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# Anticonvulsant and procognitive properties of the non-imidazole histamine H3 receptor antagonist DL77 in male adult rats

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ABSTRACT

It has become clear that histamine H3 receptors (H3Rs) are implicated in modulating epilepsy and memory in laboratory animals. The new non-imidazole H3R antagonist DL77 has excellent selectivity profile and shows high *in-vivo* potency as well as *in-vitro* antagonist affinity with $ED_{50}$ values of 2.1 ± 0.2 mg/kg and 8.4 ± 1.3 [nM], respectively. In the present study, the anticonvulsant effects of DL77 on maximal electroshock (MES)-, pentylenetetrazole (PTZ)-, and strychnine (STR)-induced seizure models were investigated. Moreover, the procognitive properties of DL77 were tested on acquisition, consolidation and retrieval processes in a one-trial inhibitory avoidance task in male Wistar rats. The results indicate that DL77 (5, 10, and 15 mg/kg, i.p.) significantly and dose-dependently reduced MES-induced seizure duration, whereas no protection was observed in PTZ- or STR-induced seizures. Importantly, the protective action observed for DL77 in MES-induced seizure was comparable to that of the reference antiepileptic drug (AED) phenytoin (PHT), and was also reversed when rats were pretreated with the CNS penetrant pyrilamine (PYR) (10 mg/kg, i.p.), or with the selective H3R agonist *R*-(α)-methyl-histamine (RAMH) (10 mg/kg, i.p.). Furthermore, the procognitive studies indicate that acute pre-training systemic administration of DL77 (2.5 mg/kg, i.p.) facilitated acquisition, whereas pre-testing acute administration of DL77 (5 and 10 mg/kg, i.p.) improved retrieval. Interestingly, the procognitive effect of DL77 on retrieval was completely abrogated when rats were pretreated with the centrally-acting H2R antagonist zolantidine (ZOL) but not the centrally acting H1R antagonist PYR, indicating that histaminergic pathways through activation of H2Rs appear to be participating in neuronal circuits involved in retrieval processes. Taken together, our results show that DL77 demonstrates anticonvulsant properties in the MES-induced seizure model and improves cognitive performance through actions on different memory stages. Therefore, H3Rs may have implications for the treatment of degenerative disorders associated with impaired memory function and may represent a novel therapeutic pharmacological target to tackle cognitive problems associated with the chronic use of antiepileptic drugs.

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## 1. Introduction

Epilepsy is a chronic disorder characterized by abnormal, excessive neuronal excitability. Pharmacological intervention with antiepileptic drugs (AEDs) represents the chief strategy for management of this ailment. Around 60–70% of patients responds to treatment, however, resistance to mono-therapy is not uncommon and drug combination is often inevitable (Brigo et al., 2013). Also, available AEDs are not well tolerated and side effects are augmented with chronic clinical use of many AEDs (Schmidt, 2002). Interestingly, cognitive impairment accompanies some types of

*Abbreviations:* H3Rs, histamine H3 receptors; MES, maximal electroshock; PTZ, pentylenetetrazole; STR, strychnine; AED, antiepileptic drug; PHT, phenytoin; PYR, pyrilamine; RAMH, *R*-(α)-methyl-histamine; ZOL, zolantidine; THLE, tonic hind limb extension; DOZ, donepezil; PIT, pitolisant; VPA, valproic acid; OFT, open field test.

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epilepsy at onset and worsens with chronic poorly controlled seizures particularly in the developing brain (Elger et al., 2004). Therefore, appropriate and early management of seizures removes the insult on the brain cognitive function, though; many AEDs are not so harmless from the same perspective (Hermann et al., 2009). More effective and safer pharmacological approaches that remove seizure complications and improve the quality of life are still warranted.

Histamine is an accepted neurotransmitter and/or neuromodulator in the central nervous system (CNS) (Orr and Pace, 1984; Schwartz et al., 1986). Cell bodies of histaminergic neurons originate from a common region in the brain that is the tuberomammillary nucleus of the posterior hypothalamus. From there, histamine neurons projection to almost every region of the brain. Therefore, it is predictable that the histamine neuronal network participates in a wide-range of homeostatic as well as higher activities, like arousal, attention, locomotion, cognition and neuroendocrine functions (Haas et al., 2008). Histamine exerts its biological activities via four distinct histamine receptors (H1R–H4R) that belong to the G-protein coupled receptor family (Panula et al., 2015). H1R and H2R are found in the brain and periphery. Although H4Rs are present in the brain, they are predominately expressed in mast cells and leukocytes, whereas H3Rs are abundant in the CNS (Schneider and Seifert, 2015). While activation of H1R and H2R mediates slow excitatory postsynaptic potentials, H3Rs are coupled to $G_i/G_o$-proteins and act as autoreceptors that control the synthesis and release of histamine in the CNS. Furthermore, H3Rs functioning as hetero-receptors can also regulate the release of other neurotransmitters like acetylcholine, glutamate, GABA, norepinephrine, serotonin, dopamine in different regions of the brain (Brown et al., 2001).

Experiments from clinical studies have provided that histamine is an endogenous anticonvulsant (Yokoyama, 2001). Also, administration of histidine (histamine precursor) or metoprine (inhibit histamine breakdown) at doses that increased brain histamine also increased the convulsion threshold in several animal models of epilepsy (Chen et al., 2002; Kamei et al., 1998; Scherkl et al., 1991; Yawata et al., 2004). Moreover, H1R activation was found to mediate the anticonvulsant action of histamine since it is reversed by centrally acting H1R antagonists. Accordingly, different brain-penetrating H1R antagonists themselves were reported to promote convulsions in epileptic experimental animals and in children with febrile convulsions (Haruyama et al., 2008; Miyata et al., 2011; Takano et al., 2010; Zolaly, 2012). Histamine in the CSF of these children was lower than their febrile non-convulsive counterparts, thus a fall in histamine might be the confounder in the fever-seizure relationship (Kiviranta et al., 1995).

