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A comparison of cholinesterase inhibitors in the treatment of quinuclidinyl benzilate-induced behavioural deficit in rats performing the multiple T-maze

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ABSTRACT

Cholinesterase inhibitors are beneficial in the treatment of Alzheimer's disease via indirect increase of cholinergic neuro-transmission. The aim of the present study was to evaluate the potency of inhibitors tacrine, rivastigmine and donepezil to reverse cholinergic depletion induced by 3-quinuclidinyl benzilate (QNB, 2 mg kg^{-1}) in Wistar rats performing the multiple T-maze test. The effect of QNB on retention was compared to the effect of standard amnesic drug, scopolamine, at the dose of 0.3 mg kg^{-1}. Well-trained rats were treated intra-peritoneally with QNB, followed by another injection containing saline or tacrine (10 mg kg^{-1}) or rivastigmine (1.2 mg kg^{-1}) or donepezil (2.65 mg kg^{-1}) 15 min later. Rats were subjected to the T-maze task 30 min and 24 h following QNB administration. The passage time and number of errors were observed. QNB significantly impaired the performance of rats in both tested times in contrast to short-lasting effect of scopolamine (30 min only). The inhibitors rivastigmine and donepezil significantly attenuated QNB-induced behavioural impairment in the 30 min tests, whereas tacrine failed to have the same effect. Moreover, the performance of tacrine-treated rats was worse due to cholinergic over-stimulation. Beneficial effects of all tested inhibitors including tacrine were evident in the 24 h test.

Introduction

Inhibitors of cholinesterase (ChEIs) are a varied group of both natural and synthetic compounds which are able to inhibit cholinesterase cleavage of the neurotransmitter acetylcholine (ACh). Decreased degradation of ACh leads to increased strength and duration of neurotransmission, thus ChEIs can be used therapeutically for the symptomatic treatment of some neurodegenerative diseases such as Alzheimer's disease (AD). Given that ACh is a key compound involved in learning and memory, ChEIs could potentially be considered as cognitive enhancers. There are a number of substances with possible beneficial effects on AD patients and their cognitive

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functions including, for example, vitamin E, selegiline, non-steroidal anti-inflammatory drugs, statin drugs or omega-3 fatty acids, however the number of approved drugs is limited (Howland, 2011). Thus current therapeutic protocols based on ChEIs, such as donepezil, galantamine and rivastigmine, are still valuable in the clinical treatment of AD (Wynn and Cummings, 2004; Isensee et al., 2007; Pohanka, 2012) even if the effect of these inhibitors is palliative rather than curative. Centrally-acting ChEIs influence the cholinergic system in the brain, which is greatly affected by the pathological processes occurring in AD. Lack of neurotransmission is correlated with progressive cognitive decline and so the elevation of ACh transmission indirectly, via drugs such as ChEIs, can reduce the progression and severity of this decline, albeit only temporarily (Giacobini, 2000; Racchi et al., 2004). The beneficial effects of ChEIs on cognition have been demonstrated several times in the laboratory rat, using either lesion- or drug-induced behavioural impairment models (Cheng and Tang, 1998; Poorheidari et al., 1998; Bejar et al., 1999). ChEIs usually demonstrate a dose-dependent beneficial effect within a limited dose range, resulting in an inverted U-shaped dose-effect curve (Braida et al., 1996; Wang and Tang, 1998; Bejar et al., 1999). Therapeutic efficacy typically declines when cholinesterase inhibition exceeds 40%, due to both central and peripheral side effects of cholinergic over-stimulation. Furthermore, the effective dose of ChEI varies considerably between these studies, which could in part be attributed to the variable doses of cognitive-impairing agents and the behavioural methods used (Bejar et al., 1999).

