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Research report

Cholinergic antagonist 3-quinuclidinyl benzilate – Impact on learning and memory in Wistar rats

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HIGHLIGHTS

- We studied the impact of cholinergic antagonist on learning and memory in rats.
- Several doses of 3-quinuclidinyl benzilate (QNB) were tested using water maze and passive avoidance.
- QNB affected specifically stage of acquisition.
- Use as a pharmacological model of Alzheimer disease is proposed.

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ABSTRACT

3-Quinuclidinyl benzilate (QNB) represents a non-selective, competitive antagonist of cholinergic receptors, which has been previously used to generate cognitive deficits in animal models of neurodegenerative disorders. The aim of this study was evaluation of QNB potency for creation of cognitive impairment during the acquisition, consolidation and retrieval stages of learning and memory in rats.

Male Wistar rats were subjected to a water maze task with hidden platform and a step-through passive avoidance task. The water maze test was carried out in two separate experiments focused on spatial learning (acquisition test) and long-term spatial memory (retrieval test). QNB doses (0.5, 1.0, 2.0 and 5.0 mg kg^{-1}) were administered to rats intraperitoneally before training sessions (acquisition test) or before probe trial (retrieval test). A QNB dose of 2.0 mg kg^{-1} was administered to rats in the passive avoidance task before training (acquisition test), immediately post-training (consolidation test) or 24 h pre-retention (retrieval test).

QNB significantly impaired the acquisition in the water maze at doses 0.5–5.0 mg kg^{-1} as well as the acquisition of passive avoidance task. In contrast, consolidation and retrieval were not affected by QNB, indicating that QNB specifically affects the stage of acquisition.

1. Introduction

The central cholinergic system plays an important role in cognition. A shortage of cholinergic transmission in target areas of the brain, predominantly in the prefrontal cortex, hippocampus, striatum or amygdala, is connected with behavioral alterations including impairments of learning and memory. These changes are typical accompanying signs for neurodegenerative diseases such as Alzheimer's disease [1]. It is generally accepted that pharmacological intervention into the central cholinergic system via agonists/antagonists of cholinergic receptors influences learning and memory tasks in the multiple stages [2–4]. Thus, anticholinergic drugs such as scopolamine, atropine, trihexyphenidyl, biperiden, or pirenzepine are extensively investigated for their behavioral and neurochemical effects [5–9]. Such pharmacological manipulation of the cholinergic system provides options for experimental modeling of neurodegenerative disorders in laboratory animals [3] and appropriate models for evaluation of new drugs such as acetylcholinesterase inhibitors, which modify cholinergic transmission [10,11].

In this study, 3-quinuclidinyl benzilate (QNB; Fig. 1) was investigated as a non-selective, competitive antagonist of muscarinic (M) receptors [12,13]. Historically, this compound was used as a military incapacitating agent due to its effects on central nervous

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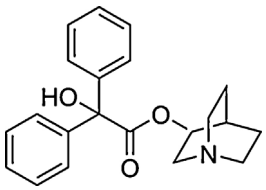

Fig. 1. Chemical structure of 3-quinuclidinyl benzilate (QNB).

system including mental slowing [14]. Although QNB has been used for identification of M receptors, primarily via the radioligand binding assay, [15,16] it has also been utilized in several studies to generate a cognitive deficit in experimental animals performing passive avoidance and the multiple T-maze task [17–19]. The aim of this study was to evaluate the potency of QNB to affect learning and memory in multiple stages – acquisition, consolidation and retrieval. Similarly, as with other anticholinergics, varying impact was expected at different learning stages [20]. In general, some previous studies have found impairment of acquisition and no effect on retrieval via cholinergic blockade in accordance with the "biphasic" cholinergic hypothesis [21]. Nevertheless, learning and memory are multiple cognitive processes depending on multiple brain structures with possible interactions between muscarinic and serotonergic, glutamatergic, or dopaminergic systems [22]. So the observed effect of particular anticholinergics might be diverse across studies, related to the type and dose of the drug. A recent study by Huang et al. [23] concluded that the cholinergic blockade via scopolamine impaired acquisition of both spatial and fear memory and retrieval of spatial memory, whereas there was no effect on the retrieval of fear memory. Conversely, impaired memory retention was found in some studies focused on avoidance learning following anticholinergic treatment [22].

