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Regulatory Peptides 147 (2008) 67–71
REGULATORY PEPTIDES
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Role of dexamethasone on vasopressin release during endotoxemic shock

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Received 13 August 2007; received in revised form 23 November 2007; accepted 2 January 2008
Available online 7 January 2008

Abstract

The present study was designed to assess the hypothesis that dexamethasone (DEX) through the control of nitric oxide (NO) synthesis could regulate the release of vasopressin (AVP), which plays an important role in the regulation of arterial pressure and plasma osmolality. Endotoxemic shock was induced by intravenous (i.v.) injection of 1.5 mg/kg lipopolisaccharide (LPS) in male Wistar rats weighing 250–300 g. After LPS administration, a group of animals were treated with DEX (1.0 mg/kg of body weight), whereas saline-injected rats served as controls. The LPS administration induced a significant decrease in mean arterial pressure (MAP) with a concomitant increase in heart rate (HR) (ΔVMAP: −16.1±4.2 mm Hg; ΔVHR: 47.3±8.1 bpm). An increase in plasma AVP concentration occurred and was present for 2 h after LPS administration (11.1±0.9 pg/mL) returning close to basal levels thereafter and remaining unchanged until the end of the experiment. When LPS was combined with i.v. administration of a low dose of DEX, we observed an attenuation in the drop of MAP (ΔVMAP: −2.2±1.9 mm Hg) and a decrease in NO plasma concentration [NO] after LPS administration (1098.1±68.1 μM) compared to [NO] after DEX administration (523.4±75.2 μM). However, this attenuation in the drop of MAP was accompanied by a decrease in AVP plasma concentration (3.7±0.4 pg/mL). These data suggest that AVP does not participate in the recovery of MAP when DEX is administered in this endotoxemic shock model.

Keywords: Endotoxemia; Nitric oxide; Endotoxin; Blood pressure; Glucocorticoids; Lipopolisaccharide

1. Introduction

Septic shock is a serious and progressive complication in the critical care unit [1,2]. This clinical syndrome is characterized by sepsis with hypotension and generally presents a hemodynamic pattern with a dramatic fall in systemic vascular resistance and generalized disturbed blood flow [3]. Although we can observe that the course of septic shock has different phases with different characteristics [4], most therapeutic interventions are based on the primary aim of combating the refractory hypotension by using aggressive fluid infusions, large doses of vasoconstrictors and glucocorticoids [5]. Glucocorticoids can reverse hemodynamic disturbances and dependence on catecholamine in septic shock. The relevant beneficial mechanisms of steroids in septic shock are unknown, although inducible nitric oxide synthase (iNOS) could account for them.

Most experimental work on sepsis has been performed using animal models with lipopolisaccharide (LPS)-induced sepsis. LPS is a Gram-negative bacteria cell membrane component, which triggers an increase in nitric oxide (NO) synthesis [6]. The iNOS is rapidly expressed in the vascular smooth muscle component of arteries and veins leading to the proposal that inhibition of NO synthesis might be a useful adjunct to septic shock therapy [7]. Under these conditions, very high levels of the vasodilator gas NO are locally formed, rendering the vessels hypo-responsive to constrictor mechanisms [8]. These observations, among others, have led to the suggestion that inhibition of nitric oxide synthase (NOS) may be of therapeutic benefit in the treatment of septic shock [9,10]. Although their use has been proposed for many years in sepsis and septic shock with controversial results [11], glucocorticoids have recently consistently demonstrated to be able to improve hemodynamics in septic shock. In humans, they were found to increase the

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doi:10.1016/j.regpep.2008.01.001

vasopressor response to adrenergic agents [12], thus reducing the time under vasopressor support [13,14]. In addition, it has been shown that NO may act as a modulator of vasopressin (AVP) release or synthesis [15,16]. This assertion is based on the fact that in normal rats, L-NAME, an unspecific inhibitor of the NOS, produces an increase in plasma AVP levels [17] and that the NO donor, sodium nitroprusside inhibits supraoptic vasopressin neuron activity as shown in an in vitro study [18].

