Pharmacology, Biochemistry and Behavior 95 (2010) 485–493

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Behavioural effects and regulation of PKCα and MAPK by huprine X in middle aged mice

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ARTICLE INFO

Article history:
Received 22 July 2009
Received in revised form 18 January 2010
Accepted 25 March 2010
Available online 2 April 2010

Keywords:
Huprine X
Acetylcholinesterase inhibitors
Learning and memory activation
Aging
Protein kinase Cα (PKCα)
Mitogen activated protein kinase (MAPK)
Amyloid precursor protein (APP)

ABSTRACT

The behavioural effects of huprine X, a new anticholinesterasic inhibitor, as well as its effects on the regulation of protein kinase C (PKC), mitogen activated protein kinase (MAPK) and α-secretase (ADAM10 and TACE/ADAM17) related to amyloid precursor protein (APP) processing remain to be established. In the present work, 12 month old 126/Sv×C57b/6 male mice which received chronic i.p. treatment with either saline, huprine X (0.04 μmol kg^{-1}) or huprine X (0.12 μmol kg^{-1}), were submitted to a battery of behavioural tests and thereafter the brains were dissected to study the neurochemical effects induced by huprine X. The results show that, in a dose dependent manner, huprine X facilitates learning and memory in the Morris water maze and improves some indicators of emotionality without inducing adverse effects, affecting motor activity nor anxiety-like behaviours, as measured in the open-field and corner tests. Moreover activation of downstream PKC/MAPK signaling pathways may underly these behavioural effects as well as the stimulation of the non-amyloidogenic processing of APP. Results obtained herein using a sample of aged animals strongly suggest that huprine X constitutes a promising therapeutic agent for the treatment of cholinergic dysfunction underlying aging and/or dementias.

1. Introduction

Experimental and neuropathological evidence support the cholinergic hypothesis of geriatric memory dysfunction postulated already 30 years ago (Bartus et al., 1982) which establishes that a serious loss of neurotransmission in the basal forebrain ascending cholinergic system contributes significantly to both age-related and Alzheimer disease induced impairments in cognitive abilities (Bartus, 2000). Accordingly, enhancement of the central cholinergic neurotransmission has been regarded as one of the most promising strategies for treatment of Alzheimer disease, mainly by means of reversible AChE inhibitors (AChEI). However, some of these drugs seem not to be exempt of CNS adverse effects, related to cholinergic stimulation in the brain, such as indirect gastrointestinal adverse effects (nausea, vomits, diarrhoea) and weight loss (Raskind et al., 2000) and extrapyramidal side effects (Carriero et al., 1997; Mayorga et al., 1997) which could be critical disadvantages when considering their therapeutic potential in some dementias (i.e. dementia with Parkinson's disease). In addition, anxiolytic-like effects and reduction of neophobia have been also reported for some AChEI such as physostigmine (Sienkiewicz-Jarosz et al., 2003). At the molecular level, it has been demonstrated that protein kinase C (PKC) plays an important role in the transduction mechanisms related to the regulation of the amyloid precursor protein (APP) metabolism (Racchi et al., 2003). Thus, in vitro studies have established the involvement of PKC and PKC-coupled receptors in the non-amyloidogenic α-secretase pathway of the APP cleavage (Buxbaum et al., 1993). Furthermore MAPK has been implicated in both PKC and tyrosine kinase receptor regulation of APP catabolism (Mills et al., 1997; Desdouits-Magnen et al., 1998). In addition, several reports seem to indicate that AChEIs may affect the secretory processes of APP via activation of both PKC, especially PKCα, and mitogen-activated protein kinase (MAPK) (Peng et al., 2006; Zhang et al., 2004; Bar-Am et al., 2004; Yogev-Falach et al., 2002). Moreover, the levels in membrane compartment of ADAM10, one of the more prominent candidates for α-secretase activity, are also increased after AChEI treatment (Zimmermann et al., 2004).

Among the different chemical species of AChEIs currently available, (±)-12-amino-3-chloro-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride, the so-called huprine X, a huperzine A-tacrine hybrid, has shown a highly potent and selective inhibitory action on acetylcholinesterase both "in vitro" and "ex vivo" (Camps et al., 2000b). Its affinity for AChE is one of the

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doi:10.1016/j.pbb.2010.03.013

highest yet reported with an inhibition constant (K_I) of 26 pM, that is, 180 times that of huperzine A, 1200 times that of tacrine, and 40 times that of E2020 (donepezil, Aricept) the most selective AChEI currently approved for therapeutical use (Camps et al., 2000a). Recently some new huprines have been synthetized with similar activities against human recombinant AChE (Ronco et al., 2009). In addition huprine X has an agonistic action on muscarinic M_1 and nicotinic receptors (Román et al., 2002, 2004) and exhibited a "tight binding" character in experiments of reversibility of bovine AChE inhibitory activity (Camps et al., 2000b). However, the 'in vivo' effects of huprine X remain to be established.

