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Additive counteraction by α7 and α4β2-nAChRs of the hypotension and cardiac sympathovagal imbalance evoked by endotoxemia in male rats

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ABSTRACT

The cholinergic antiinflammatory pathway favorably influences end organ damage induced by inflammatory conditions. Here, we hypothesized that α7 and/or α4β2-nicotinic acetylcholine receptors (nAChRs) protect against cardiovascular and autonomic imbalances induced by endotoxemia in rats. We assessed dose-effect relationships of i.v. nicotine (25, 50, or 100 μg/kg), PHA-543613 (α7-nAChR agonist; 0.2 or 2.0 mg/kg), or 5-iodo-A-85380 (5IA, α4β2-nAChRs agonist; 0.01 or 0.1 mg/kg) on cardiovascular and inflammatory responses elicited by lipopolysaccharide (LPS, 10 mg/kg i.v.). The two lower doses of nicotine caused dose-dependent attenuation of hypotensive and tachycardic responses of LPS. Nicotine also reversed LPS-evoked reductions in time-domain indices of heart rate variability (HRV) and spectral measure of cardiac sympathovagal balance. Alternatively, hypotensive and tachycardic effects of LPS were (i) partly and dose-dependently reversed after selective activation of α7 (PHA) or α4β2-nAChRs (5IA), and (ii) completely eliminated after co-treatment with the smaller doses of the two agonists. Further, PHA or 5IA abolished the reducing effect of LPS on time and spectral measures of HRV. Elevations in serum tumor necrosis factor-α (TNF-α) observed in LPS-treated rats were compromised upon co-administration of nicotine, PHA, or 5IA. In conclusion, monomeric α7 or heteromeric α4β2-nAChRs favorably and additively influence inflammatory and associated cardiovascular anomalies induced by endotoxemia.

1. Introduction

The treatment of animals with lipopolysaccharide (LPS), the cell-wall component of gram-negative bacteria, is a recognized way of inducing experimental endotoxemia (Borovikova et al., 2000). Several studies support the concept that the proinflammatory cytokines produced during endotoxemia are responsible for most of the cardiovascular deleterious effects of endotoxemia including hypotension (Sallam et al., 2017, 2016a; Tunctan et al., 2010) and cardiac autonomic dysfunction (Hajiasgharzadeh et al., 2011; Sallam et al., 2017; Tateishi et al., 2007). The cholinergic antiinflammatory pathway is a key physiologic mechanism for combating inflammation (Tracey, 2007) that can be stimulated by nAChR agonists, including nicotine (de Jonge et al., 2005; Saeed et al., 2005).

The evidence that nicotine exhibits antiinflammatory effects is compelling. For example, nicotine attenuates LPS-induced decreases and increases in blood pressure (BP) and heart rate (HR), respectively, in telemetered rats, changes that are associated with significant inhibition of TNF-α and IL-1β generation (Kojima et al., 2011). In other studies, nicotine attenuates the increase in NF-κB protein expression in mice with hepatic ischemia reperfusion injury (Park et al., 2013), improved kidney injury caused by LPS in mice (Chatterjee et al., 2012), and reduces the severity and onset of the experimental autoimmune encephalomyelitis (Naddafi et al., 2013).

nAChRs have many protein subunits (α2–10 and β2–4) that assemble into hetero- and homo-pentameric ion channels whose opening is gated in response to the binding of the neurotransmitter acetylcholine (Buckingham et al., 2009). nAChRs are differentially expressed in central and peripheral nervous systems and on other cell types throughout the body, including immune cells (Matsunaga et al., 2001). The most prevalent nAChR subtypes in the nervous system are the homopentameric α7-nAChRs and heteropentameric α4β2-nAChRs (Saika et al., 2015). The antiinflammatory response elicited by nAChR activation on macrophages has been solely attributed to activation of α7-nAChRs (de Jonge et al., 2005; Li et al., 2011; Wang et al., 2003). α7-nAChR activation has been shown to inhibit nuclear factor (NF)-κB transcriptional activity (Wang et al., 2004) and proinflammatory cytokine production (Wang et al., 2009). However, several studies have

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identified the expression of α4β2 and but not α7-nAChR in alveolar macrophages (Galvis et al., 2006; Matsunaga et al., 2001).

Most, if not all, of the reported studies that investigated the therapeutic potential of nicotine in endotoxemia focused mainly on its antiinflammatory activity and ability to suppress cytokine production (Borovikova et al., 2000; van Westerloo et al., 2005; Wang et al., 2004). We are not aware of any study that investigated the effects of selective or nonselective nAChRs activation on cardiovascular and autonomic anomalies of endotoxemia. Therefore, the current study attempted to investigate the dose-effect relationships of nicotine or drugs that selectively activate monomeric α7 or heteromeric α4β2-nAChRs on the inflammatory, cardiovascular, and autonomic responses elicited by endotoxemia in conscious rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (180–230 g; Faculty of Pharmacy animal facility, Alexandria University, Alexandria, Egypt) were used in this study. All experiments were performed in strict accordance with the guidelines of the Animal Care and Use Committee of the Faculty of Pharmacy, Alexandria University, Egypt (ACUC project # 28/2014).