The exact mechanisms by which glucocorticoids may act in septic shock are unknown. It is however noteworthy that among several inhibitor effects on proinflammatory cascade, they are potent inhibitors of iNOS expression [19]. Therefore, the present study was designed to confirm the hypothesis that dexamethasone through the control of NO synthesis could regulate the release of AVP, which plays an important role in the regulation of arterial pressure.

2. Materials and methods

2.1. Animals and preparation

Experiments were performed on adult male *Wistar* rats weighing 250–300 g at the time of surgery. All experiments were performed in accordance with institutional ethical guidelines. The animals were housed at controlled temperature (25.0±2 °C) and exposed to a daily 12:12 h light dark cycle.

Lipopolisaccharide (LPS) from *Escherichia coli* serotype 0111:B4 and Dexamethasone were obtained from Sigma (St. Louis, MO, USA) and dissolved in pyrogen-free sterile saline. Animals were submitted to general anaesthesia with intraperitoneal injection of 2.5% (1.0 mL/100 g b.w.) 2,2,2-tribromoethanol (Aldrich, Milwaukee, WI, USA) and implanted with a polyethylene catheter in the femoral artery for direct blood pressure recording and in the jugular vein for intravenous (i.v.) drug administration.

Rats were injected i.v. by bolus injection with 1.5 mg/kg of LPS in a final volume of 0.5 mL. The beginning of the experimental protocols, time "zero", was defined as the point at which the rats received the LPS injection. Control rats were injected intravenously with 0.5 mL sterile saline.

2.2. Determination of the effect of LPS on mean arterial pressure (MAP), heart rate (HR), NO and plasma AVP concentration

MAP and HR of un-anaesthetized freely moving rats were recorded using a polygraph (Grass P122), connected to a pressure transducer (Grass P23XL-1) and using the software PolyView (Astro-Med, West Warwick, RI, USA), over a period of 6 h after LPS or saline i.v. injection.

The rats were decapitated 1, 2, 4 and 6 h after LPS or saline administration (control rats). Blood was collected into chilled plastic tubes, containing heparin (200 U), centrifuged for 20 min at 2000 g at 4 °C for plasma separation and stored at −70 °C before dosage. On the day of the assay, plasma samples were thawed and deproteinized with 95% ethanol (at 4 °C) for 30 min, subsequently centrifuged, and the