Therefore, the behavioural effects of huprine X on both aging and/or animal models for Alzheimer's disease are of special interest as well as those studies investigating its effects on the regulation of PKC, MAPK and secretase levels related to APP processing. Thus, the first aim of the present work was to describe the 'in vivo' effects of chronic administration of two range of doses on normal aged animals by means of a battery of tests assessing spatial reference learning and memory, locomotor activity, anxiety-like behaviour, neophobia and emotionality. Thereafter, in the same animals, we determined the effects of huprine X on the above mentioned molecular substrates. Thus, we studied both cytosolic and membrane fractions obtained from the hippocampus and the cortex of control and huprine X treated mice and investigated the effects of huprine X on the expression and distribution of the PKC, especially PKCα, the MAPK levels as well as its effects on APP processing and the trafficking of both α-secretases (ADAM 10 and TACE/ADAM 17).

2. Methods

2.1. Animals

Twenty-nine 12-month-old 129/Sv×C57b/6 male mice were maintained and behaviourally assessed in the facilities of the Medical Psychology Unit. Four to five animals were housed per cage in standard plastic type Macrolon cages (35×35×19 cm, with 2 l of wood cuttings as bedding) up to the time of the experiment. They were maintained at room temperature (22±2 °C) with 60±10% relative humidity and a 12 h light/dark (LD) schedule with lights on at 08:00 h. The animals had lab chow and tap water "ad libitum" until the moment of the test. All the research was conducted in compliance with the Spanish legislation on 'Protection of Animals Used for Experimental and Other Scientific Purposes' and in accordance with the EU Directive 08-88 on this subject.

2.2. Drug treatment

(±)-12-Amino-3-chloro-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride (huprine X) was obtained from the Laboratori de Química Farmacèutica (Facultat de Farmàcia, Universitat de Barcelona). The drug was dissolved in a vehicle of 0.9% saline solution for chronic intraperitoneal injection in a volume of 1 ml kg^{-1}. At the age of 12 months, the animals received chronic treatment with either huprine X (0.04 and 0.12 μmol kg^{-1}) or saline at 15.00 h daily for 14 days and then the drug treatment continued throughout an additional 7-day period for behavioural testing. In each cage, animals were randomly selected to receive one of the doses of huprine X or saline.

2.3. Assessment of behavioural effects induced by huprine X

During the daily sessions of drug treatment presence or absence of adverse effects such as diarrhoea, tremulous jaw movements was recorded by visual observation of each single animal. Weight was monitored daily to control putative weight loss. Fourteen days after the start of the chronic treatment animals were successively confronted with the following battery of behavioural tests (modified from Giménez-Llort et al., 2002): 1) several spatial learning and memory tests in the Morris water maze: place learning for reference memory, removal and cue learning; 2) open-field test; 3) corner test. Behaviour was evaluated by both direct observation and analysis of video-tape recorded images by an observer unaware of the animal's treatment. The experiments were performed under dim white light (20 lx) during their light phase of the LD cycle (from 10:00 h to 13:00 h).

2.3.1. Morris water maze tests

Two paradigms in the Morris water maze were carried out (Giménez-Llort et al., 2002). The mice were trained to locate a platform (7 cm diameter) in a circular pool (Intex Recreation Corp. CA, USA; 91 cm diameter; 20 cm height, 25 °C opaque water) located in a black test room with distal cues.

2.3.1.1. Days 1–5, place learning. This place task consisted of progressive training of animals to find the location of the platform until all the three experimental groups performed equally. The procedure involved four trial sessions per day, with trials spaced 15 min apart. The mouse was gently released (facing the wall) from one starting point randomly selected (N, S, E or W) and allowed to swim until they located the platform submerged 1.5 cm in a fixed position (SW quadrant and 10 cm away from the wall). The escape latency was recorded. Mice that failed to find the platform within 60 s were placed on it for 10 s, the same period that was allowed for the successful animals.

2.3.1.2. Day 5, removal. The retention and level of accuracy of the precise location of the platform position achieved were measured in a probe trial or 'removal', one and a half hour after the end of the last session of place task. The procedure consisted of removing the platform from the maze and release the mouse from the north starting point and let the animal navigate for 60 s. The time spent in each quadrant and the navigation trajectories (Lang et al., 2003) were measured by analysis of the video-tapes.

2.3.1.3. Day 6, cue learning or visual platform. In this task, the platform was elevated 1 cm above the water level, with its position in the NW and indicated by a visible stripped flag (5×8×15 cm), whereas external maze cues were removed from the walls. Four trials spaced 15 min apart were administered in one single day. The escape latency was recorded.

2.3.2. Open field test

Animals were assessed for locomotor activity and anxiety/emotionality in an open field (woodwork, white, 50×50×35 cm height). The animals were placed in the centre of the apparatus and were observed for 5 min. The latencies to leave the centre (5×5 cm central square), to reach the peripheral zone (a 5 cm wide square ring next to the walls) and to perform the first rearing were noted. Horizontal (number of crossings) and vertical (number of rearings) locomotor activity, the number and duration of groomings, the number of defecation boli and the presence of urination were also recorded. The apparatus was cleaned thoroughly before testing the following animal.

2.3.3. Corner test

Animals were weighted and placed in the centre of a cage filled with 2 l of clean wood cuttings. One cage was used per animal. Number of visited corners and number of rearings were recorded for 30 s. The number of defecation boli was also recorded, while urination was undetectable because of the beddings.