Chemico-Biological Interactions 186 (2010) 9–15

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Gender-related differences in circadian rhythm of rat plasma acetyl- and butyrylcholinesterase: Effects of sex hormone withdrawal☆

Gracielle Alves-Amaral, Marcelo Pires-Oliveira, Ana Luiza Andrade-Lopes, Tiago Chiavegatti, Rosely Oliveira Godinho*

Department of Pharmacology (INFAR), Universidade Federal de São Paulo, 04044-020 São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 12 January 2010
Received in revised form 14 March 2010
Accepted 2 April 2010
Available online 5 May 2010

Keywords:
AChE, acetylcholinesterase
AP-1, activator protein-1
ASCh, acetylthiocholine
BuChE, butyrylcholinesterase
BuSCh, butyrylthiocholine
DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid)
EDTA, ethylenediaminetetraacetic acid
ERE, estrogen responsive element
Iso-OMPA, tetraisopropyl pyrophosphoramide
Oct-1, octamer-motif-binding factor 1
PEA-3, polyoma enhancer activator 3

Keywords:
Acetylcholinesterase
Butyrylcholinesterase
Sex hormones
Circadian cycle
Blood

ABSTRACT

The role of acetylcholinesterase (AChE) in the termination of the cholinergic response through acetylcholine (ACh) hydrolysis and the involvement of plasma butyrylcholinesterase (BuChE), mainly of hepatic origin, in the metabolism of xenobiotics with ester bonds is well known. Besides, BuChE has a crucial role in ACh hydrolysis, especially when selective anticholinesterases inhibit AChE. Herein, we analyzed the gender-related differences and the circadian changes of rat plasma cholinesterases. Plasma and liver cholinesterase activities were evaluated in control or 2–30-day castrated adult male and female rats. Plasma and liver AChE activities did not differ between genders and were not influenced by sex hormone deprivation. BuChE plasma activity was 7 times greater in female, reflecting gender differences in liver enzyme expression. Castration increased liver and plasma BuChE activity in male, while reduced it in female, abolishing gender differences in enzyme activity. Interestingly, female AChE and BuChE plasma activities varied throughout the day, reaching values 27% and 42% lower, respectively, between 2 p.m. and 6 p.m. when compared to the morning peaks at 8 a.m. Castration attenuated daily female BuChE oscillation. On the other hand, male plasma enzymes remained constant throughout the day. In summary, our results show that liver and plasma BuChE, but not AChE, expression is influenced by sex hormones, leading to high levels of blood BuChE in females. The fluctuation of female plasma BuChE during the day should be taken into account to adjust the bioavailability and the therapeutic effects of cholinesterase inhibitors used in cholinergic-based conditions such Alzheimer's disease.

1. Introduction

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are members of the cholinesterase family, which catalyze the hydrolysis of choline esters with different substrate specificities. Although the essential role of AChE in termination of acetylcholine signaling at central and peripheral synapses is well recognized, the physiological significance of high amounts of AChE and BuChE that are found in plasma [1] remains unclear.

Blood BuChE catalyses the hydrolysis of a wide variety of choline and non-choline esters and toxins [2], and it is clinically important because it metabolizes the short-acting muscle relaxant succinylcholine as well as ester-type local anesthetics and cocaine [3–6]. Some patients have an 'atypical' variant of BuChE with reduced catalytic activity, which explains prolonged apnea after administration of usual therapeutic doses of succinylcholine [3]. However, the relevance of BuChE has gained a new dimension because of its ability to hydrolyze ACh. Indeed, BuChE activity is responsible for the survival of AChE knockout mice that lack AChE activity [7], demonstrating that BuChE can totally replace AChE in ACh hydrolysis. In fact, past as well as recent studies suggest that AChE and

☆ *Financial support*: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP); R.O.G project grant 05/59006-1, Brazil; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil; Conselho de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES), Brazil.

* Corresponding author at: Department of Pharmacology, Universidade Federal de São Paulo, Rua Três de Maio, 100, 3rd floor, 04044-020 São Paulo, SP, Brazil. Tel.: +55 11 55764447; fax: +55 11 50813849.
E-mail address: godinho@unifesp.br (R.O. Godinho).

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doi:10.1016/j.cbi.2010.04.002

BuChE may exhibit additional functions; for review see [8–11]. For example, both AChE and BuChE exhibit intrinsic aryl acylamidase activity [12–15] which might be involved in degradation of melatonin [16]. Besides, plasma BuChE progressively increases in patients with Alzheimer's disease [17] and in experimental models of diabetes, hypertension, insulin resistance and hyperlipidemia [18,19] for review see [20]. Interestingly, the incidence, prevalence, and progression of these pathologies are influenced by gender and sex hormones [21].

Trophic influences of gender and sex hormones on central and peripheral cholinergic synapses, especially with regard to AChE activity, are well known [22–26]. On the other hand, BuChE activity in plasma and liver is higher in female than male adult rats [27] and it appears to be much more sensitive to hormonal factors such as glucocorticoids than AChE [28]. Taking into account the hepatic source of blood BuChE and the high sensitivity of liver to sex hormones [29], in the present study we evaluated the gender-related differences and the influence of castration over hepatic and plasma cholinesterases of male and female adult rats.

2. Materials and methods

2.1. Animals and sample preparation

Adult female and male Wistar rats from the institutional animal care facility were maintained on a 12/12 h light–dark cycle, with food and water ad lib. Vaginal smears were taken for histological determination of estrous cycle in female rats [30] for 5 days, between 8:00 a.m. and 10:00 a.m. Female rats were divided into 3 groups: controls in proestrus, and females castrated either for 15 (C15) or 30 days (C30). Male rats were divided into 5 groups: controls and rats castrated for 2 (C2), 7 (C7), 15 (C15) or 30 days (C30). At 120 days of age, animals were anesthetized between 2:00 p.m. and 4:00 p.m., aortic arterial blood was collected and liver, uterus and levator ani (LA) muscle were removed after aortic exsanguination. Controls were non-operated, age- and gender-matched rats, since preliminary data showed that plasma AChE and BuChE activities did not differ between those and sham-castrated female and male rats.

