Experimental Neurology 236 (2012) 28–33

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

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

Selective down-regulation of α4β2 neuronal nicotinic acetylcholine receptors in the brain of uremic rats with cognitive impairment

Juan J. Ballesta [a,*], Carlos del Pozo [b], Juan Castelló-Banyuls [a], Clara C. Faura [a]

[a] Institute of Neurosciences, CSIC-Universidad Miguel Hernández, Avenida Ramon y Cajal s/n. 03550 San Juan de Alicante, Spain
[b] Department of Nephrology, Hospital Verge dels Lliris, Alcoi, Alicante, Spain

ARTICLE INFO

Article history:
Received 27 October 2011
Revised 31 January 2012
Accepted 29 March 2012
Available online 5 April 2012

Keywords:
Renal failure
Cognitive impairment
Brain
Nicotinic receptors

ABSTRACT

Cognitive impairment is common in patients with chronic kidney disease. Brain nicotinic acetylcholine receptors modulate cognitive functions, such as learning and memory. Pharmacological cholinergic enhancement is useful in patients with cognitive dysfunction. The major nicotinic acetylcholine receptor subtypes in the brain are heteromeric α4β2 and homomeric α7 receptors. To study the involvement of neuronal acetylcholine receptors in cognitive impairment in uremic rats, bilateral nephrectomy was performed. 24 weeks after nephrectomy, memory was assessed using the one trial step-down inhibitory avoidance test. Neuronal nicotinic acetylcholine receptors in the brain were studied by radioligand binding, immunoprecipitation, Western blot and sucrose gradient experiments. We demonstrated that rats with severe renal failure show disorders of short term memory. Long term memory was not altered in these rats. The number of functional α4β2 heteromeric neuronal nicotinic receptors was decreased in the brains of rats with severe renal failure. There was a significant correlation between the degree of renal impairment and the number of heteromeric nicotinic acetylcholine receptors in the brain. The down-regulation of functional α4β2 receptors in the brains of rats with severe renal failure was not due to a reduction of α4 or β2 subunit proteins. The number of α7 homomeric neuronal nicotinic acetylcholine receptors was not altered. These findings may have important clinical significance for the management of cognitive impairment in patients with chronic kidney disease.

© 2012 Elsevier Inc. All rights reserved.

Introduction

Neuronal nicotinic receptors (nAChRs) are a heterologous class of cationic channels present throughout the central nervous system. nAChRs are involved in cognitive functions, such as learning and memory, attention and executive function, both in humans and animals (Levin et al., 2006). It has been shown that nicotinic agonists may improve cognitive symptoms in conditions such as Alzheimer's disease, age-associated memory impairment, and Parkinson's disease (Levin et al., 2006).

nAChRs are classified on the basis of selectivity for the snake toxin, α-bungarotoxin (α-Bgtx). The nAChRs that do not bind α-Bgtx are formed from combinations of either α2, α3, α4 or α6 agonist-binding subunits along with β2, β3, β4 and/or α5 structural subunits (Millar and Harkness, 2008). They bind the alkaloid epibatidine (EB) with high affinity (Gerzanich et al., 1995). α7 and α8 subunits bind α-Bgtx and usually form homopentamers (Millar and Harkness, 2008). As for α7 and α8, the α9 subunit is able to form homopentamers that bind α-Bgtx, but it is usually co-assembled with α10 subunits in native nAChRs (Millar and Harkness, 2008). The most important nAChRs subtypes in the brain are heteromeric α4β2 heteromeric receptors and α7 homomeric receptors.

Distinct nAChRs modulate different cognitive functions. β2* nAChRs modulate executive functions and exploratory behaviour in rodents (Granon et al., 2003; Maskos et al., 2005). α4β2 and α7 nAChRs are involved in working memory (Levin et al., 2006; Picciotto et al., 1995). Hippocampal α7 but not β2* nAChRs modulate spatial memory (Picciotto et al., 1995; Ren et al., 2007). α4β2 nAChRs regulate associative memory (Marti-Barros et al., 2004; Picciotto et al., 1995).

