Blocking central leukotrienes synthesis affects vasopressin release during sepsis

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Abstract—Recent studies revealed that vasopressinergic neurons have a high content of cysteiny1 leukotriene C4 (LTC4) synthase, a critical enzyme in cysteiny1 leukotriene synthesis that may play a role in regulating vasopressin secretion. This study investigates the role of this enzyme in arginine vasopressin (AVP) release during experimentally induced sepsis. Male Wistar rats received an i.v. injection of 3-[1-(p-chlorobenzyl)-5-(isopropyl)-3-tert-butylthioindol-2-yl]-2, 2-dimethylpropanoic acid (MK-886) (1.0 μg/kg), a leukotrienes (LTs) synthesis inhibitor, or vehicle, 1 h before cecal ligation and puncture (CLP) or sham operation. In one group of animals the survival rate was monitored for 3 days. In another group, the animals were decapitated at 0, 4, 6, 18 and 24 h after CLP or sham operation, and blood was collected for hematocrit, serum sodium and nitrate, plasma osmolality, protein and AVP determination. A third group was used for blood pressure measurements. The neurohypophysis was removed for LTC4 synthase analysis by Western blot. Morbidity measurements. The neurohypophysis was removed for AVP determination. The central administration of MK-886. The increase in plasma AVP levels and hypothalamic LTC4 synthase content but did not alter the plasma protein and osmolality was not affected by the LTs blocker. In the final phase of sepsis, the plasma AVP level and the hypothalamic LTC4 synthase content were at basal levels. The central administration of MK-886 increased the hypothalamic LTC4 synthase content but did not alter the plasma and neurohypophysis AVP levels observed, or the blood pressure during this phase. These results suggest that the central LTs are involved in the vasopressin release observed during sepsis. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: cecal ligation and puncture, plasma, neurohypophysis, hypothalamus, LTC4 synthase.

Leukotrienes (LTs) have been implicated in pathological manifestations of inflammatory diseases, including asthma, rheumatoid arthritis, pulmonary fibrosis and infectious disease (Henderson, 1994; Nicosia et al., 2001; Flamand et al., 2007; Peters-Golden and Henderson, 2007). A growing body of evidence suggests that they play a major role in sepsis (Sprague et al., 1989; Morlion et al., 2000; Benjamim et al., 2005; Baenktler et al., 2006). Leukocytes have the capacity to generate large amounts of LTs derived from arachidonic acid via the 5-lipoxygenase (5-LO) pathway. The enzyme 5-LO, in conjunction with its helper protein 5-lipoxygenase-activating protein (FLAP), oxygenates arachidonic acid to form LTA4. This unstable intermediate can be hydrolyzed to form LTB4 or it can be conjugated with glutathione by cysteiny1 leukotriene C4 (LTC4) synthase to produce the cysteiny1 leukotrienes (cys-LTs) LTC4, cys-leukotriene D4 (LTD4) and cys-leukotriene E4 (LTE4) (Peters-Golden and Brock, 2003; Peters-Golden and Henderson, 2007). Although cells other than leukocyte generally do not have sufficient 5-LO and FLAP to synthesize appreciable amounts of LTs they can take up leukocyte-derived LTA4 and metabolize it into bioactive LTs by transcellular synthesis (Folco and Murphy, 2006). LTB4 is known to be a powerful chemotactic agent that stimulates the adhesion of leukocytes to the endothelium and their emigration across the endothelial wall and stimulates phagocytosis and microbial killing (Canetti et al., 2003; Coffey et al., 2004; Serezani et al., 2005, 2006; Flamand et al., 2007). The cys-LTs cause vasoconstriction, increase in vascular permeability, and hypotension and can contribute to host defense (Smadegard et al., 1982; Anderson and Blumer, 2000; Peres et al., 2007; Peters-Golden and Henderson, 2007).