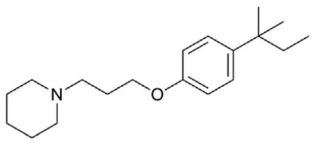
Interestingly, H3R antagonists/inverse agonists earned a growing interest since the past decade with their copious possible applications in treatment of neuropsychiatric disorders (Gemkow et al., 2009). Results from previous experimental work revealed that blocking H3Rs increases the release of histamine and other neurotransmitters like acetylcholine and GABA (Bhowmik et al., 2012). Supported by positive outcomes in preclinical epilepsy (electrically- and chemically-induced convulsions in rodents), H3R antagonists/inverse agonists progressed to clinical phase III with the recent success of pitolisant (PIT) (Schwartz, 2011). Dose-dependently, PIT, alone or in combination with other AEDs, showed a favorable EEG profile in a proof of concept human photosensitivity model (Kasteleijn-Nolst Trenite et al., 2013). Importantly, PIT is one of the first non-imidazole-based H3R antagonists/inverse agonists that, compared to imidazole-based agents like thioperamide, have lower drug-interaction and increased brain penetration (Sander et al., 2008).

Notably, H3R antagonists/inverse agonists have a unique feature by their potential cognition-enhancing property when compared to other available AEDs and as indicated by several lines of evidence from preclinical studies. Accordingly, it has been previously found that scopolamine- or dizocilpine-induced memory deficits in rodents ameliorated by a variety of H3R antagonists/inverse agonists (Witkin and Nelson, 2004). Furthermore, in a previous study scopolamine-treated rats that were pretreated with thioperamide (10 mg/kg, i.p.) had an improved memory consolidation score compared to saline-treated rats in an inhibitory avoidance task (Bernaerts et al., 2004). Charlier et al., were the first to report that in normal adult mice, the H3R antagonists/inverse agonist thioperamide can boost learning and memory at all memory stages from acquisition to retrieval in inhibitory avoidance task (Charlier et al., 2013). Given their localization and their ability to affect multiple neurotransmitter systems, the central H3Rs represent an attractive target for the development of new H3R antagonists/inverse agonists that might have a potential role in multi-neurotransmitter disorders including epilepsy and cognitive disorders (Bhowmik et al., 2012; Harada et al., 2004; Uma Devi et al., 2010; Witkin and Nelson, 2004; Yokoyama, 2001; Yokoyama et al., 1993). Therefore, the earlier described and non-imidazole-based H3R antagonist/inverse agonist DL77 with high H3R *in vitro* affinity (Lazewska et al., 2006), excellent *in-vitro* selectivity profile, and *in vivo* antagonist potency has been investigated on its anticonvulsant effects in MES-, PTZ-, and STR-induced convulsion models in male Wistar rats (Fig. 1). Moreover, the behavioral effects of DL77 on acquisition, consolidation and retrieval processes in a one-trial inhibitory avoidance paradigm in rats were tested. Given that motor activity could mask the effects of DL77 on learning and memory, we also used an open field test (OFT) to evaluate activity and anxiety in the same animals.

## 2. Methods and materials

### 2.1. Animals

Male Wistar rats (bred at the Central Animal Facility of the UAE University) at a body weight of 180–200 g were used. All animals were maintained in an air-conditioned room with controlled temperature (24 ± 21C) and humidity (55 ± 15%) under a 12-h light/dark cycle (lights on at 07:00 h). The animals were given free access




**DL77**

$ED_{50}$[a] = 2.1 ± 0.2 mg/kg, p.o.;

$pK_i$(hH3R)[b] = 8.08; $pK_i$(hH4R)[c] = 4.31; $pK_i$(hH1R)[d] = 6.18; $pK_i$ (α2R)[e] = 2.10

**Fig. 1.** Chemical structure, *in vitro* affinities, and *in-vivo* potency of DL77. [a]Central histamine $H_3$R assay *in vivo* after p.o. administration to mice, n = 3(Garbarg et al., 1992; Garbarg et al., 1989; Lazewska et al., 2006; Ligneau et al., 1998). [b][$^{125}$I]Iodoproxyfan binding assay at human $H_3$R stably expressed in CHO-K1 cells, n = 3(Lazewska et al., 2006; Ligneau et al., 1994; Ligneau et al., 2000). [c][$^3$H]Histamine binding assay performed with cell membrane preparation of Sf9 cells transiently expressing the human histamine H4R and co-expressed with $G_{\alpha i2}$ and $\beta_{1\gamma 2}$ subunits n = 3(Amon et al., 2007; Isensee et al., 2009; Meier et al., 2001; Tomasch et al., 2013). [d][$^3$H]Pyrilamine binding assay performed with cell membrane preparation of CHO-hH1R cells stably expressing the human H1R; n = 3(Schibli and Schubiger, 2002; Schlotter et al., 2005; van Staveren and Metzler-Nolte, 2004). [e]The affinity for α(2)-adrenoceptors was evaluated by radioligand binding assays to rats cortex membrane using [$^3$H]clonidine (Barbaro et al., 2002; Betti et al., 2002; Handzlik et al., 2008).