Moderate to severe cognitive impairment is highly prevalent in chronic kidney disease (CKD) and end stage renal disease (ESRD) patients (Griva et al., 2010; Khatri et al., 2009; Murray et al., 2006). Cognitive domains relevant for daily function, as executive function, memory and language, are deteriorated in these patients. Indeed, cognitive impairment is associated with increased mortality of dialysis patients (Griva et al., 2010). Nevertheless, presently there is no treatment of cognitive impairment in CKD and ESRD patients.

Abbreviations: α-Bgtx, α-bungarotoxin; Bmax, density of binding sites; CKD, chronic kidney disease; EB, epibatidine; ERAD, endoplasmic reticulum associated degradation; ESRD, end stage renal disease; IA, inhibitory avoidance; LTM, long-term memory; nAChRs, neuronal nicotinic receptors; RF, renal failure; STM, short-term memory.

* Corresponding author. Fax: +34 965919561.
E-mail address: jj.ballesta@umh.es (J.J. Ballesta).

0014-4886/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2012.03.020

The aim of the present work was to assess if there are any changes in brain nAChRs associated with cognitive impairment in nephrectomized uremic rats.

Materials and methods

Abdominal surgery

All procedures were approved by the Institutional Animal Care and Use Committee. Male Wistar rats (Harlan, Barcelona, Spain) weighting about 250 g were used. 4/5 nephrectomy was performed in the moderate renal failure (RF) group. (Okada et al., 2005). 5/6 nephrectomy was performed in the severe RF group (Schaeffer et al., 2001). In control animals, sham procedures were performed. Each animal was initially anesthetized with 5% isofluorane. Anesthesia was then maintained with 1.5–2% isofluorane in a 60–70% O_2/air mixture.

Behavioural procedures

24 weeks after nephrectomy, memory was assessed using the one trial step-down inhibitory avoidance (IA) test (Bernabeu et al., 1997). Rats were placed on a platform (2.5 cm-high, 8 cm-wide and 25 cm-long) on the left end of a plexiglas cage (50 cm × 25 cm × 25 cm) with a series of metallic bars which constituted the floor of the cage. In the training sessions, immediately upon stepping down putting their four paws on the grid, the rats received a 0.4 mA scrambled footshock for 2 s. At 1 h (short-term memory, STM) and 24 h (long-term memory, LTM) after training, the animals were tested again, but the footshock was omitted and step-down latencies (to a ceiling of 3 min) were measured.

Binding of [^{3}H]-EB and [^{125}I]-α-Bgtx

25 weeks after nephrectomy, rat brain microsomal fraction was prepared (Whiting and Lindstrom, 1986). The binding of [^{3}H]-EB (0.025–2.5 nM) (Perkin Elmer, Boston, MA, USA) to the microsomal brain membranes was performed as described by Houghtling et al. (1995). Non-specific binding was determined in the presence of 0.5 μM unlabelled EB. Saturation of [^{125}I]-α-Bgtx (0.05–6 nM) (Perkin Elmer, Boston, MA, USA) was performed as before. To reduce non-specific binding, [^{125}I]-α-Bgtx incubation buffer contained 10% BSA and filters were presoaked in 2% polyethyleneimine. Non-specific binding was determined in the presence of 0.5 mM nicotine. Binding data were fitted to a one-binding site saturation model using GraphPad Prism 5 software (Graphpad Software, La Jolla, CA, USA).

Immunoprecipitation of nAChRs

25 weeks after nephrectomy, solubilized rat brain microsomal fraction (Whiting and Lindstrom, 1986) was used to perform immunoprecipitation experiments (Vicente-Agullo et al., 2001). Extracts were incubated with 2.5 nM [^{3}H]-EB and immunoprecipitated with either anti-α4 subunit (1:1000, mAb 299, Sigma, USA), anti-β2 subunit (1:1000, mAb 270, Sigma, USA) or anti-α3 subunit antibody (1:1000, mAb 313, Sigma, USA), or with 5 nM [^{125}I]-α-Bgtx and immunoprecipitated with anti-α7 subunit antibody (1:1000, mAb 319, Sigma, USA).