Blood samples (1 ml) were collected in vials containing 30 μl of 0.1 M EDTA. After centrifugation (3000 × *g*, 4 °C) for 20 min, plasma was collected and kept at −20 °C until the determination of cholinesterase activity. Livers were kept at −70 °C and then homogenized (30–100 ml/mg of tissue) in 20 mM borate buffer, pH 9.0, containing 1 M NaCl, 5 mM EDTA, 0.5% Triton X-100, 5 mM N-ethylmaleimide, 2 mM benzamidine and 0.7 mM bacitracin for cholinesterase extraction [31]. Homogenates were centrifuged (15,000 × *g*, 4 °C) for 30 min and supernatants were maintained at −70 °C until cholinesterase activity determination.

The wet weights of uterus and levator ani muscle, tissues highly sensitive to changes in plasma estrogen [32] and androgens [33], were used as index of female estrogens and male androgens plasma levels, respectively. All experimental procedures were approved by the institutional ethics committee (protocol #0688/2006).

2.2. Daily rhythm in cholinesterase activity

Circadian cycles of plasma AChE and BuChE activity were evaluated in control or C15 female and male rats. Animals were anesthetized and 100 μl of blood from the caudal vein were collected in 2 h intervals for 24 h, in vials containing 200 μl PBS, pH 7.4, with 10 μl of 100 IU heparin and then centrifuged (3000 × *g*, 4 °C) for 20 min. Plasma was collected and kept at −20 °C until the determination of cholinesterase activity. Harvesting was performed during the dark period (06:00 p.m. to 06:00 a.m.), under focal illumination of the tail with a dim red light source.

2.3. Determination of AChE and BuChE activities

Enzymatic assays were performed using an adaptation of the colorimetric method [34], as described by Pereira et al. [25]. Enzyme activity of AChE and BuChE was measured in plasma diluted 1:5 in 0.1 M sodium phosphate buffer which allowed the measurement of linear formation of product for at least 90 min, at 3 min intervals. Plasma cholinesterase activity was detected using as substrate butyrylthiocholine (BuSCh) or acetylthiocholine (ASCh). Briefly, samples (20 μl) were incubated with 3.2 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and excess of substrate (2.4 mM) in 0.1 mM phosphate buffer, pH 8.0 at 30 °C.

Initially, we evaluated the effects of selective inhibitors of BuChE (Iso-OMPA; 0.1–100 μM) and AChE (BW284c51; 0.1–100 μM) on ASCh and BuSCh hydrolysis in plasma samples. Iso-OMPA partially inhibited the hydrolysis of ASCh by up to 35% at ≥1 μM, indicating that plasma BuChE significantly contributes to this reaction. At same range of doses, Iso-OMPA completely inhibited hydrolysis of BuSCh. BW 284 C 51 (0.1–100 μM) reduced by 50% the hydrolysis of ASCh but did not change the hydrolysis of BuSCh. Since it was found that plasma ASCh is hydrolyzed by either plasma BuChE or AChE, whereas BuSCh is selective degraded by BuChE, BuChE activity was determined using butyrylthiocholine (BuSCh) as substrate, whereas AChE activity was evaluated using acetylthiocholine (ASCh), in the presence of the selective inhibitor of BuChE, tetraisopropylpyrophosphoramide (Iso-OMPA, 10 μM).

Kinetic parameters (K_m and V_{max}) were determined using a substrate range from 0.01 mM to 2.0 mM. Synthesis of the reaction product was followed in a microplate reader (BioTek FL600, BioTek Instruments, Inc, Winooski, VT, USA) at 405 nm for up to 150 min. Velocity of the reaction for each sample was determined in triplicate and expressed as arbitrary units (AU) per min per ml of plasma or per mg of tissue. All drugs and reagents were purchased from Sigma Chemical Co, St. Louis, MO, USA, unless otherwise noted.

2.4. Statistical analysis

Data were presented as mean ± S.E.M. Statistical significance was tested by Student's *t*-test or one-way ANOVA with Newman–Keuls post hoc test, using GraphPad Prism® for Windows 5.01. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Gender differences and effect of castration on body and organ weight

Mean body weight of control male rats (360.5 ± 9.4 g; Fig. 1c) was significantly greater than that of female animals (221.4 ± 3.9 g, Fig. 1a) and EDL weight (115.5 ± 2.8 mg) was proportionally higher in male than in female rats (74.5 ± 5.7 mg). Uterus wet weight decreased by 74% and 81% in C15 and C30 female rats, respectively (female controls = 423.3 ± 45.3 mg; Fig. 1b). LA wet weight also decreased progressively following castration of male rats, reaching 51% of control values (305.4 ± 18.2 mg; Fig. 1d) after 30 days.

3.2. Effect of castration on plasma AChE and BuChE activity of female and male rats

Fig. 2 shows plasma AChE and BuChE activity profile of male and female rats. AChE activity was not significantly different between genders (female = 10109.2 ± 268.6 AU/min/ml of plasma, $n = 9$; male = 9079.7 ± 480.7 AU/min/ml of plasma, $n = 7$). On the other hand, female BuChE activity (15720.0 ± 774.2 AU/min/ml of plasma, $n = 16$) was 6.2 times higher than male enzyme activity (2178.0 ± 120.0 AU/min/ml of plasma, $n = 14$).