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Cardiorespiratory effects induced by 2-nitrate-1,3-dibuthoxypropan are reduced by nitric oxide scavenger in rats

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ABSTRACT

The search for new nitric oxide donors is warranted by the limitations of organic nitrates currently used in cardiology. The new organic nitrate 2-nitrate-1,3-dibuthoxypropan (NDBP) exhibited promising cardiovascular activities in previous studies. The aim of this study was to investigate the cardiorespiratory responses evoked by NDBP and to compare them to the clinically used organic nitrate nitroglycerine (NTG). Arterial pressure, heart rate and respiration were recorded in conscious adult male Wistar rats. Bolus i.v. injection of NDBP (1 to 15 mg/kg; n = 8) and NTG (0.1 to 5 mg/kg; n = 8) produced hypotension. NDBP induced bradycardia at all doses, while NTG induced tachycardia at three lower doses but bradycardia at higher doses. Hydroxocobalamin (20 mg/kg; HDX), a NO scavenger, blunted hypotension induced by NDBP (15 mg/kg), and its bradycardic effect (n = 6). In addition, HDX blunted both hypotension and bradycardia induced by a single dose of NTG (2.5 mg/kg; n = 6). Both NDBP and NTG altered respiratory rate, inducing a biphasic effect with a bradypnea followed by a tachypnea; HDX attenuated these responses. Our data indicate that NDBP and NTG induce hypotension, bradycardia and bradypnea, which are mediated by nitric oxide release.

1. Introduction

Nitric oxide (NO) is a simple gas that can be formed in vivo by the action of the NO synthase, an enzyme that converts L-arginine to L-citrulline. NO plays a role in multiple physiological and pathological effects, including vasodilation, angiogenesis, immunity, platelet aggregation and neurotransmission (Moncada et al., 1991; Bolotina et al., 1994; Ignarro, 2000). In the central nervous system (CNS), especially in the brainstem, NO has been implicated in the control of cardiovascular function (Krowicki et al., 1997; Kamendi et al., 2006). To accomplish its physiological effects, the classic signaling pathway induced by NO involves activation of the soluble guanylyl cyclase (sGC) catalyzing the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), which in turn activates the cGMP-dependent protein kinase (PKG) (Arnold et al., 1977; McDonald and Murad, 1996).

The simplicity and versatility of the NO molecule allow the synthesis of a great variety of NO donors (Muscará and Wallace, 1999; Miller and Megson, 2007; Munhoz et al., 2012). NO donors are substances that mimic the role of endogenous NO. These substances have been used for several cardiovascular diseases, mainly hypertension and coronary artery disease (Harrison and Bates, 1993; Naseem, 2005; Paulo et al., 2012). Two of the most known NO donors are the sodium nitroprusside (SNP) and NTG. Despite their beneficial effects in cardiovascular system, they have some restrictions for their clinical applications due to undesirable side effects (Laursen et al., 1996). For instance, NTG has its effect attenuated during continuous treatment; it also promotes tolerance to other nitrates, a phenomenon known as cross-tolerance (Kosmicki, 2009). SNP has limited use due to the development of tolerance; furthermore, prolonged administration of this NO donor has been restricted due to the high toxicity caused by the release of cyanide (Ignarro et al., 2002). These limitations of commonly used NO donors could be potentially overcome with the synthesis of new NO donors. The 2-nitrate-1,3-dibuthoxypropan (NDBP) is a synthetic compound obtained in our research center from glycerin (Santos, 2009). Previous study from our research group showed that NDBP induces hypotension predominantly due to the reduction in cardiac output by direct vagal activation (França-Silva et al., 2012b). Since the respiratory pattern generating network is located in close proximity, we hypothesized that NO donors might have respiratory effects. Our overall aim was to further investigate the cardiovascular responses evoked by NDBP and to compare this effect with the well-known NO donor, nitroglycerine.

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2. Material and methods

2.1. Animals

Adult male Wistar rats were obtained from the University of Paraiba Biotechnology Center animal house. They were weighing 200–250 g and were kept in conditions of controlled temperature (21 ± 1 °C) at a 12 h light–dark cycle (lights on 8 am) with free access to food (Labina®, Purina, São Paulo, Brazil) and tap water. All procedures described in the present study were approved by the Institutional Animal Care and Use Committee of the Federal University of Paraiba (CEPA/LTF protocol nº 0209/10). All rats were anesthetized with ketamine and xylazine (75 and 10 mg/kg, i.p., respectively) and the lower abdominal aorta and inferior vena cava were catheterized via left femoral artery and vein using polyethylene tubing catheters. The catheters were filled with heparinized saline and tunneled under the skin to the interscapular region.

2.2. Experimental protocol

One day after the surgical procedures, blood pressure and heart rate were evaluated in one group of conscious rats (n = 8) before and after administration of NDBP (1, 5, 10, 15 and 20 mg/kg) (França-Silva et al., 2012b). In another group of animals (n = 8), effects of NTG (0.1, 0.25, 0.5, 1, 2.5 and 5 mg/kg) (Queiroz et al., 2013) were assessed. Blood pressure signal was obtained from intra-arterial catheter; animals remained in their home cages during recordings. The drugs were administered via intravenous catheter; injection volume was 0.25–0.3 ml. The submaximal doses of 15 mg/kg and 2.5 mg/kg for NDBP and NTG, respectively, were chosen for the following experiment conducted in two other groups of rats (n = 6 each). Either NDBP or NTG was administered twice, with hydroxocobalamin (HDX, a NO scavenger, 20 mg/kg) given 15 min before the addition of the second dose of the nitrate. In this second experiment, in addition to arterial pressure and heart rate measurements, we also recorded respiratory rate (see below).

