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Aortic butyrylcholinesterase is reduced in spontaneously hypertensive rats

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Short title:

Cholinesterases in rat aorta

Summary:

Despite the fact that vessels have sparse cholinergic innervation, acetylcholine (ACh), the primary neurotransmitter of parasympathetic nervous system, has been commonly used in physiological experiments to assess vascular function. ACh is hydrolysed by two cholinesterases (ChE), namely acetylcholinesterase and butyrylcholinesterase (BChE). However, little is known about these enzymes in blood vessels. The aim of the project was to characterize the expression and activity of ChE in rat aorta. As the effect of ACh on vascular tone depends on the presence of endothelium, Wistar rats were used as a model with intact endothelium and spontaneously hypertensive rats as a model of impaired endothelial function. Relative expression of both ChE in different parts of the aorta were determined using RT-qPCR. Enzyme activities were assessed in tissue homogenates by Ellman's assay. Here we showed that both ChE are present in each part of rat aorta, while mRNA is more abundant for BChE than for AChE, irrespective of aortic compartment or genotype. Normotensive Wistar rats possess higher aortic mRNA expression and activity of BChE compared to SHR.. We concluded that BChE is the dominant type of ChE in rat aorta and it might play an important role in the regulation of vascular tone.

Key words:

Rat aorta • Acetylcholinesterase • Butyrylcholinesterase • Spontaneously hypertensive rats

Introduction: Acetylcholine (ACh) is frequently used to assess endothelial function of isolated vessels. ACh increases arterial pressure during normal vascular tone, whereas the opposite is observed during increased vascular tone (Furchgott 1991; Norel et al. 1996). Notably, ACh has the ability to relax a precontracted aorta, provided an intact endothelium (Furchgott 1991; Norel et al. 1996; Félétou and Vanhoutte 2006). Despite routine use of ACh in vascular physiological experiments, little is known about its degradation enzymes in blood vessels, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).

Here, we address this knowledge gap by examining the mRNA expression and activity of both enzymes, AChE and BChE, in three aortic segments: *arcus aortae*, *aorta thoracalis* and *aorta abdominali*s. Additionally, we compared healthy vessels from Wistar rats to the corresponding regions of the vessels from spontaneously hypertensive rats (SHR), which we and others (Félétou and Vanhoutte 2006) have observed to display differential responses to ACh.

Methods: Male Wistar rats, a model of the intact endothelium (n = 6) and SHR, a model with impaired endothelium (n = 6), 12 weeks of age were euthanized by CO_2 and the aorta was dissected into three parts (*arcus aortae, aorta thoracalis* and *aorta abdominalis*), frozen immediately in liquid nitrogen and stored at –80°C. All experiments were approved by the State Veterinary and Food Administration of the Slovak Republic. The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies (Tveden-Nyborg, Bergmann, and Lykkesfeldt 2018).

Tissue extractions and activity Ellman's assays were performed as described before (Dingova et al. 2014), using an equal amount of protein from each part of the aorta quantified by Pierce™ BCA Protein Assay. As substrates, 1 mM acetylthiocholine (for both ChE) or 1 mM butyrylthiocholine (selective for BChE) were used. For AChE activity, the samples were preincubated with the BChE inhibitor, tetraisopropyl-pyrophosphoramide (1 mM), for 30 min prior to substrate. BChE activity was determined in the presence of butyrylthiocholine. Enzyme activity was detected at 30 s intervals for 30 min and expressed as ΔO.D./min (Dingova et al. 2014).

Total RNA was isolated from intact aorta samples with TRI Reagent (Sigma Aldrich) and 335 ng was used for RT-qPCR employing primers and reaction conditions as described before (Kilianova et al. 2020, Targosova et al. 2021). Gene expression was normalized to Actb and Hprt1 and relative expression values were normalized to rat brain due to high expression of AChE and BChE. Amplification efficiencies were determined for each primer pair using the software LinRegPCR according to the methods of Ruijter (Ruijter et al. 2009). The amplification efficiency values for AChE and BChE were 1.864 and 1.809, respectively.

Two-factor ANOVAs (rat model x aorta segment) with Tukey's post-hoc test was used. We computed effect sizes (Cohen's f) for each ANOVA to ensure that significant main effects and interaction effects exceeded the minimum effect size determined by a sensitivity power analysis.

