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Original Article

Effect of propofol on vascular reactivity in thoracic aortas from rats with endotoxemia

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Abstract

Background: This study examined the effect of propofol on thoracic aortas isolated from endotoxic rats to assess endothelium-dependent and -independent relaxant responses.
Methods: Adult male Wistar rats were assigned randomly to one of two groups, a saline control group or an experimental group treated with lipopolysaccharide (LPS, 10 mg/kg intravenously). At 6 hours after saline or LPS infusion, the thoracic aorta was excised and cut into 3-mm rings. Aortic rings with or without endothelium were suspended in organ baths for isometric tension recording.
Results: Both norepinephrine (NE)-induced vascular contraction and acetylcholine-induced vasodilation were attenuated in aortasfrom LPS-treated rats. Furthermore, preincubation with propofol caused a rightward shift in the NE concentration–response curve for aortasfrom LPS-treated rats compared to sham controls. The slow and sustained, but not the initial fast, contractile response to NE was significantly suppressed by propofol in LPS-treated aortas. In addition, vascular relaxation induced by propofol in LPS-treated aortas was partially suppressed by inhibitors of either nitric oxide (NO) synthase or soluble guanylate cyclase, but not bypotassium channel inhibitors.
Conclusion: These data suggest that propofol reduces the sensitivity to NE in aortic rings from endotoxic rats. This appears to be caused by (i) blockade of the extracellular calcium influx rather than a reduction in intracellular calcium release and (ii) an increased response to, at least in part, NO–cGMP in rat aortas.

Keywords: calcium influx; nitric oxide; propofol; sepsis; vascular smooth muscle

1. Introduction

Septic shock has detrimental effects leading to circulatory failure and abnormal tissue perfusion. Altered tissue perfusion may be caused by vascular hyporeactivity to adrenergic agonists. It is believed that enhanced formation of nitric oxide (NO), principally because of activation of the calcium-independent, inducible isoform of NO synthase (iNOS), contributes to the hyporeactivity to endogenous and exogenous vasoconstrictor agents in septic shock and thus underlies hypotension.[1] In addition, our previous studies have shown that K^+ channels are involved in the mechanism of abnormal relaxation of arteries in rats during endotoxic shock induced by lipopolysaccharide (LPS).[2–4]

Propofol (2,6-diisopropylphenol) is a short-acting intravenous anesthetic/hyponotic agent. It has been shown that propofol causes hypotension via myocardial depression,[5,6] direct vascular relaxation,[7,8] and/or a decrease in sympathetic activity.[9] In isolated arteries, propofol decreased vascular tone and adrenoceptor agonist-induced vasoconstriction.[10–12] There are three NOS isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS), and iNOS.[13] The release of NO induced by eNOS in the vascular endothelium partially contributes to propofol-induced relaxation.[11,12,14] By contrast, vasodilation in response to propofol has been attributed to

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a decrease in intracellular Ca^{2+} availability within vascular smooth muscle cells, reflecting inhibition of the Ca^{2+} influx through voltage- or receptor-gated Ca^{2+} channels.[12,15,16]

Propofol is widely used not only in anesthesia but also in critical care units.[17] Therefore, whether arterial sensitivity to vasoconstrictors and/or vasodilators can be modulated by propofol is an important issue in ill patients.In this study, we investigated the effect of propofol on norepinephrine (NE)-induced contractile responses in thoracic aortas isolated from Wistar rats treated with LPS. In addition, we assessed whether propofol can modulate the action of NE by studying endothelium-dependent and -independent relaxant responses.

2. Methods

Sixty male Wistar rats (250−300 g) were purchased from BioLASCO Taiwan (Taipei, Taiwan). The animals were maintained on a 12-hour light/dark cycle and were given free access to water and standard rat chow. Animal experiments were approved by our institutional and Committee on the Care and Use of Animals (National Defense Medical Center, Taipei, Taiwan, ROC) and all animals received humane care according to the criteria of the National Academy of Sciences.