2.3. Data acquisition and analysis

Arterial pressure was measured by connecting the arterial catheter to a pre-calibrated pressure transducer (MLT0380/D, ADInstruments, Sydney, Australia). Respiratory rate was acquired using a custom-built whole-body plethysmograph (Kabir et al., 2010; Carnevali et al., 2013). This consisted of a sealed Perspex cylinder (i.d. 95 mm, length 260 mm, volume 2.5 l) with medical air constantly flushed through it at a flow rate of 2.5 l/min. The output flow was divided into two lines using a T-connector. One line was attached to a differential pressure amplifier (model 24PCO1SMT, Honeywell Sensing and Control, Golden Valley, MN, USA), while the other line was open to the room air. Arterial and venous catheters were passed through the plethysmograph wall via a sealed port. Respiratory and AP signals were digitized at 1 kHz and acquired using PowerLab4/35 (ADInstruments, Australia) and a computer running the LabChart 7.0 software (ADInstruments, Australia). Respiratory rate, heart rate, systolic, diastolic and mean arterial pressure were computed online with subsequent off-line verification.

2.4. Drugs

NDBP was synthetized in the Department of Chemistry at the Federal University of Paraiba as described earlier (França-Silva et al., 2012b). It was dissolved in a mixture of saline and cremophor and diluted to the desired concentrations with saline as previously described (França-Silva et al., 2012a). NTG was obtained from Cristal Pharm® (Brazil) and diluted to the desired concentrations with saline. Hydroxocobalamin was obtained from Merck® (USA).

2.5. Statistical analysis

Results are expressed as mean ± SEM. Data were analyzed by the Student's t-test or by the two-way repeated measures ANOVA followed by a Dunnett post-hoc test for multiple comparisons whenever appropriate. All statistical analyses were performed using GraphPad Prism (v. 5.0, GraphPad Software, Inc.). Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Effects NDBP and NTG on blood pressure and heart rate

Vehicle injections had no effect on any of measured parameters. Administration of NDBP elicited hypotensive and bradycardic responses as illustrated in Fig. 1A. These effects were dose-dependent as shown in Fig. 3A. Intravenous bolus injection of NTG provoked similar hypotensive effect (Fig. 2A), also in a dose-dependent manner (Fig. 3B). Interestingly, NTG at three lower doses induced tachycardia while at three higher doses it caused bradycardia whereas NDBP caused bradycardia at all doses (Fig. 3B). The mean arterial pressure and heart rate values returned to basal levels within the first minute after injection of all doses of the nitrates. NTG was found to be more potent than NDBP in inducing a decrease in mean arterial pressure and heart rate ($P > 0.05$).

3.2. NDBP and NTG induce a more potent reduction in diastolic than in systolic arterial pressure

As during profound bradycardia, mean arterial pressure is not an adequate descriptor of pressure changes, we also analyzed their effects separately on systolic and on diastolic arterial pressure values (SAP and DAP). The maximal variations in these parameters induced by NDBP and NTG are presented in Fig. 4. After administration of NDBP (1 to 20 mg/kg), there was a reduction in the SAP and DAP, the latter being substantially more prominent (Fig. 4A). Likewise, bolus injection of NTG (0.1 to 5 mg/kg) produced a dose-dependent decrease in both SAP and DAP, with more prominent effect on the latter one (Fig. 4B).

3.3. Hypotension and bradycardia induced by NDBP and NTG are blunted by NO scavenger

In order to evaluate the NO involvement in the responses induced by NDBP or NTG, the cardiovascular effects were assessed before and after a bolus injection of a NO scavenger, HDX (20 mg/kg). Administration of HDX blunted hypotension induced by NDBP (−11 ± 5 vs. −66 ± 6 mm Hg, $P < 0.05$, n = 7) and converted its bradycardic effect into the tachycardic (+65 ± 13 vs. −356 ± 24 bpm, $P < 0.05$, n = 7) as shown in Fig. 1B (raw data) and Fig. 5A (mean group values). HDX also blunted both hypotension (−47 ± 5 vs. −82 ± 8 mm Hg, $P < 0.05$, n = 6) and bradycardia (−191 ± 28 vs. −378 ± 30 bpm, $P < 0.05$, n = 6) evoked by a single dose of NTG as illustrated in Fig. 2B (raw data) and Fig. 5B (mean group values). HDX alone had no effect on any of the recorded variables (data not shown).

3.4. NDBP and NTG induce biphasic changes in respiratory rate

To determine whether NO donors change the respiratory function, we monitored the respiratory rate in conscious rats using whole-body plethysmography. NDBP induced a short-lasting reduction in respiratory rate (−78 ± 9 cpm; $P < 0.05$, n = 6) followed by a prolonged tachypnea (231 ± 28 cpm; $P < 0.05$, n = 6) as shown in Fig. 1A (raw data) and in Fig. 6A&C (mean group values). Similar biphasic effects were found following NTG administration: short-latency bradypnea (−95 ± 12 cpm; $P < 0.05$, n = 6) followed by a tachypnea (159 ± 19 cpm; $P < 0.05$, n = 6) as shown in Fig. 1B (raw data) and in Fig. 6B&D (mean group values).