2.2. Intravascular cannulation

Full details of this procedure were described in our previous studies (El-Mas et al., 1997, 2009, 2012; El-Mas and Abdel-Rahman, 1995, 1999). Briefly, rats were anesthetized with ketamine/xylazine (80/10 mg/kg, i.p.) and polyethylene catheters were inserted in the abdominal aorta and vena cava via the femoral artery and vein for BP measurement and i.v. drug administration, respectively. The arterial catheter was connected to a BP transducer (model P23XL; Astro-Med, West Warwick, RI) that was attached through MLAC11 Grass adapter cable to a computerized data acquisition system with LabChart-7 pro software (Power Lab 4/35, model ML866/P; AD Instruments Pty Ltd., Castle Hill, Australia). Catheters were tunneled subcutaneously, exteriorized at the back of the neck between the scapulae, flushed with heparin (100 U/ml), and plugged by stainless steel pins. Each rat received an i.m. injection of benzathine benzyl penicillin (60,000 U). Experiments started 2 days later in conscious rats.

2.3. Time-domain analysis of HRV

Two time-domain measures of the cardiac autonomic activity were employed, the standard deviation of beat-to-beat intervals (SDNN) and the root mean square of successive beat-to-beat differences in R-R interval durations (rMSSD) (Omar and El-Mas, 2004; Stein et al., 1994). The RR intervals were computed from the heart rate (i.e. the reciprocal of heart rate in ms). The SDNN is comparable to the total power of the spectrum of RR variability, which measures the overall autonomic balance of the heart. The rMSSD is largely validated as a measure of the parasympathetic input to the heart and, therefore, correlates with the high frequency power of the spectrum (Sgoifo et al., 1997; Stein et al., 1994) SDNN and rMSSD were measured before (baseline) and at 15 min intervals after drug treatments. For each time point, the 5-min values of each variable were averaged.

2.4. Frequency-domain analysis of HRV

Spectral hemodynamic fluctuations, quantitative indices of cardiac autonomic control, were used to reflect changes in sympathetic and vagal outflows. Hemodynamic variability was analyzed in the frequency domain using FFT algorithms of R-R data series (El-Mas and Abdel-Rahman, 2007, 2013; Stein et al., 1994). Spectra were integrated into 2 specific frequency bands, LF (0.25–0.75 Hz) and HF (0.75–3 Hz) bands and expressed in normalized units (LF_{nu} and HF_{nu}). The LF/HF ratio is taken as a measure of the cardiac sympathovagal balance. Spectral data were estimated before (baseline) and at 15 min intervals after drug treatments.

Table 1
Baseline values of mean arterial pressure (MAP, mmHg) and heart rate (HR, beats/min).

Group	n	MAP	HR
Saline/ Saline	8	104 ± 3	370 ± 10
LPS/Saline	9	102 ± 3	354 ± 11
LPS/NIC 25 μg/kg	8	103 ± 3	396 ± 15
LPS/NIC 50 μg/kg	8	106 ± 4	354 ± 8
LPS/NIC 100 μg/kg	9	104 ± 4	358 ± 7
LPS/PHA 2 mg/kg	9	103 ± 3	333 ± 12
LPS/PHA 0.2 mg/kg	8	102 ± 4	329 ± 15
LPS/ 5IA 0.1 mg/kg	9	99 ± 3	339 ± 11
LPS/5-IA-0.01 mg/kg	9	102 ± 5	328 ± 11
LPS/PHA 0.2/5IA 0.01	7	104 ± 4	341 ± 8

Values are means ± S.E.M. of 7–9 observations. LPS, lipopolysaccharide, NIC, nicotine.

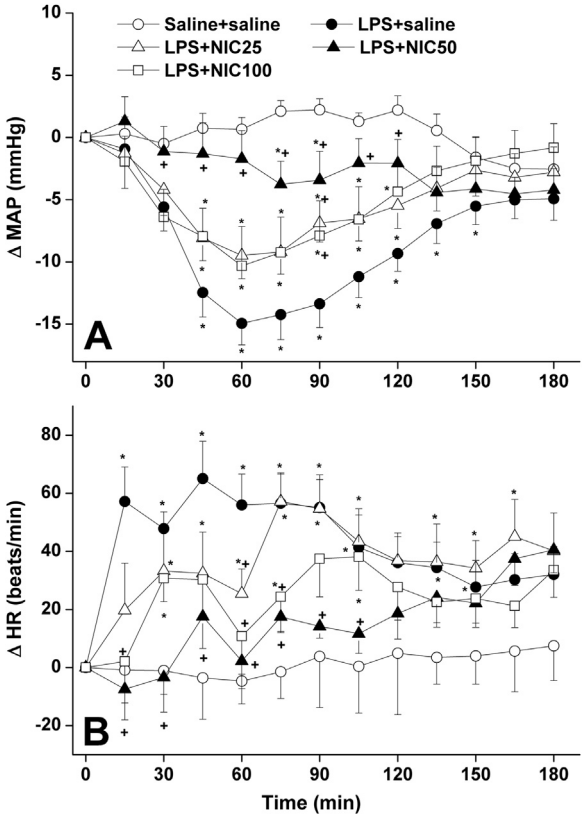

Fig. 1. Dose-related effects of i.v. nicotine (25, 50, or 100 μg/kg) on the hypotensive and tachycardic responses to LPS in male rats. Values are means ± S.E.M. of 7–9 observations. *P < 0.05 vs. "saline + saline", +P < 0.05 vs. "LPS + saline".

2.5. Protocols and experimental groups

2.5.1. Effects of nicotine on inflammatory and cardiovascular effects of endotoxemia

This experiment investigated the dose-effect relationship of nicotine on endotoxic cardiovascular manifestations. Five groups of conscious male rats (n = 7–9 each) were allocated to receive one of the following i.v. regimens: (i) saline + saline, (ii) LPS (10 mg/kg) (Mori et al., 2010) + saline, (iii) LPS + nicotine 25 μg/kg, (iv) LPS + nicotine 50 μg/kg,