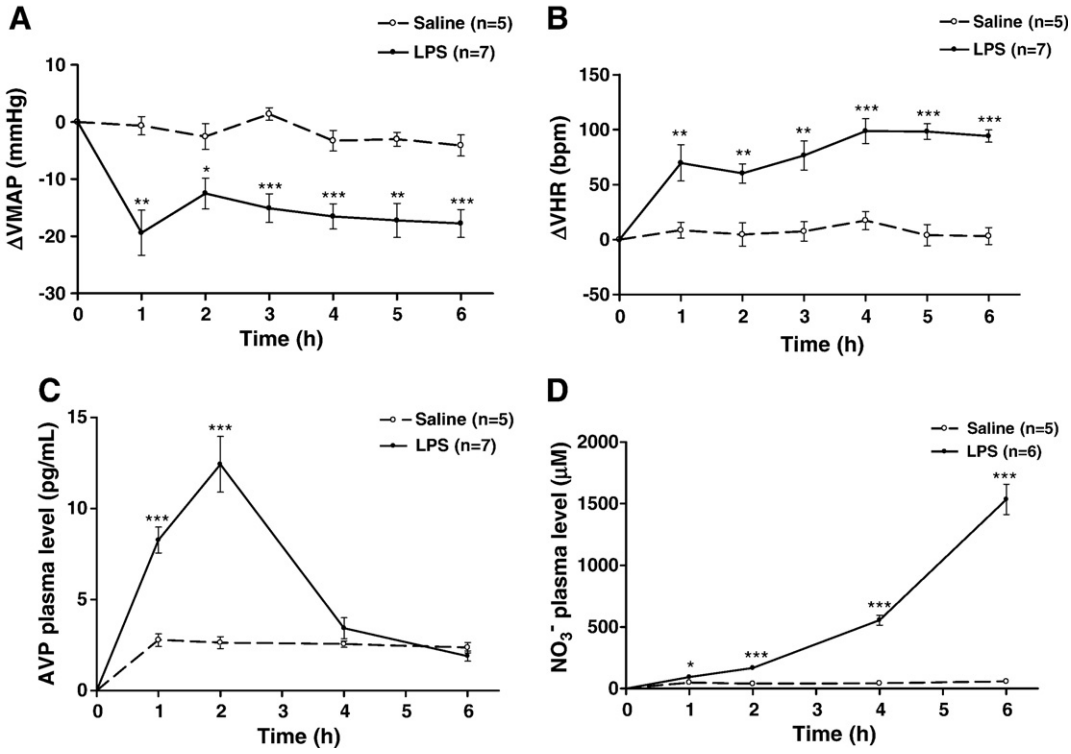

Fig. 1. Effect of saline or LPS on A: change in mean arterial pressure (ΔVMAP); B: change in heart rate (ΔVHR); C: AVP plasma level; D: NO_3^- plasma level. Values are means±SEM. *$p<0.05$, ** $p<0.01$ and *** $p<0.001$ significant difference when compared with saline group. In all the experiments the LPS injection was administered at zero time.

supernatant was used for measurement of nitrate according to the NO/ozone chemiluminescence technique [20], using a Sievers NO Analyzer 280 (GE Analytical Instrument, Boulder, CO, USA). Sodium nitrate (Sigma, St. Louis, MO, USA) was used as standard reference.

AVP was extracted from 0.7–1.5 mL of plasma using acetone and petroleum ether. Dried sample extracts were stored at −20 °C until the time for radioimmunoassay.

2.3. Determination of the effect of the combined effects of LPS and dexamethasone

In a separate set of experiments, rats received an i.v. injection of dexamethasone (1.0 mg/kg of body weight in a final volume of 0.5 mL). Control animals were injected with the same volume of saline. Thirty minutes after the dexamethasone injection, animals received LPS and were decapitated 1, 2, 4, and 6 h later for the determination of plasma NO and AVP concentration. A separate set of animals were used to determine MAP and heart rate in the same experimental period.

2.4. Data analysis

Results are expressed as means±S.E.M. Statistical analyses were performed on these data using one-way analyses of variance (ANOVA) followed by the Tukey multiple comparisons test. Values of $p<0.05$ were considered to be statistically significant.

3. Results

To validate our experimental model, we determined the MAP, HR, AVP and plasma levels. Fig. 1A and B show the effect of LPS administration on MAP and heart rate. When the animals were injected with LPS, a reduction ($p<0.001$) in MAP and an increase ($p<0.01$) in heart rate occurred. The effect of LPS administration on NO and AVP plasma concentration are observed in Fig. 1C and D respectively. Plasma nitrate levels increased gradually from 1 to 6 h and were significantly higher than those observed in the control group throughout the experiment after the 2nd hour of LPS treatment ($p<0.001$), in addition a significant ($p<0.001$) increase in plasma AVP concentration occurred. However, 4 h after LPS administration, plasma AVP concentrations were similar to those observed in saline-treated rats.

To evaluate the role of dexamethasone during endotoxemic shock we determined the MAP, HR, AVP and plasma levels in animals after the combination of dexamethasone and LPS administration. We found that dexamethasone significantly attenuated the drop ($p<0.01$) in MAP and brought about no changes in heart rate ($p<0.01$) induced by LPS treatment (Fig. 2A and B).

