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Adrenal α_2-adrenergic receptors in the aging normotensive and spontaneously hypertensive rat

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Abstract

This study investigates α_2-adrenergic receptor (α_2AR) mediated feedback inhibition of catecholamine release from the adrenal medulla of adult (52 weeks) and old (98 weeks) spontaneously hypertensive rats (SHR) and normotensive controls Wistar Kyoto (WKY) rats. Adrenal epinephrine content as well as the spontaneous and the nicotinic-evoked release of epinephrine were similar between adult SHR and WKY rats. Aging produced a significant reduction in epinephrine synthesis in WKY rats. In contrast, in SHR aging produced a significant increase in epinephrine release without significant changes in epinephrine synthesis. The α_2AR agonist medetomidine abolished (80–90% inhibition) the nicotinic-evoked release of epinephrine in adult SHR and WKY rats. With aging, this effect was unaltered in WKY rats but was significantly decreased in SHR (30% inhibition). Adrenal α_{2A}AR mRNA levels were significantly reduced in old SHR compared with age matched WKY rats. In conclusion, in aging the α_2AR mediated feedback inhibition of epinephrine release from the adrenal medulla is preserved in WKY rats but compromised in SHR, resulting in increased epinephrine release.

Keywords: Aging; Alpha2-adrenergic receptor; Adrenal medulla; Epinephrine; Spontaneously hypertensive rat; Nicotinic receptors

1. Introduction

The sympathetic nervous system (SNS) plays a critical role in the maintenance of physiological homeostasis in general and arterial blood pressure in particular. The catecholamines norepinephrine and epinephrine, play an important role in the sympathetic control of arterial blood pressure and cardiac function (Westfall and Westfall, 2006). Circulating epinephrine and norepinephrine derive from two major sources in the whole organism: the sympathetic nerve-endings, which release norepinephrine on effector organs upon stimulation, and the chromaffin cells of the adrenal medulla, which synthesize, store and release epinephrine and norepinephrine on acetylcholine stimulation of nicotinic cholinergic receptors (Young and Landsberg, 1998). Both norepinephrine and epinephrine act at G protein–coupled receptors of the adrenergic receptor family, comprising α_1, α_2, or β, to mediate sympathetic effects. Molecular cloning has led to the identification of three α_2-adrenergic receptors (α_2AR) subtypes: α_{2A}, α_{2B}, and α_{2C} (Bylund et al., 1994). Presynaptic α_2ARs are known to play a critical role in regulating norepinephrine release and synthesis from sympathetic nerve terminals by a negative feedback mechanism (Gilsbach et al., 2009; Vieira-Coelho et al., 2009). Moreover, α_2ARs are solely responsible for autocrine feedback inhibition of norepinephrine and epinephrine secretion from cromaffin cells of the adrenal medulla (Knaus et al., 2007).

Aging is associated with a variety of changes in cardiovascular function, at the level of the cardiovascular autacoid receptors, at post receptor levels, in neuromuscular function, in circulating levels of autacoids, and in autonomic reflexes (Docherty, 2002), leading to an increased incidence of cardiovascular disorders, including hypertension and heart failure (Esler et al., 2002). The effect of aging on

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presynaptic α_2ARs has been studied in detail, and most studies report a decreased responsiveness in aging (Docherty, 1990, 2002). Notably, essential hypertension and heart failure have also been shown to be accompanied by elevated sympathetic nervous activity both in man (Kaye et al., 1994; Schlaich et al., 2004) and in an animal model of hypertension the spontaneously hypertensive rat (SHR) (Okamoto and Aoki, 1963; Trippodo and Frohlich, 1981).

In contrast to the increased SNS activity, tonic epinephrine secretion from the adrenal medulla is markedly reduced with age in man (Kaye and Esler, 2008). However, the mechanism(s) contributing to the reduction in epinephrine secretion from the adrenal medulla with advancing age have not been investigated to date. The adrenal medulla in combination with the SNS has also been reported to play a role in the epigenesis of SHR hypertension (Borkowski and Quinn, 1983, 1985; Borkowski, 1991; Korner et al., 1993; Lee et al., 1991), although epinephrine plasma levels in SHR remain similar to normotensive rats before (6 weeks) and during development of high blood pressure (12 and 22 weeks of age) (Moura et al., 2005).