A tropane alkaloid, scopolamine, is extensively used in the preclinical testing of new cognitive enhancers, as a standard model of 'cholinergic' amnesia in experimental animals (D'Hooge and De Deyn, 2001; De-Mello and Carobrez, 2002; Gacar et al., 2011; Falsafi et al., 2012). Scopolamine affects cholinergic neurotransmission via the blockade of muscarinic (M) receptors, resulting in the failure of cognitive functions; the reversal of this cognitive impairment is predictive of the beneficial pharmaco-dynamic effects of cognitive enhancers (Lenz et al., 2012). As an alternative to scopolamine, the cholinergic antagonist 3-quinuclidinyl benzilate (QNB) has also been used as a model of 'cholinergic' amnesia in rats (Krejcova et al., 2004; Kunesova et al., 2008) and was used as an amnesic agent in this current study. Similar to scopolamine, QNB acts as a non-selective competitive antagonist of M receptors (Kinney et al., 1995; Haga et al., 2012). The doses of both anticholinergic drugs have been established in previous studies, producing an amnesic effect on reference memory in rats performing several behavioural tests, including the multiple T-maze, elevated T-maze, water maze and passive avoidance tasks (De-Mello and Carobrez, 2002; Krejcova et al., 2004; Kunesova et al., 2008; Gacar et al., 2011).

The current study assesses the potential beneficial effects of a single dose of the three ChEIs tacrine, rivastigmine and donepezil on QNB-induced cholinergic depletion in male Wistar rats performing the multiple T-maze, a behavioural test of spatial learning and memory (Kunesova et al., 2008; Sharma et al., 2010; Falsafi et al., 2012; Sase et al., 2012). The ChEIs were administered 15 min after the dose of QNB, and their efficacy was evaluated 30 min and 24 h after the administration of QNB.

Materials and methods

Animals

Male Wistar rats (8–10-week-old, 150–200 g b.w.) were obtained from Velaz (Czech Republic). The animals were housed in groups of 6 in temperature- and light-controlled breeding units at an approved animal facility. The animals received standard rodent diet (Cerea corp.) and drinking water *ad libitum*. The food supply was limited to approximately 75% of the free feeding rate and the acclimatization period was a minimum of 10 days. The use of animals in this study was formally approved by the Ethics Committee of the Faculty of Military Health Sciences, Czech Republic. All procedures involving animals were performed in accordance with current legislation.

Chemicals

QNB HCl^{-} was synthesized *de novo* at the University of Defence (Faculty of Military Health Sciences, Department of Toxicology) and was of 90% purity (HPLC determination). Scopolamine hydrobromide and the ChEIs tacrine (9-amino-1,2,3,4-tetrahydroacridine hydrochloride), rivastigmine ((S)-N-ethyl-3-[(dimethyl-amino)ethyl]-N-methyl-phenylcarbamate hydrogentartarate) and donepezil (1-benzyl-4-[(5,6-dimethox-1-indanon)-2-yl] methylpiperidine hydrochloride) were purchased from Sigma–Aldrich Ltd. (Czech Republic) as well as other chemicals for assessment of brain cholinesterase inhibition [Tris–HCl buffer, acetylthiocholine, 5,5′-dithiobis-(2-nitrobenzoic acid)]. Cholinergic antagonists and ChEIs were diluted in normal saline (0.9% natrium chloride, B. Braun Medical Ltd., Czech Republic) immediately before administration to the experimental animals.

Inhibition of brain cholinesterase

The doses of ChEIs were chosen on the basis of previous studies (Kirkby et al., 1996; Kosasa et al., 1999; Bruins Slot et al., 2003); tacrine at 10 mg kg^{-1}, rivastigmine at 1.2 mg kg^{-1} and donepezil at 2.65 mg kg^{-1}. The *in vivo* inhibitory potential of these doses was verified by evaluating the inhibition of brain cholinesterase in the rats after *i.p.* administration. Rats were administered with the ChEIs at the same dose and via the same route as those rats utilized for the behavioural testing and euthanized by CO_2 15 min after administration of the drug. The brains were removed immediately and homogenized using a homogenizator Ultra-Turrax T25 Basic (IKA® – WERKE, Germany) in Tris–HCl buffer (0.02 mol/l, pH 7.6, 1:10) and the cholinesterase activity was determined by standard spectrophotometric methods (Ellman et al., 1961), using acetylthiocholine as a substrate (Tris–HCl buffer, N = 0.1 mol/l, pH 7.6). The Helios Alpha spectrophotometer was used for determination of absorbance at 436 nm and the cholinesterase activity was expressed as μkat/kg (μmol substrate hydrolyzed/kg wet tissue within 1 s). Untreated control values for brain cholinesterase activity were obtained from rats administered with saline instead of ChEI (saline control).