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Interleukin 1-beta (IL-1β) enhances contractile responses in endothelium-denuded aorta from hypertensive, but not normotensive, rats

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Abstract

Background: The chronic effects of interleukin 1-beta (IL-β) on vascular reactivity include augmentation of contraction and relaxation. Few studies have assessed the acute effects of IL-1β in vessels from hypertensive and normotensive rats. We hypothesized that IL-1β would enhance constriction in aorta from stroke prone spontaneously hypertensive rats (SHRSP).
Methods: Endothelium denuded aortic rings from 12 week-old SHRSP and Wistar Kyoto (WKY) rats were mounted in a myograph and incubated with IL-1β (20 ng/ml) for 1 h before construction of a phenylephrine dose response curve. Indomethacin (1 μM) and PP-2 (1 μM) were utilized to inhibit cyclooxygenase (COX) and Src-kinase respectively.
Results: In aorta from SHRSP, IL-1β caused a significant increase in the force generated over the hour incubation; inhibition of COX or Src-kinase prevented this. The maximum phenylephrine-induced contraction was greater in aorta from SHRSP incubated with IL-1β than control. COX or Src-kinase inhibition prevented this. IL-1β had no effect on the vessels from WKY rats.
Conclusions: These novel data suggest that IL-1β has rapid effects on vascular smooth muscle from hypertensive rats to produce constriction and to enhance phenylephrine-induced constriction. The COX and Src-kinase pathways appear to be involved in this response.

Keywords: Inflammation; Hypertension; Vasoconstriction

1. Introduction

There is clear evidence that chronic inflammation plays an important role in the pathogenesis of hypertension (Bautista, 2003). Hypertensive subjects have higher circulating levels of, and an enhanced capacity to produce proinflammatory cytokines (Dalekos et al., 1997; Peeters et al., 2001). Similarly, rodent models of hypertension exhibit an inflammatory phenotype. The levels of IL-1β and interleukin 6 are increased in the aorta and plasma of spontaneously hypertensive rats (SHR) compared to WKY rats (Sanz-Rosa et al., 2005). Similarly, kidneys from SHRSP have increased levels of inflammatory markers and inflammatory cell infiltration (Sironi et al., 2004).

Reports of the effects of IL-1β on vascular reactivity are conflicting. *In vivo* treatment with IL-1β increases the responsiveness of mesenteric arteries to vasoconstrictors (De Salvatore et al., 2003) and causes constrictive remodeling and vasospasm in coronary arteries (Morishige et al., 2001). Similarly, ex vivo incubation of temporal arteries with IL-1β for two days causes increased vessel responsiveness to endothelin B receptor agonists (White et al., 2000). In these studies, the duration of the incubation of IL-1β was sufficient for IL-1β to alter gene transcription and translation. However, studies have also shown that a short-term exposure of vessels to IL-1β increases the contractile response to angiotensin II (Vicaut et al., 1996).

Conversely, other studies suggest that IL-1β renders vessels hyporesponsive to vasoconstrictors. Overnight incubation of aortic rings with IL-1β reduces their ability to contract in response to phenylephrine (Soler et al., 2003); this may be due to increased nitric oxide (NO) availability in the IL-1β treated vessels (Dinarello, 2002). This increase in NO availability may be caused by increased inducible NO synthase (NOS) expression

Abbreviations: COX, cylcooxygenase; IL-1β, interleukin 1-beta; SHR, spontaneously hypertensive rat; SHRSP, stroke prone spontaneously hypertensive rat; VSMC, vascular smooth muscle cell; WKY, Wistar Kyoto; PP-2, 4-Amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-D]pyrimidine.

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(Yang et al., 2004) or increased levels of tetrahydrobiopterin, a co-factor for endothelial NOS (Shi et al., 2004).

Few studies have assessed the direct acute effects of IL-1β on vascular smooth muscle cell (VSMC) contractility, and none have compared its effects in hypertensive and normotensive rats. We hypothesized that acute IL-1β treatment would cause constriction in endothelium-denuded aorta from SHRSP and augment the phenylephrine-induced contraction. We also tested the involvement of the COX and Src-kinase pathways in the IL-1β-induced response; both of these pathways have previously been implicated in the pathogenesis of hypertension (Touyz et al., 2001a,b; Touyz et al., 2002; Hermann et al., 2003; Wu et al., 2005).

2. Methods

2.1. Animals

Twelve-week-old male SHRSP were obtained from the breeding colony at the Medical College of Georgia. Age-matched male WKY rats were purchased from Harlan (Indianapolis IN). Rats were maintained on a 12-hour light dark cycle, housed two per cage and allowed access to normal chow and water *ad libitum*. These studies complied with the protocols for animal use outlined by the American Physiological Society.