Fig. 2 (C and D) shows the effect of systemically administered dexamethasone on plasma AVP and nitrate concentration after LPS injection. We found no significant changes in plasma AVP concentration when dexamethasone alone was injected when compared to saline-injected animals

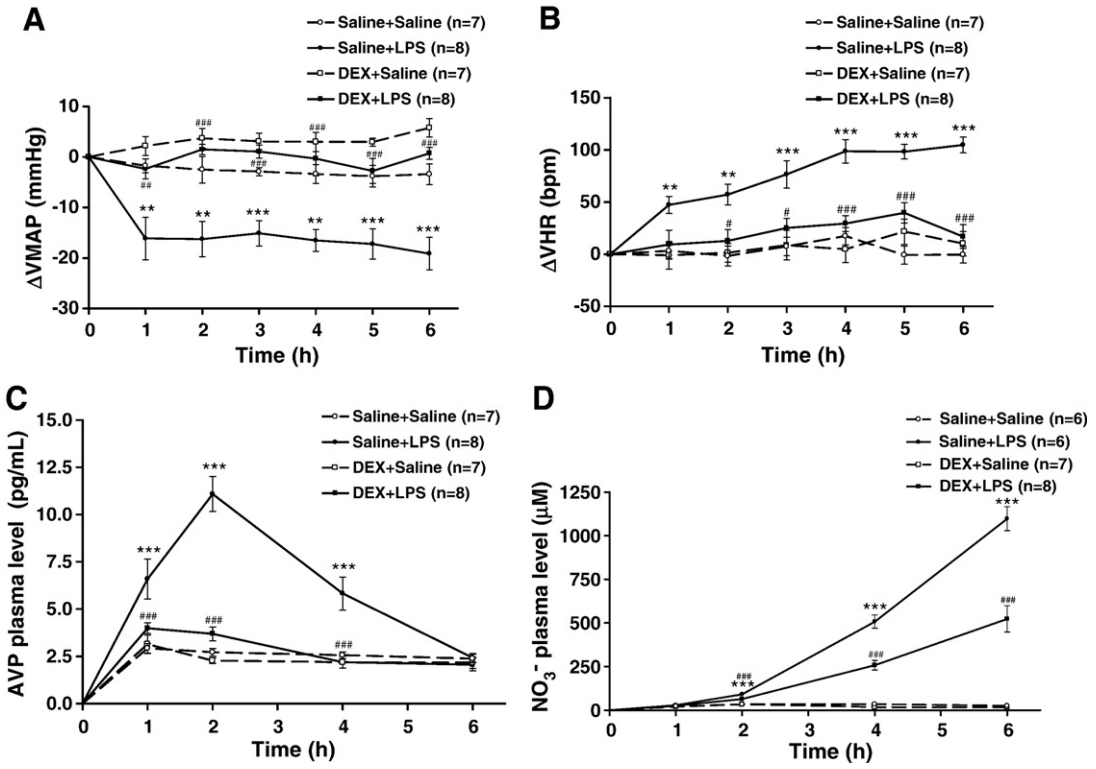

Fig. 2. Effect of intravenously administration of dexamethasone (1.0 mg/kg) during the endotoxemic shock on A: change in mean arterial pressure (ΔVMAP); B: change in heart rate (ΔVHR); C: AVP plasma level; D: NO_3^- plasma level. Values are means±SEM. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ significantly different compared with saline+saline group; $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$ and $^{\#\#\#}$ $p<0.001$ significantly different compared with saline+LPS group.

($p<0.001$). We also found a significant decrease in plasma AVP levels after the second and fourth hour of LPS plus dexamethasone injection, when compared to the rats treated with LPS plus saline ($p<0.001$). Plasma nitrate levels decreased in LPS plus dexamethasone treated animals when compared to LPS plus saline animals ($p<0.001$).

4. Discussion

The present study provides evidence that administration of low doses of dexamethasone attenuated the hypotension-induced by LPS and it seems that this recovery may not be due to the release of AVP, since the dexamethasone injection induced a decrease in plasma AVP levels.

The use of corticosteroids as adjunctive therapy for severe sepsis and septic shock has been a source of controversy for the past 35 years. There have even been reports suggesting the potential for harm associated with the administration of early high-dose corticosteroids in patients with severe sepsis and septic shock [21]. Recent trials have reported hemodynamic and survival benefits associated with the use of more physiological steroid replacement therapy in patients with vasopressor-dependent septic shock [22,23]. The rationale for the use of glucocorticoids in this setting of severe systemic inflammatory response has been the potent anti-inflammatory properties of these drugs [24]. Such effects include inhibition of cytokine production and prevention of the migration of circulating inflammatory cells into tissues [25]. It is evident from literature that there are more questions than answers in this important field and more pre-clinical data is needed.