Despite the importance of adrenal α_2ARs in the control of epinephrine release and cardiovascular function, their role in the control of adrenal catecholamine release with age and in the SHR have not been previously tested. Therefore, in the present study we evaluated adrenal α_2ARs responsiveness in male adult (52 weeks) (Cabassi et al., 1996; Gomes et al., 2009) and old (98 weeks) (Boluyt et al., 1995; Loch et al., 2009; Maemura et al., 1982) SHR and Wistar Kyoto (WKY) rats.

2. Methods

2.1. Animals

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and the experiments were performed according to the Portuguese law on animal welfare. Male WKY rats and outbred SHR were obtained from Harlan Interfauna Ibérica (Barcelona, Spain) and left to age in our animal facilities. The animals were kept under controlled environmental conditions (12 : 12-h light/dark cycle and room temperature 22 ± 2 °C). All animals were fed ad libitum throughout the study with standard rat chow (PANLAB, Barcelona, Spain), containing 1.9 g/kg of sodium. Having reached the age of 52 and 98 weeks of age, rats were anesthetized with sodium pentobarbital (60 mg/kg ip). In the experiments carried out to evaluate the release of catecholamine from the adrenal medulla we proceeded as follows. The right and left adrenal glands were rapidly removed through an abdominal midline incision and immediately weighed. The right adrenal gland was placed in a modified Krebs–Henseleit solution of the following composition (mmol/L): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.57, disodium ethylenediaminetetraacetic acid 0.03, oxygenated with a mixture of 95% O_2 and 5% CO_2 in the presence of a monoamine oxidase inhibitor (pargyline, 100 μmol/L) and a catechol-*O*-methyltransferase inhibitor (tolcapone, 1 μmol/L). To evaluate mRNA or protein expression the left adrenal glands were rapidly removed, weighed and immediately frozen at −80 °C. For the catecholamine assay the left adrenal glands were weighed and immediately placed in 1 ml of 0.2 mol/L perchloric acid (PCA).

2.2. Norepinephrine and epinephrine release

The adrenal medullae were isolated from the gland and then placed in superfusion chambers, one per chamber, where they were superfused with Krebs–Henseleit solution at a rate of 0.5 ml/min, at 37 °C. After a 90-minute period of stabilization, successive 5-minute samples of the superfusate were collected into tubes containing 0.3 ml of PCA (2 mol/L), from t = 90 min to t = 150 min (t = 0 min being the start of superfusion). At the end of the experiments, the adrenal medullae were placed in 1 ml of PCA (0.2 mol/L) and catecholamines determined in superfusates and tissues. The effect of the nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) on catecholamine release was determined by addition a single concentration of DMPP (500 μmol/L) delivered at t = 125 min. The inhibitory effect of the α_2AR medetomidine (MED) on catecholamine release was determined by addition of a single concentration of MED (100 nmol/L) (K_i values in nM α_{2A}: 0.8 ± 0.1; α_{2B}: 3.8 ± 1.1; α_{2C}: 8.8 ± 3.2) (Jansson et al., 1994). When used, the α-adrenergic receptor antagonist rauwolscine (300 nmol/L) was present from t = 90 min until the end of nicotinic stimulation. The concentrations of DMPP, MED and rauwolscine were based on those used in previous studies designed to characterize adrenal α_2AR function in mice (Gilsbach et al., 2007; Moura et al., 2006). The spontaneous outflow of norepinephrine and epinephrine was calculated as a fraction of the norepinephrine or epinephrine tissue content at the onset of the respective collection period (fractional rate; min^{-1}), as a means of correcting for adrenal norepinephrine or epinephrine tissue content variability. The overflow elicited by nicotinic stimulation was calculated as the difference "total norepinephrine or epinephrine outflow during and after stimulation" minus "basal outflow", and was then expressed as a percentage of the norepinephrine or epinephrine content of the tissue at the onset of stimulation. Overflow ratios obtained after addition of a drug were also calculated as a percentage of the corresponding ratio in controls in which no drug was added.

2.3. Tyrosine hydroxylase activity and expression

Tyrosine hydroxylase (TH) activity was measured as described previously (Moura et al., 2005). In brief, the adrenal was homogenized in phosphate buffer solution (50 mmol/L, at pH = 7.0) containing 0.2% Triton x-100, the