, University of Otago, Dunedin, New Zealand
[b] School of Pharmacy, University of Otago, Dunedin, New Zealand
[c] Brain Health and Research Centre, University of Otago, Dunedin, New Zealand

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ABSTRACT

Recent evidence suggests that polyamines putrescine, spermidine and spermine are essential in maintaining normal cellular function. The present study investigated the effects of chronic treatment of difluoromethylornithine (DFMO, 3% in drinking water), a potent inhibitor of putrescine synthesis, for 54 consecutive days on animals'behavior and neurochemical levels in the CA1, CA2/3 and dentate gyrus sub-regions of the hippocampus and the prefrontal cortex. The DFMO group showed performance impairments in the place navigation and the probe test conducted 24 h after the training in the reference memory version of the water maze task, but not in the elevated plus maze, open field, object recognition, cued navigation and the working memory version of the water maze task when compared to the control group (drinking water only). DFMO treatment resulted in approximately 80–90% and 20% of reductions in the putrescine and spermidine levels, respectively, in the four brain regions examined, and a small reduction in agmatine level in the CA2/3, with no effects on spermine, glutamate and γ-aminobutyrate. The DFMO group showed decreased body weight relative to the control one. However, there were no significant differences between groups in the normalized brain, kidney and liver weights. The present study demonstrates that chronic treatment of DFMO depletes putrescine and decreases spermidine levels in the brain, inhibits growth, and impairs spatial learning and memory in the reference memory version of the water maze specifically. These findings merit further investigation to fully understand the functional role of endogenous polyamines in learning and memory.

1. Introduction

Polyamines putrescine, spermidine and spermine are the downstream metabolites of L-arginine. In mammalian cells, putrescine is mainly derived from L-ornithine (the product of arginase) by ornithine decarboxylase (ODC). Putrescine combines with decarboxylated *S*-adenosylmethionine to produce spermidine via spermidine synthase, and spermine through a second aminopropyltransferase reaction involving spermine synthase. Spermidine/spermine N^1-acetyltransferase is the key enzyme involved in polyamine interconversion (Wallace et al., 2003). Agmatine, decarboxylated arginine, can be converted to putrescine by agmatinase (Halaris and Piletz, 2007; Wu and Morris, 1998), and hence is considered as a member of the polyamine family (Moinard et al., 2005).

The polyamines are widely distributed in mammalian cells, and their concentrations at physiological levels are important in maintaining normal cellular function (for reviews see Oredsson, 2003; Wallace, 2000; Williams, 1997). Difluoromethylornithine (DFMO) is a potent and irreversible inhibitor of ODC, and depletes putrescine mainly among the three polyamines (Gupta et al., 2009; Jänne et al., 1991; Malaterre et al., 2004; Metcalf et al., 1978; Slotkin et al., 1982; Sparapani et al., 1996). Because the polyamines are essential for cancer cell proliferation during tumorigenesis, DFMO has been used as a therapeutic and chemopreventive agent for cancers clinically (Babbar and Gerner, 2011; Meyskens et al., 2008; Simoneau et al., 2008). It has also been used as a pharmacological tool to investigate the effects of altered polyamine levels (Gupta et al., 2009; Malaterre et al., 2004).

The hippocampus is the key structure of the medial temporal lobe, and is important for certain types of learning and memory (Squire et al., 2004). There is a functional dissociation across its major subregions CA1, CA3 and dentate gyrus (DG). For example, the DG region creates a metric spatial representation and is involved in spatial pattern separation, whereas the CA3 and CA1 regions are important for pattern completion and sequence encoding, and temporal pattern association/completion and intermediate-term memory, respectively (Hoge and Kesner, 2007; Kesner, 2007; Kesner et al., 2004, 2008).

Abbreviations: DFMO, difluoromethylornithine; DG, dentate gyrus; GABA, γ-aminobutyric acid; HPLC, high performance liquid chromatography; i.c.v., intracerebroventricular; LC/MS, liquid chromatography/mass spectrometry; NO, nitric oxide; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; PFC, prefrontal cortex; SVZ, subventricular zone.

* Corresponding author at: Department of Anatomy, School of Medical Sciences, University of Otago, Dunedin, New Zealand. Tel.: +64 03 4797536; fax: +64 03 4797254.
E-mail address: ping.liu@otago.ac.nz (P. Liu).

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Neurogenesis is found in the DG in the adult brain, and these newborn cells can mature into functional neurons and play a role in maintaining hippocampal function (Aimone et al., 2011; Altman and Bayer, 1990; Clelland et al., 2009; Gage, 2002; Kempermann et al., 2004; Van Praag et al., 2002). Malaterre et al. (2004) reported that 3% DFMO in drinking water depleted putrescine mainly in the hippocampus, and significantly impaired adult neurogenesis in the DG in young rats. These findings suggest a novel role of endogenous putrescine in hippocampal neurogenesis.