Results: The mRNA expression of both ChEs was detected in each segment of the aorta of both animal models (Fig 1). Surprisingly, BChE is more abundant than AChE in all aortic segments, ranging from a 240- to 425-fold increase in Wistar rats and a 140- to 220-fold increase in SHR. AChE relative expression revealed differences between aorta segments ($F_{2,22} = 14.21$, $p = 0.0001$). The main effect of rat model ($F_{1,22} = 2.777$, $p = 0.109$) and the interaction ($F_{2,22} = 0.837$, $p = 0.446$) were not significant. Relative expression of AChE in *arcus aortae* was higher in comparison to the other segments in Wistar rats (Fig 1a). For BChE, we observed main effects for both aorta segments ($F_{2,22} = 21.1$, $p < 0.0001$) and rat model ($F_{1,22} = 12.93$, $p < 0.01$), although the interaction was not significant ($F_{2,22} = 1.229$, $p = 0.312$). The relative expression of BChE was lower in *aorta thoracalis* than *arcus aortae* and *aorta abdominalis* in both animal models (*arcus aortae vs. aorta thoracalis* in SHR showing trend) (Fig 1b). Overall, the expression of BChE is lower in the aorta of SHR regardless of the anatomical division.

Both ChE activities were detected in all segments of aorta in both studied models, with BChE activity markedly exceeding AChE activity (Fig 2). Both ChE activities revealed significant main effects for aorta segment (AChE: $F_{2,28} = 80.50$, $p < 0.0001$; BChE: $F_{2,29} = 174.0$, $p < 0.0001$), rat model (AChE: $F_{1,28} = 23.14$, $p < 0.0001$; BChE: $F_{1,29} = 132.8$, $p < 0.0001$) and the interaction (AChE: $F_{2,28} = 10.04$, $p = 0.0005$; BChE: $F_{2,29} = 46.24$, $p < 0.0001$). Activity of AChE in Wistar rats decreased in the following order: *arcus aortae* > *aorta abdominalis* > *aorta thoracalis* (Fig 2a). AChE in SHR aorta segments were comparable to Wistar, with the exception of *aorta abdominalis*, where AChE activity was increased in SHR. BChE activity in *arcus aortae* was roughly twice the activity observed in *aorta thoracalis* and *aorta abdominalis* in Wistar rats (Fig 2b). Similar to mRNA, BChE activity was decreased in *arcus aortae* and *aorta thoracalis* of the SHR in comparison to Wistar.

Discussion: BChE activity exceeds that of AChE in numerous tissues (Li et al. 2002), supporting our finding that BChE is the dominant ChE in the rat aorta. The physiological importance of high BChE activity, however, remains uncertain. Vascular BChE may compensate for low BChE activity in rat plasma, compared to other species (Li et al. 2002. Indeed, in contrast to rats, where we detected low AChE and substantial BChE activities, the *aorta thoracalis* of mice showed very low levels of both AChE and BChE activities (data not shown).

The presence of muscarinic receptors in the aorta suggests that the primary function of vascular ChE is to regulate local ACh to prevent over-excitation of muscarinic receptors. The origin of ACh that would affect vascular ACh receptors is ambiguous. Aortic BChE may buffer the vessel from ACh arising from the blood, produced for example by T-cells (Kawashima et al. 2015). The more distant neuronal or non-neuronal sources are, despite a low rat plasma BChE activity, unlikely due to a rather high plasma AChE activity (Li et al. 2002). Aortic BChE may also degrade spilled-over ACh (Nervo et al. 2019) from the heart, which would explain our observation that the highest ChE activities reside in the *arcus aortae*, the aortic region proximal to the heart. Nevertheless, “cholinergic contamination” arising from parasympathetic innervation at the base of the heart is plausible.

Another source of ACh that could serve as a substrate for vascular BChE could be the vessel itself. Recently, the existence of many non-neuronal cholinergic tissues were described, playing important roles in physiological and pathological processes (Beckmann and Lips 2013). The action of non-neuronal ACh is usually autocrine/paracrine (Wessler and Kirkpatrick 2008). We speculate that the aorta may represent another non-neuronal cholinergic tissue, although additional experiments examining the presence of enzymes and transporters necessary for local ACh synthesis, release, and degradation in the aorta are required.

