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# Blood pressure variability provokes vascular β-adrenoceptor desensitization in rats

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ABSTRACT

Spontaneous variation in blood pressure is defined as 'blood pressure variability' (BPV). Sinoaortic denervation (SAD) is characterized by BPV without sustained hypertension. In the present study, we investigated whether BPV could be related to vascular β-adrenoceptor desensitization in rats. Three days after surgery (SAD and control), aortic rings were placed in an organ chamber and the relaxation stimulated by β-adrenoceptor agonists, isoprenaline, terbutaline, BRL37344 and cyanopindolol was verified. The participation of intracellular nucleotides signaling pathways was also verified using forskolin, sodium nitroprusside and acetylcholine to induce relaxation. The effects of BPV on the increase in endothelial cytosolic $Ca^{2+}$ concentration stimulated by the $\beta_2$-adrenoceptor agonist was examined by confocal microscopy. In addition, the vascular expression of the $\beta_2$-adrenoceptor was also examined by immunohistochemistry. The results show that isoprenaline and terbutaline-induced relaxation was lower in the aortas of rats with BPV. Relaxation responses to other vasorelaxant compounds were similar in both groups of rats. Histological analysis revealed a lower level of $\beta_2$-adrenoceptor and confocal microscopy showed minor cytosolic $Ca^{2+}$ concentration in endothelial cells stimulated by the $\beta_2$-adrenoceptor agonist in rats with BPV. In conclusion, BPV leads to desensitization of the $\beta_2$-adrenoceptor, which could contribute to worse β-adrenoceptor agonist-induced relaxation in isolated aortas.


## 1. Introduction

β-adrenoceptors (β-ARs) activation in the blood vessels leads to relaxation through cellular signaling pathway activation, mainly adenosine 3′,5′-cyclic monophosphate/protein kinase A (cAMP/PKA) and guanosine 3′,5′-cyclic monophosphate/protein kinase G (cGMP/PKG), depending on the type of vessel studied [1–3]. β-ARs are known to be expressed in the vascular smooth muscle and endothelial cells [4] and at least three populations of β-AR subtypes have been shown to be involved in the β-adrenergic response in blood vessels: $\beta_1$-, $\beta_2$-, $\beta_3$-AR [5,6]. It has been shown that another subtype, $\beta_4$-AR, actually corresponds to a low-affinity state of $\beta_1$-AR [7]. In addition, β-ARs stimulated vascular relaxation is impaired in hypertensive humans and animals [8–12].

Despite being elevated, many studies have shown that blood pressure is not constant in hypertensive subjects, but may vary spontaneously. Spontaneous variation in blood pressure is defined as 'blood pressure variability' (BPV). In general, BPV is positively related to the severity of organ damage in hypertensive humans and animals [13–18]. To determine BPV, a method widely employed is that of calculating the standard deviation of the blood pressure values continuously recorded over a certain time range. With this method, investigators have found that BPV is increased in hypertensive patients [13,19–21] and in several hypertension models in rats [14–22,23]. Therefore, certain research groups have hypothesized that BPV could be a common phenomenon in hypertension.

Sinoaortic denervation (SAD) is a procedure that interrupts the arterial baroreceptor reflex system. The haemodynamic alterations produced by SAD have been extensively studied in many mammals, especially in rats. There is general agreement that SAD causes a substantial increase in BPV with no changes in the mean levels of blood pressure [24–27]. Therefore, SAD rats can be used to study the physiological changes caused by high BPV without sustained hypertension.

This study examines whether BPV provoked by SAD (i.e., BPV in the absence of hypertension) influences the relaxation induced by β-ARs-activation. The basis for this proposal and the experimental approach are based on the following evidences: (a) β-ARs-induced relaxation of arteries in hypertensive humans and animals is impaired; (b) hypertension is often accompanied by BPV; and, (c) SAD rats present BPV without hypertension. We have applied these findings to test our hypothesis that β-

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ARs-induced relaxation could be impaired as a consequence of BPV and not just by hypertension.

## 2. Methods

### 2.1. Sinoaortic denervation

All the procedures were carried out in accordance with 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). Male Wistar rats (180–210 g) were submitted to SAD or to a sham-operation, according to the method previously described by members of our group [26,27]. In brief, the aortic and superior laryngeal nerves were isolated and dissected under anesthesia with a solution of ketamine (50 mg/kg/i.p.) and xylazine (5 mg/kg/i.p.). The bifurcation and all the carotid branches were stripped of fibers and connective tissues. These procedures were completed bilaterally. Control rats were sham-operated (SO). After the SAD or sham-surgery, polyethylene catheters (PE-10 connected to PE-50) were implanted in the lower abdominal aorta via the left femoral artery in order to measure blood pressure and heart rate, and in the left femoral vein for the subsequent administration of phenylephrine. Catheters were tunneled subcutaneously and exteriorized at the dorsal neck region. Three days after surgery, the blood pressure and heart rate were measured by connecting the arterial catheter to a pre-calibrated pressure transducer coupled with an amplifier recorder, and the responses were recorded using a computerized system and Chart software 4.0 (PowerLab, ADInstruments). Total SAD was evaluated by determining the change in heart rate response to a 40 ± 10 mm Hg increase in the mean blood pressure produced by the intravenous injection of phenylephrine (3–4 μg/kg). Only rats presenting a bradycardia of <20 beats/min were considered to be SAD rats.

### 2.2. Blood pressure recordings

The experiments were performed on the third day for the following reasons: (i) this period allows for animal adaptation; (ii) the frequency of the oscillatory contractions in SAD rat aortas is higher in this period [26,27]; and (iii) the levels of blood pressure are similar in both groups, even though the SAD group presented high BPV [25–27].