Western blots

25 weeks after nephrectomy, the prefrontal cortex, enthorinal cortex, hippocampus and amygdala were dissected and homogenized and Western blots were performed (Berbel et al., 2010). Primary antibodies used were mAb 299 (1:20,000), mAb 270 (1:20,000) and mAb 319 (1:20,000). After washing, the membranes were incubated with goat anti-rat IgG-HRP (1:2000, Santa Cruz, USA). The bands were detected using ECL-Plus Western blotting detection reagents (Amersham Pharmacia Biotech Ltd, Barcelona, Spain). Bands were digitalized and quantified using an LAS-1000 Bioimager (Fujifilm Co., Barcelona, Spain) and data analysis was performed using the Image Gauge 4.0 software (Fujifilm Co.).

Sucrose gradients

Sucrose gradient analysis of α4β2 AChRs was performed (Alves et al., 2011). Solubilized brain microsomal fractions were layered onto 11 ml sucrose gradients (5–20%). After sedimentation of nAChRs, 300 μl-aliquots were collected and incubated with 2.5 nM [^{3}H]-EB and mAb 299 (1:1000) and the radioactivity was measured. Control gradients with purified *Torpedo* nAChRs were run in parallel.

Statistical analysis

Nonlinear mixed models (Davidian and Giltinan, 2003) were used to compare [^{3}H]-EB and [^{125}I]-α-Bgtx saturation data from control and nephrectomized rat brains using the library nlmn from the R 2.8.0 software. As a ceiling of 3 min was used in behavioural experiments, nonparametric statistics was used. Data were expressed as median and interquartile intervals test-session latency in the step-down inhibitory avoidance task. Kruskal–Wallis analyses of variance were followed by Mann Whitney *U* test when appropriate. In all other cases data were expressed as means ± S.E.M. and the Student's *t*-test for unpaired data was used when appropriate. A $p<0.05$ was considered to be statistically significant.

Results

Biological parameters

Before surgery, serum creatinine was similar in all groups of rats. 25 weeks after nephrectomy, serum creatinine was significantly increased in nephrectomized as compared to control rats, demonstrating that the procedures of nephron mass reduction used here led to a significant renal injury (sham: 0.38 ± 0.01 mg/dL, moderate RF: 0.64 ± 0.02 mg/dL, severe RF: 1.19 ± 0.07 mg/dL, $p<0.0001$ sham vs moderate RF, sham vs severe RF and moderate RF vs severe RF). Indeed, a significant negative correlation was observed between the percentage of renal mass remaining after surgery and the levels of serum creatinine 25 weeks after the surgery in the group of severe RF ($r=-0.45$; $p<0.01$). In contrast, no significant correlation was observed in the moderate RF group ($r=-0.06$; $p>0.05$).

Cognitive function

Memory was assessed using the one trial step-down IA test, because it discriminates between STM and LTM. IA is a form of learning that engages several sensorial stimuli, sensitivity to pain and emotional, fear driven components. Kruskal–Wallis analysis of the IA acquisition trial data revealed no significant differences among groups (Fig. 1A). One hour after the IA acquisition trial step-through latencies were significantly reduced in severe RF rats as compared to the control group (Fig. 1B). In contrast, comparisons of step-through latencies between control and moderate RF rats showed no significant differences (Fig. 1B). Thus, STM is impaired in uremic rats with severe RF. Twenty four hours after the IA acquisition trial step-through latencies were not different between groups (Fig. 1C). Thus, LTM is not altered in uremic rats with either moderate or severe RF.

Download English Version:

https://daneshyari.com/en/article/3055662

Download Persian Version:

https://daneshyari.com/article/3055662

Daneshyari.com