LTs are extensively produced in several organs including the CNS (Wolfe, 1982; Hulting et al., 1985). The production of these substances in the brain is associated with the expression of 5-LO and FLAP in neurons of several brain regions, such as the hippocampus, cerebellum, thalamus, hypothalamus and brain stem (Lammers et al., 1996). LTB4 is produced relatively uniformly in the entire rat brain but the highest concentrations of LTC4 were detected in the hypothalamus and the median eminence (Lindgren et al., 1984; Miyamoto et al., 1987). Recent studies revealed that LTC4 synthase is selectively local-
ized in the hypothalamic and extrahypothalamic vasopressin systems, indicating a possible involvement of cys-LTs in neuroendocrine system and, especially vasopressinergic neural functions (Shimada et al., 2005). During sepsis, the pituitary gland is activated via blood-borne proinflammatory mediators and through a complex interaction between the autonomic nervous system and immune cells. Clinical and experimental studies report that in the initial phase of sepsis, high plasma vasopressin levels can be found in plasma of patients or experimental animals, and that this may help to restore blood pressure that tends to decrease due to cytokines and nitric oxide (NO) release. However, in the late phase, when the observed hypotension would normally stimulate vasopressin secretion, plasma levels can be inappropriately low (Landry et al., 1997; Landry and Oliver, 2001; Sharshar et al., 2003). Low plasma vasopressin levels are considered a deleterious consequence of an abnormal pituitary response, because they will contribute to the progression of sepsis, septic shock, multiple organ failure and death (Landry et al., 1997; Holmes et al., 2001; Maxime et al., 2007).

Vasopressin analogs in association or not with corticoids have been used therapeutically in the treatment of septic shock in humans (Annane et al., 2005; Delmas et al., 2005). Understanding the mechanisms responsible for the release of this hormone during sepsis is, therefore, also of clinical importance. The objective of this work was to investigate the effects of a centrally administered LTs blocker on the arginine vasopressin (AVP) release during sepsis induced by cecal ligation and puncture (CLP) in rats.

EXPERIMENTAL PROCEDURES

Animals

Wistar male rats (200–300 g) from the Animal Care Facility of Universidade de São Paulo (USP), Campus Ribeirão Preto were used in the present experiments. The rats were housed in controlled temperature (25 ± 1 °C) and photoperiod (12-h light/dark cycle) conditions, with food (Nuvilab CR-1, Nuvital Nutrientes, Paraná, Brazil) and water available ad libitum. All experimental protocols were performed according to the international guidelines on the ethical use of animal and approved by the Ethics Committee of USP (CEUA)–Campus Ribeirão Preto, minimizing the number of animals used and their suffering. Humane endpoints in shock research (Nemzek et al., 2004) were used as criteria to euthanize the CLP animals in high suffering immediately before or soon after the time-points defined in this study.

Experimental protocol

I.v. injections were performed using a 10 µl Hamilton syringe and a dental injection needle (200 µm outer diameter; Becton Dickinson do Brasil, São Paulo, Brazil). The volume of each injection was 2 µl for all protocols. Injection was performed over a period of 1 min, and an additional minute was allowed to elapse before the injection needle was withdrawn from the guide cannula, to avoid reflux.

The animals received an i.v. injection of dimethyl sulfoxide (DMSO) 5% as vehicle or 3-[(p-chlorobenzyl)-5-(isopropyl)-3- tert-butylthiindolyl-2-yl]-2, 3-dimethylpropanoic acid (MK-886) (Merck Frost Canada Ltd., Quebec, Canada), a FLAP and LTC4 inhibitor (Lam et al., 1994), 1 h before the CLP or sham operation. The dose of 1.0 µg/kg was established by previous work in our laboratory. In a first set of experiments the survival of the animals in each group was monitored for 3 days. In a second set, the animals were decapitated at 0, 4, 6, 18 or 24 h after surgery and the blood collected for determination of hematocrit, serum nitrate and sodium, plasma osmolality, protein and AVP measurements. The neurohypophysis was carefully removed and stored frozen at −70 °C for further measurement of vasopressin content by radioimmunoassay (RIA). The hypothalamus was dissected at 0, 4, 6 and 24 h time-points, rapidly frozen and stored for LTC4 synthase detection by Western blot. The blood pressure was determined in conscious and freely moving rats of the third group.

I.v. cannulation

Animals were anesthetized with 2,2,2-tribromoethanol (Acros Organics, Geel, Belgium, 250 mg/kg i.p.) and fixed in a stereotaxic frame. A stainless steel guide cannula (0.7 mm outer diameter) was introduced into the right lateral ventricle (coordinates: A: −0.8 mm, L: 1.4 mm, D: 3.2–3.7 mm). The cannula was attached to the bone with stainless steel screws and acrylic cement. A tight-fitting style was kept inside the guide cannula to prevent occlusion. After surgery, animals were treated with 100,000 U of benzyl-penicillin and allowed to recover for 5–7 days.