2.2. Measurement of isometric force generation

Rats were euthanized with sodium pentobarbital (50mg/kg IP) and the thoracic aorta was removed and placed in ice cold PSS (in mmol/L; NaCl: 130, KCl: 4.7, KH_2PO_4: 1.18, $MgSO_4$: 1.17, $CaCl_2$: 1.6, $NaHCO_3$: 14.9, dextrose: 5.5, EDTA: 0.03). The aorta was cleaned and cut into 3 mm rings. To assess the direct effects of IL-1β on the vascular smooth muscle, the endothelium was removed by gentle rubbing of the luminal surface and the rings were mounted on stainless steel pins in a modified myograph system (DanishMyo Technologies, Aarhus, Denmark). The organ baths contained PSS warmed to 37 °C and gassed with 95% O_2–5% CO_2. A passive tension of 36–37 mN was placed on each ring and the rings were allowed to equilibrate for 45 min. The rings were then challenged with phenylephrine (10^{-7}M) to ensure tissue viability and with acetylcholine (10^{-5} M) to ensure the absence of an endothelium. A maximum contraction to phenylephrine (10^{-5} M) was obtained, and then vessels were incubated with IL-1β (20ng/ml) (R&D Systems, Minneapolis MN) for 1 h. When required, indomethacin (1 μM) or PP-2 (1 μM) (Biomol, Plymouth Meeting PA) were added to the bath 10 min prior to the addition of the IL-1β. After the incubation with IL-1β a cumulative phenylephrine does response curve was constructed (10^{-10}–10^{-5}M). Two rings were used per treatment for each experiment and the results obtained from these rings were averaged.

2.3. Statistics

Results are represented as the mean±standard error of the mean. The contractions in response to IL-1β and phenylephrine are depicted as the increase in force generated from baseline. The baseline for the phenylephrine dose response was taken immediately prior to the addition of the first dose of phenylephrine. Dose response curves were compared by two-way repeated measures ANOVA. EC50 values were calculated using GraphPad Prism (GraphPad Software Inc. San Diego

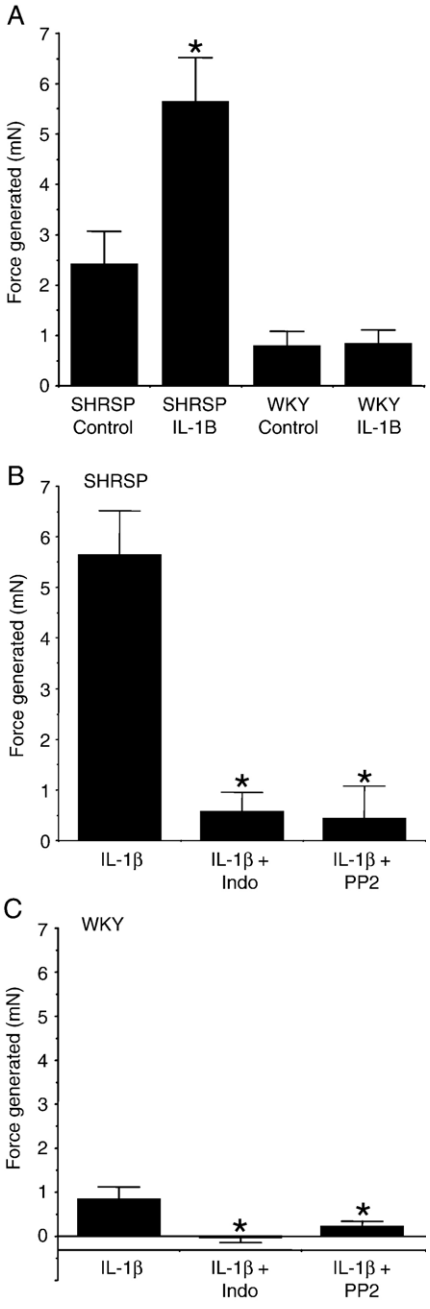

Fig. 1. (A) IL-1β caused contraction in aorta from SHRSP ($n=9$) but not WKY ($n=6$) rats. Contraction was measured as the mN change in force generation from baseline. *Indicates a significant difference ($p<0.05$) from the appropriate strain control. (B) Indomethacin ($n=5$) and PP-2 ($n=5$) inhibit the IL-1β-induced contraction in aorta from SHRSP, *indicates a significant difference from the IL-1β treated vessels. C. Indomethacin ($n=6$) and PP-2 ($n=6$) reduced the force generated by the vessels from WKT rats incubated with IL-1β. WKY—Wistar Kyoto, SHRSP—stroke prone spontaneously hypertensive rat, IL-1β—interleukin 1-beta, indo—indomethacin.