Aging leads to cognitive decline and impairs neurogenesis in hippocampal DG (Driscoll et al., 2006; Wati et al., 2006). Interestingly, the putrescine level in the DG decreases with age (Liu et al., 2008c). Given the link between putrescine, hippocampal neurogenesis and hippocampal function (Aimone et al., 2011; Clelland et al., 2009; Malaterre et al., 2004), polyamine system dysfunction may contribute to cognitive decline during aging. Hence, it is important to understand how reduced level of putrescine affects animals' behavioral function, including learning and memory. Gupta et al. (2009) reported that acute depletion of putrescine (80–90% reduction in the hippocampus and prefrontal cortex) by DFMO administered intracerebroventricularly (i.c.v.) resulted in anxiety-like behavior and impaired memory for the object displacement in young adult rats, without affecting animals' locomotor and exploratory activities and spatial learning and memory. The present study was designed to investigate how chronic putrescine depletion affects animals' behavioral function in a number of commonly used behavioral tasks and the levels of polyamines, as well as glutamate and γ-aminobutyrate (GABA), the major excitatory and inhibitory neurotransmitters in the central nervous system. DFMO (3% in drinking water) decreased putrescine and spermidine levels in the sub-regions of the hippocampus and prefrontal cortex dramatically, and impaired spatial learning and memory in the reference memory version of the water maze task mainly, with mild or no effects on anxiety, exploration, locomotion and object recognition memory.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats at age of 3 months, weighing between 320 and 380 g, were housed one per cage ($33\times21.5\times17.5$ cm^3), maintained on a 12-h light–dark cycle (lights on 8 a.m.) and provided *ad lib* access to food and water. Animal's body weight and water intake were monitored and recorded every day. Behavioral procedures were conducted during the light period of the light–dark cycle. All experimental procedures were carried out in accordance with the regulations of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals (Otago, New Zealand). Every attempt was made to limit the number of animals used and to minimize their suffering.

2.2. Drug and treatment

DFMO was a generous gift from Dr. Patrick Woster (Wayne State University, USA), who received it from Genzyme (Cambridge, MA). Rats were randomly divided into the control (drinking water only, n = 10) and DFMO (3% DFMO in drinking water, n = 10) groups. The dose of DFMO was based on Malaterre et al. (2004) and a pilot study. DFMO was freshly prepared every two days, and the animals were treated for 54 consecutive days. Animal's body weight and water intake were measured and recorded daily from 6 days prior to the treatment till the end of the study. The mean body weight and water intake across the first 6 days were used as baseline (shown as day 0 in Fig. 1, and block 0 in Fig. 2A and B). The behavioral tests started after 36 days of treatment and lasted for 18 days, and the animals were sacrificed on day 54 (Fig. 1). The same animals were used for all of the behavioral tests throughout the study.

2.3. Behavioral apparatus

All behavioral experiments were conducted in a windowless room with three clear and one red 75 W bulbs mounted on the ceiling. A video camera was mounted at ceiling height in the centre of the room used for recording the performance during the experimental period. A radio speaker was located adjacent to the video camera at ceiling height to provide background masking noise. The extramaze cues (the laboratory furniture, lights and several prominent visual features, as well as the location of the experimenter) were held constant throughout the experiments.

2.3.1. Elevated plus maze task

The elevated plus maze (to assess animal's anxiety level) was shaped like a plus sign in black-painted wood, with two unwalled (open) arms (50×13.5 cm^2), surrounded by a clear plexiglass of 4 cm and two walled (closed) arms ($50\times13.5\times29$ cm^2). The central area of the maze measured 13.5×13.5 cm^2. The maze was elevated 60 cm above the floor, and the arm locations were kept constant with north and south being the closed arms.

2.3.2. Open field

An experimental chamber, which consisted of a 60×60 cm^2 hardboard box with walls 20 cm high, was used for the exploration and object recognition experiments. All four of the chamber walls and the floor of the box were painted black. The floor of the box was divided into 36 equal-sized squares by white marking tape. The box was elevated approximately 1 m above the floor.

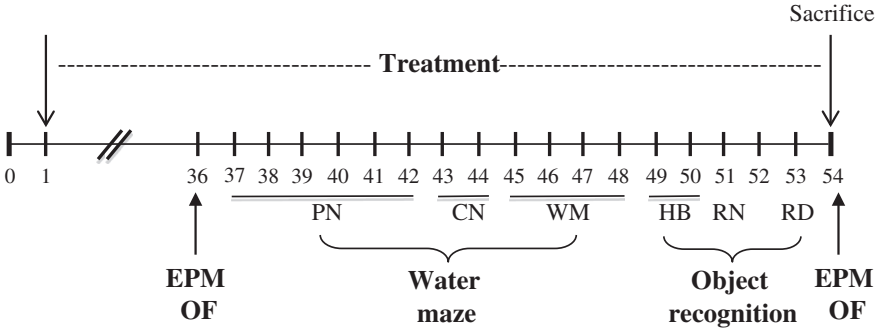

Fig. 1. Experimental timeline. Animals were given drinking water (Control, n = 10) or 3% DFMO in drinking water (DFMO, n = 10) for 54 consecutive days. Animals were tested in the elevated plus maze (EPM) and open field (OF) on days 36 and 54, in the place navigation (PN; days 37–42) and cued navigation (CN; days 43 and 44) of the reference memory version and the working memory version (WM; (days 45–48) of the water maze task, and the object recognition task (HB: habituation on days 49 and 50; RN: reaction to novel object on day 51; RD: reaction to displaced objects on day 53). All animals were sacrificed on day 54 after completion of the EPM and OF tests, and the brain tissues were harvested for the neurochemical analysis.