In this study QNB treated rats were subjected to learning and memory tasks in two behavioral tests – water maze (WM) and step-through passive avoidance. The water maze (WM) is a common and widely used behavioral test for investigating spatial learning and memory [3,24]. Spatial learning in the WM task requires recollection of experience and information, which, in rodents, is considered analogous to human episodic memory ("episodic-like memory") [20,25]. Passive avoidance is based on aversive learning that requires suppression of the innate response. Using both behavioral tests, impairment of learning and memory was observed in previous studies after chronic or single administration of anticholinergic drugs [20,23,26,27].

The QNB was administered to rats in order to assess its impact on specific stages of the learning process, assuming that drug treatment before training is responsible for effects on acquisition, whereas the treatment of trained individuals before the final delayed test could be interpreted as affecting memory retrieval [20,23]. In retrieval tests, QNB was administered 24 h after the last training session, and this is considered to be sufficient for complete consolidation [28]. Finally, QNB was also administered immediately after training of the passive avoidance task to determine its impact on the consolidation stage.

Cholinergic antagonists usually affect multiple systems and the drug-induced peripheral or central side effects such as motor/attention impairment observed after scopolamine administration may be primarily responsible for impaired cognition [29–31]. Thus, it was necessary to identify any side effects that may have been responsible for the observed behavioral impairment. Additional tests such as visible platform test [30], accelerating rotarod [32] and measurement of spontaneous locomotor activity were performed to disclose potential "non-cognitive" side effects of QNB.

2. Methods

2.1. Animals

Male Wistar [Crl:(WI)BR] rats (10–12-week-old, 250–300 g b.w.) were obtained from Velaz (Czech Republic). The animals were housed in groups of 6 in an environmentally controlled breeding facility (temperature 21 ± 1 °C, 12/12 h light/dark cycle). The animals received standard rodent diet (Cerea corp.) and drinking water ad libitum. The time of acclimatization was at least 10 days prior to use. 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 in accordance with contemporary legislation (2010/63/EU).

2.2. Chemicals

Quinuclidinyl benzilate (QNB) hydrochloride was synthesized de novo at the University of Defense (Faculty of Military Health Sciences, Department of Toxicology) and was of 90% purity (HPLC determination). Several doses of QNB ranging from 0.5 to 10.0 mg kg^{-1} QNB were assigned to individual behavioral tests (Table 1) and were much below median lethal dose of QNB which is considered to be 281 mg kg^{-1} (male Wistar rats, intramuscular administration [14]). The 2.0 mg kg^{-1} dose of QNB, which has been previously used as behavior-impairing dose in other studies [18,19], was used as the initial dose for all tests. In accordance with reducing animal numbers for experimentation, lower/higher doses were applied only in selected tests (WM, rota-rod) according to behavioral response to 2 mg kg^{-1} dose (Table 1). QNB diluted in saline (0.9% (w/v) sodium chloride, Noviko Ltc., Czech Republic) was administered to animals as a standard volume of 1 mL kg^{-1} via intra-peritoneal (*i.p.*) injection. Saline also served as vehicle control.

2.3. Water maze

The WM apparatus consisted of a black circular pool with a diameter of 180 cm, filled with water (25 °C) to a depth of 25 cm. The maze was virtually divided into four concordant quadrants numbered 1–4 clockwise. The circular escape platform with diameter of 15 cm was placed in the middle of quadrant no. 3, 37.5 cm from the proximate edge of the pool. The location of the platform was constant during the experiment. The platform was the same color as the maze with an antireflective surface and was 1 cm below the water level. Thus, the platform was invisible to swimming rat. There were no spatial conditional cues on the wall of the maze and thus the rats were required to navigate completely according to extra-maze cues, represented by two adjacent windows (south and west side), a desktop computer (northwest side), furniture and other equipment of the experimental room. The daily training sessions were carried out from 8 a.m. to 11 a.m. for 4 consecutive days, with 8 swims per day, eventually followed by the probe trial on the fifth day (retrieval test). At the end of day 4, each rat had undergone a total of 32 swims. The rats were released from 8 variable start positions around the maze within a one day session. Starting positions were clearly defined as the midpoints (4 starting positions) of all quadrants and the border points among all adjacent quadrants of the maze (remaining 4 starting positions). All starting positions were separated by a constant distance of 70 cm. The order of starting positions varied randomly among trials and daily training sessions, and no position was repeated within the same daily session. This experimental design had the advantage that animals were forced to use only extra-maze cues for navigation in contrast to stable start positions, where they can navigate according to body position or swimming direction.