Glucocorticoids are potent inhibitors of iNOS [19]. It has been demonstrated that nitrite/nitrate concentrations were markedly reduced in glucocorticoids-treated animals and similar findings have been observed in septic shock patients treated with hydrocortisone in a randomized crossover study [26].

The mechanisms related to the profound drop in blood pressure during septic shock have been extensively studied and partly explained by the induction of vasoactive enzymes. Some studies have demonstrated that the drop in MAP can be inhibited by using specific iNOS inhibitors that decrease NO production [27,28,6]. However, the benefits observed in animal models were not reproducible in humans [29,30]. These findings led the scientists to re-valuate the mechanisms by which NO may affect the vasculature during septic shock.

AVP is a nonapeptide with disulfide bridge between two cysteine amino acids. It is synthesized as a large prohormone in magnocellular neurons located in the paraventricular and supraoptic nuclei of the hypothalamus [31]. The hormone and neurophysin, an axonal carrier protein, then migrate via the supraoptic-hypophyseal tract to the axonal terminals of the magnocellular neurons, located in the pars nervosa of the posterior pituitary, where vasopressin is stored in granules. The regulation of vasopressin release is complex and can be classified into osmotic and nonosmotic stimuli (osmotic regulation) and severe hypovolemia and hypotension (hypovolemic regulation) are the most potent stimuli to vasopressin release. However, AVP has also been shown to be involved in the difficult mechanism of stress. AVP and the corticotrophin-releasing hormone are synthesized at the parvocellular neurons of the paraventricular nuclei (PVN), with each neuropeptide stimulating the secretion of the other. In nonstressfull situations, both CRH and AVP are secreted in the portal system in a circadian, pulsatile and highly concordant fashion. During acute stress there is an increase in the amplitude and synchronization of the PVN CRH and AVP pulsatile release into the hypophyseal portal system [31].

Tsuneyoshi and col. (2001) have reported a group of patients with septic shock in whom severe hypotension refractory to infusion of catecholamine was reversed using AVP infusion. In this way, it is reasonable to speculate that AVP deregulation contributes to the pathophysiology of septic shock [32].

A recent study from our laboratory has shown an increase in plasma AVP levels in endotoxemic rats during the first and second hours after LPS administration followed by a rapid decrease over the next few hours. This occurred at the time when the rats continued to have profound hypotension secondary to LPS administration. When animals were pre-treated with intracerebroventricular administration of aminoguanidine, a specific inhibitor of the iNOS, AVP plasma concentration was maintained well beyond the initial increase and blood pressure remained higher when compared to endotoxemic shock rats not treated with the iNOS blocker [6]. These data suggest an inhibitory effect of NOS in AVP release during this stage of the sepsis. In this study we found that Dex induced a decrease in nitrate plasma levels and an increase in MAP. However, the increase in AVP induced by LPS injection was attenuated by DEX administration. This may be explained by a different effect of NO into the central nervous system and systemically.

There have been recent developments in supportive care suggesting a role for AVP infusion as an alternative therapy for patients with septic shock refractory to standard vasopressor therapy [33]. This use is based on the fact that in septic patients, plasma AVP levels remain close to baseline physiological levels despite prominent reductions in blood pressure [34]. These findings led the authors to suggest that AVP levels in patients with septic shock might be inappropriately low [34]. Considering that there is an interrelation between the pathways which control the release of glucocorticoids and AVP, we may expect that the glucocorticoid replacement could act through the AVP release to maintain the hemodynamic function. However, in our study, we have observed a significant decrease in the plasma AVP level after LPS plus dexamethasone injection, when compared to the rats treated with LPS plus saline. These data suggest that the improvement in hemodynamics induced by dexamethasone administration is not mediated by AVP release in this septic shock model.

Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We thank Juliana Zanetti and Márcia Adriana Salles for their excellent technical assistance.

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