Hydrolysis of ACh may not be the sole role of aortic BChE, considering its broad substrate specificity, ability to cleave bulkier substrates, and relationship to lipid metabolism (Lockridge 2015; Chen et al. 2016; Santarpia et al. 2013, Hrabovska et al. 2006, Dingova et al. 2016). Moreover, there is clinical evidence of a strong correlation between plasma BChE activity and triacylglycerol and LDL cholesterol levels (Iwasaki et al. 2007), markers associated with risk of endothelial dysfunction and subsequent atherosclerosis. Thus, the presence of BChE in blood vessels could function as a detoxification-defence mechanism to prevent deposition of harmful substances as well as lipid components in the blood vessels and consequently the development of atherosclerosis.

SHR are characterized by progressive deterioration of the anatomical, morphological, and physiological characteristics of vessels. Moreover, SHR develop endothelial dysfunction and subsequently the vasodilatory response to ACh is modified (Félétou and Vanhoutte 2006). Our results confirmed an impaired cholinergic system in SHR aorta. While SHR had normal aortic AChE mRNA levels and activity, BChE mRNA levels and activity were reduced, suggesting involvement of BChE in the regulation of ACh signalling. Additionally, participation of BChE in vascular pathogenesis is supported by the aforementioned non-cholinergic catalytic properties and clinical evidence. Endothelial dysfunction is associated with essential hypertension and cardiovascular risk factors such as aging, hypercholesterolemia and diabetes mellitus (Hadi, Carr, and Al Suwaidi 2005). These conditions are linked to ChE, primarily BChE levels in related tissues (Iwasaki et al. 2007). Endothelial dysfunction, also characterized by reduced bioavailability of NO (Bonetti, Lerman, and Lerman 2003), increases oxidants and the expression of adhesion molecules involved in the initiation and progression of atherosclerotic plaque formation and the incidence of cardiovascular events (Félétou and Vanhoutte 2006). We hypothesize that vascular BChE acts as a detoxifying enzyme for these molecules and is involved in the prevention of atherosclerosis. This is consistent with the fact that plasma BChE activities correlate with higher LDL and triacylglycerol levels, known risk factors for atherosclerosis.

Our results reveal that BChE is the dominant ChE in the rat aorta, with the highest activity in the *arcus aortae*. BChE levels are lower in the aorta of SHR. We assume that aortic BChE is responsible for the regulation of ACh signalling and participates in angioprotection. Future studies should use selective AChE/BChE inhibitors to delineate the roles of each ChE in vasodilatory responses to ACh in compartments of the aorta.

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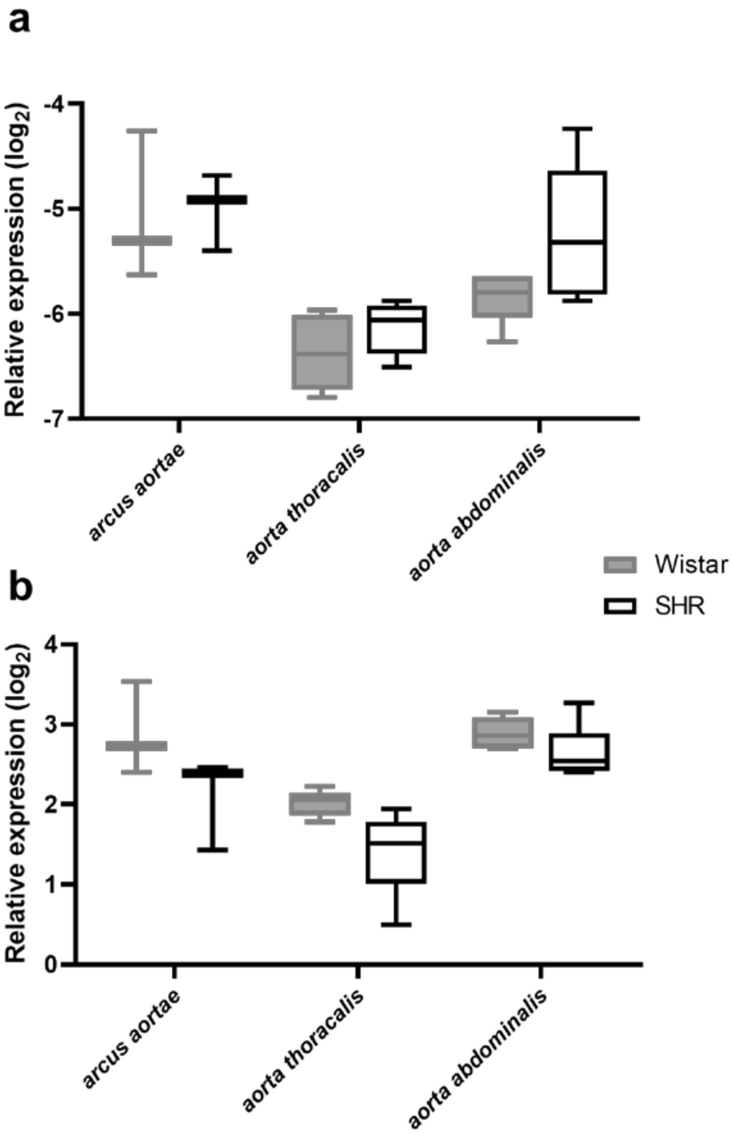