The rats were anesthetized by intraperitoneal injection of urethane (1.2 g/kg) and body temperature was maintained at approximately 36 °C with a heating pad. The trachea was cannulated to facilitate respiration. The right carotid artery was cannulated and connected to a pressure transducer (P23ID, Statham, Oxnard, CA, USA) for measurement of mean arterial blood pressure and heart rate, which were displayed on a polygraph recorder (MacLab/4e, ADInstruments, Castle Hill, Australia). The left jugular vein was cannulated for administration of endotoxin or vehicle. On completion of the surgical procedure, cardiovascular parameters were allowed to stabilize for 20 min. After recording baseline hemodynamic parameters, animals received *Escherichia coli* LPS (10 mg/kg intravenous infusion for 10 minutes) or normal saline (same volume as LPS) and were monitored for 6 hours. Bacterial LPS (*E. coli* serotype 0127:B8, L3127) was obtained from Sigma Chemical (St. Louis, MO, USA).

At 6 hour after injection of saline or LPS, thoracic aortas were isolated from sham controls and rats treated with LPS under anesthesia. The thoracic aorta was cleaned of adhering periadventitial fat in ice-cold (4 °C) Krebs solution and was cut into rings of approximately 3 mm in length, with four aortic rings assessed from one rat.The Krebs solution (pH 7.4) consisted of (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 11. To analyze endothelium-independent effects of the drug, the endothelium was removed from some by gently rubbing the intimal surface.[2] For the remaining rings, care was taken not to touch the inner surface of the blood vessel. Aortic rings were mounted in 20-mL organ baths containingoxygenated (95% O_2−5% CO_2) Krebs solution kept at 37 °C. Two S-shaped stainless steel hooks were inserted through the lumen; the lower hook was fixed and the upper one was attached to anisometric force displacement transducer (Grass FT03 transducer, Grass Technologies, Quincy, MA, USA). The resting tension was set to 2 g (determined to be optimal in preliminary length−tension experiments) and preparations were allowed to equilibrate for at least 60 minutes. During this incubation period, each ring was washed three times with fresh Krebs solution. Endothelial integrity or denudation was confirmed in each ring by testing relaxation induced by acetylcholine (ACh, 1 μM) after precontraction with the alpha-adrenergic agonist NE (100 nM). Lack of a relaxation response to ACh was considered as evidence that the endothelium had been removed. After this procedure, the rings were washed and allowed to re-equilibrate to baseline tension for 45 minutes.

In the first series of experiments, eight rats were used in each sham control groupand each group of rats treated with LPS.Cumulative NE concentration−response curves were calculated without propofol. After washing three times with fresh Krebs solution for 30 minutes, aortic rings were then incubated with or without propofol (10 μM) in fresh Krebs solution for 20 minutes, and cumulative NE concentration−response curves were calculated for all vessels with or without endothelium. It was noted that vascular reactivity was not altered by repetition of agonist stimulation, and that propofol did not modify the basal tension of rings from sham and LPS-treated rat aortas during the time course of incubation before agonist addition.

In the second series of experiments, eight rats were also used in each sham control groupand each group of rats treated with LPS. To study the role of propofol in extracellular calcium influx and intracellular store release induced by NE, endothelium-intact aortic rings were incubated with or without propofol (10 μM) in fresh Krebs solution for 20 minutes and then the rings were contracted with NE (1 μM). When maximal contraction was attained, rings were washed three times with Ca^{2+}-free Krebs solution and the protocol was repeated after a 45-minute equilibration period in Ca^{2+}-free solution. The calcium-free solution was of the same composition as Krebs solution except that $CaCl_2$ was omitted. The maximal NE-induced contraction of aortic rings in normal and Ca^{2+}-free Krebs solution was termed S and F phase, respectively, as previously described.[18] The fast initial phase (F phase) of NE-induced contraction depends on a common intracellular Ca^{2+} store, and the slow tonic phase (S phase) of NE-induced contraction mostly depends on the extracellular Ca^{2+} influx.[19−21]

In the third series of experiments, eight rats were used in each sham control groupand each group of rats treated with LPS. Cumulative concentration−response curves for ACh (1 nM to 1 μM) and propofol (1 nM to 10 μM) were obtained for aortic rings precontracted with NE in endothelium-intact and -denuded preparations. The NE-induced contraction level in preparations obtained from the LPS-treated group was adjusted to the same level as that in the sham group. Therefore, 100 nM NE was used in the sham group and 1 μM NE in the LPS-treated group. The vasodilatory response to propofol was expressed as a percentage of the initial contraction induced by NE.