Three days after surgery (SAD and SO), blood pressure recordings, lasting at least 45 min, were taken from conscious rats. The blood pressure and heart rate signals were digitalized by a microcomputer, and the values were determined and averaged. A widely used method for determining BPV is that of calculating the standard deviation of the arterial pressure recorded continuously over a certain time range [22–27]. BPV was expressed by the standard deviation of the mean value of the arterial pressure.

## 3. Vascular reactivity study

The rats were anaesthetized and sacrificed by abdominal aortic exsanguination. Thoracic aorta was quickly removed, dissected free, and cut into 4-mm-long rings. These aortic rings were placed between two stainless-steel stirrups and connected to an isometric force transducer (Letica Scientific Instruments) coupled with a PowerLab data acquisition unit. The responses were recorded using a computerized system and a Chart software 4.0 (PowerLab, ADInstruments) to measure tension in the vessels. The aortic rings were placed in a 10 mL organ chamber containing Krebs solution with the following composition (mmol/L): NaCl 130.0, KCl 4.7, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 14.9, glucose 5.5, $CaCl_2$ 1.6. The solution was maintained at pH 7.4, and gassed with 95% $O_2$ and 5% $CO_2$ at 37 °C. Rings were initially stretched to their optimum resting tension of 1 g before being allowed to equilibrate in the bathing medium. In some preparations, the endothelium was mechanically removed by gentle abrasion and its removal was validated by lack of relaxation to acetylcholine (1 μmol/L) following contraction to phenylephrine (0.1 μmol/L).

After the equilibrium period, phentolamine (α-adrenoceptor antagonist, 0.75 μmol/L) was added to the organ bath to avoid interaction between β-ARs agonist and α-ARs. After 20 min, the arteries were contracted with prostagladin $F_{2\alpha}$ ($PGF_{2\alpha}$, $EC_{50}$: 3 μmol/L) and cumulative concentration–response curves (0.1 nmol/L to 10 μmol/L) to isoprenaline (non-selective β-ARs agonist), terbutaline (selective $\beta_2$-ARs agonist), cyanopindolol (agonist of low-affinity state of the $\beta_1$-AR), BRL37344 (selective $\beta_3$-ARs agonist) were carried out.

In another series of experiments, cumulative concentration–response curves to forskolin (FSK, adenylyl cyclase activator), acetylcholine (ACh, muscarinic receptor agonist) and sodium nitroprusside (SNP, nitric oxide donor) were carried out in isolated arteries precontracted with $PGF_2\alpha$ ($EC_{50}$). All experiments were performed in the intact-endothelium preparations, with the exception of isoprenaline and FSK (denuded and intact endothelium) and SNP (denuded arteries).

### 3.1. Confocal microscopy

The SAD and Sham rats were sacrificed under anesthesia, and the thoracic aorta was quickly removed, cut longitudinally, and maintained in a Hanks solution with the following composition (in mmol/L): NaCl 145.0, $CaCl_2$ 1.6, KCl 5.0, $MgCl_2$ 1.0, $NaH_2PO_4$ 0.5, dextrose 10.0, and HEPES 10.0, pH 7.4. The inner surfaces of the vessels were gently scraped with a plastic stem in order to isolate the endothelial cells. The cell suspension was centrifuged at 1000 rpm for 5 min. The cell pellet was then suspended in a 0.5 mL Hanks solution and maintained in a humidified incubator (37 °C) until the experiment was accomplished. The cells were placed on glass coverslips covered with poly-L-lysine. The $Ca^{2+}$-sensitive fluorescent probe Fluo-3 AM (10 μmol/L, Sigma Probes) was used to determine the intracellular $Ca^{2+}$ concentration. Fluo-3 fluorescence was excited (band-pass 460–480 nm) using a mercury lamp fitted to a multislit disk confocal scanner (IX-DSU; Olympus), and the light emitted was filtered using a band-pass filter (BA 495–540 nm). Data were stored for offline analysis by means of image-processing software Image-J. The isoprenaline or terbutaline-induced increase in fluorescence, which represents the $Ca^{2+}$ concentration in the endothelial cells isolated from Sham- and SAD-operated rats, was calculated. Intracellular $Ca^{2+}$ signals were expressed as the ratio of the Fluo-3 fluorescence intensity changes to the baseline (F/F0), F0 = before the agonist and F = after the agonist. Signals were monitored in endothelial cells by taking measurements every 3 s. All data are presented as mean ± standard error of the mean.

### 3.2. Histological analysis

The rats were anaesthetized, sacrificed and the vessels fixed *in situ* by constant pressure fixation with formalin (10%) through a 22-gauge butterfly angiocatheter in the left ventricle. Aortas were harvested, embedded in paraffin, and cross-sectioned (4 μm). The preparations were stained with haematoxylin and eosin or submitted to immunohistochemical analysis. In brief, 4-μm-thick sections mounted on poly-L-lysine-coated slides were deparaffinized, rehydrated, immersed in 10 mmol/L citrate buffer, pH 6.0, and submitted to heat-induced epitope retrieval using a vapor lock for 45 min. The slides were rinsed with phosphate-buffered saline (PBS) and immersed in 3% hydrogen peroxide for 20 min to block endogenous peroxidase. Non-specific protein binding was blocked with normal serum (Vectastain Elite ABC Kit, Universal, Vector Laboratories Inc., Burlingame, CA, USA) for 30 min. The sections were then incubated with monoclonal primary antibodies specific for the $\beta_2$ adrenergic receptor (clone H-20; Santa Cruz Biotechnology, Santa Cruz, CA, USA; dilution 1:50), for 2 h at room temperature (25 °C) in a humid chamber. After rinsing in PBS, biotinylated pan-specific universal secondary antibody (Vectastain Elite ABC Kit, Universal, Vector Laboratories Inc.) was applied for 30 min. Next the slides