Fig. 1. Relative expression of AChE in different segments of rat aorta assessed by RT-qPCR:

Box plots show the relative expression of AChE (a) and BChE (b) in different segments of rat aorta. Boxes extend from the 25th to 75th percentiles with the line representing the median and the whiskers displaying the minimum and maximum values. Individual values are normalized to brain expression levels (values set to 0 on log_2 scale). The number of animals used for mRNA extraction and RT-qPCR in each group is: *arcus aortae* (n = 3), *aorta thoracalis* (n = 5), *aorta abdominalis* (n = 6). $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***).

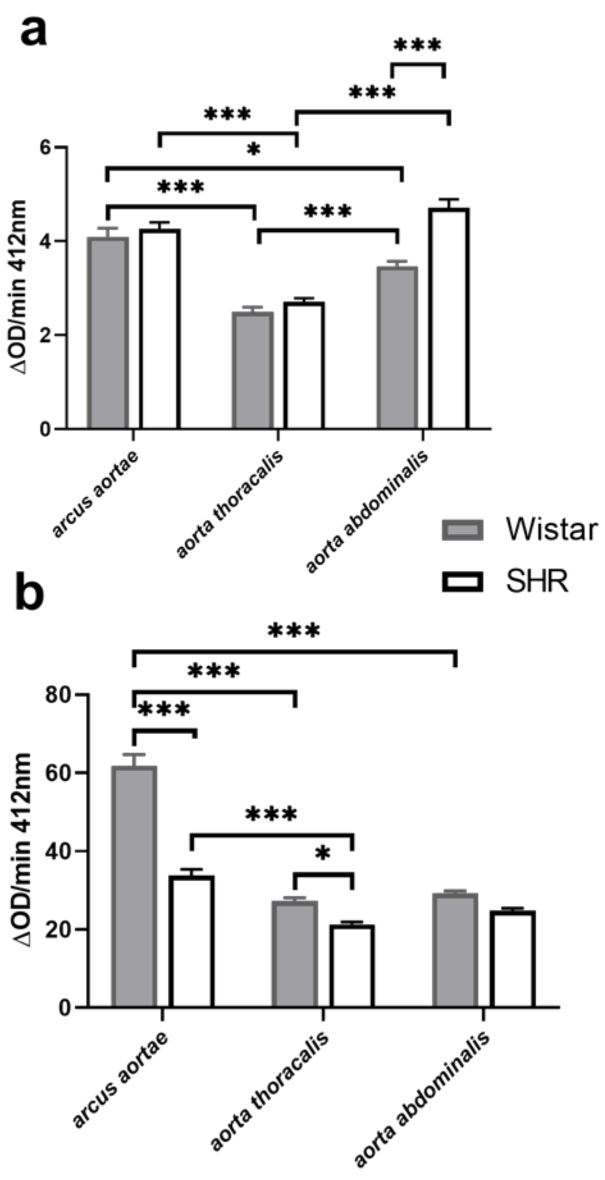

Fig. 2. AChE and BChE activity in different segments of rat aorta assessed by Ellman's assay:

Bar graphs depict enzyme activity of AChE (a) and BChE (b) in different segments of rat aorta. Data are expressed as mean + SEM. The number of animals used for Ellman's assay in each group is: *arcus aortae* (n = 6), *aorta thoracalis* (n = 6), *aorta abdominalis* (n = 6). $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***).