CLP

Induction of severe sepsis was performed by a CLP model as previously described (Wichterman et al., 1980; Torres-Dueñas et al., 2006; Corrêa et al., 2007; Oliveira-Pelegrin et al., 2009). Briefly, rats were anesthetized with 2,2,2-tribromoethanol (Acros Organics, Geel, Belgium, 250 mg/kg i.p.). Under sterile surgical conditions, a 2 cm midline incision was made on the ventral surface of the abdomen, and the cecum was exposed and ligated below the ileocecal junction without causing bowel obstruction. The cecum was punctured 10 times with a 18-gauge needle, and fecal contents were allowed to spill into the peritoneum. The cecum was repositioned in the abdomen, and the peritoneal wall and skin incisions were closed. Sham-operated animals underwent an identical laparotomy, but did not undergo ligation and puncture and served as controls. All animals received a subcutaneous injection of normal saline (20 ml/kg body weight) immediately after the surgery. The animals were allowed to recover in their cages with free access to food and water. All rats subjected to CLP developed early clinical signs of sepsis, including lethargy, piloerection, and tachypnea.

Determination of hematocrit, serum nitrate and sodium, plasma osmolality and protein

Hematocrit was measured by centrifugation, serum sodium by flame photometry (Micronal, São Paulo, Brazil) and plasma osmolality by freezing point depression (Precision System, Inc., Natick, MA, USA). Plasma protein was determined by a Bradford colorimetric assay (Microplate reader, Bio-Rad Laboratories, Hercules, CA, USA) and nitrate was quantified by chemoluminescence (Sieves 280 NOA, Sievers, Boulder, CO, USA).

Radioimmunoassay (RIA) for vasopressin

An RIA for AVP was performed as previously described (Corrêa et al., 2007). Briefly, plasma samples (1.0 ml) were extracted using the acetonе/petroleum ether method, lyophilized and stored at −70 °C until analysis. Neurohypophyses were homogenized in 0.1 N HCl. A 50 µl aliquot of the homogenate was used to measure protein content by the Bradford method and the remainder was used for RIA. At the moment of the assay the homogenate was diluted 1:4,000 in assay buffer. Standard reagents and incubation protocols were used for the peptide assays. AVP measurements were carried out with a commercial antisera (Peninsula Laboratories, Inc., San Carlos, CA, USA) at a final dilution of 1:40,000. The antisera is specific and shows essentially no
cross-reactivity with other known peptides. The buffer was Na₂HPO₄ (0.062 M) and Na₂EDTA (0.013 M) with 0.5% bovine serum albumin (BSA), pH 7.5. For peptide labeling, ¹²⁵I was purchased from a commercial supplier (Amersham Biosciences, Pittsburgh, PA, USA). A non-equilibrium assay was used with an incubation volume of 500 μl and an incubation time of 4 days at 4 °C. Bound hormone was separated from unbound by a secondary antibody produced in the laboratory of José Antunes-Rodrigues and Lucía L. K. Elias (Faculty of Medicine of Ribeirão Preto, USP, São Paulo, Brazil) where the RIA was performed. The minimum detection limit was 0.9 pg/ml and the coefficients of intra- and inter-assay variations were 7% and 11%, respectively. Neurohypophyseal hormone content was normalized by the protein content of each sample.

**LTC₄ synthase determination by Western blotting**

Each dissected hypothalamus was homogenized in 500 μl Tris buffer (0.25 M Tris–HCl, pH 6.8, containing 2% sodium dodecyl sulfate [SDS]). After incubation for 3 h at 25 °C, the sample was centrifuged (12,000 rpm, 70 °C) for total protein and LTC₄ synthase quantification. Total protein content of the hypothalamic precipitate was determined spectrophotometrically using the bicinchoninic acid assay (Smith et al., 1985). The quantification was done in 1 μl at 592 nm in a Nanodrop fluorospectrophotometer (Nanodrop Products, Wilmington, DE, USA). Equal amounts of protein in the hypothalamic extracts were separated by SDS-PAGE in a 12% acrylamide gel. Molecular weight markers of 10–250 kDa (Full-Range Rainbow, Amersham Biosciences, Pittsburgh, PA, USA) were applied in a lateral lane of the gel for visualization of separation and transfer quality. Following electrophoresis, the proteins were blotted to a nitrocellulose membrane (0.45 μm; Amersham Biosciences, Pittsburgh, PA, USA) in a tank blotting system. Western blotting was performed under a current of 350 mA during 1.5 h in a Tris–glycine transfer buffer (25 mM Tris, 400 mM glycine, containing ethanol in water) (1:5 v/v). After blocking the nitrocellulose membrane by incubation for 1 h under light agitation with defatted dried milk (5%, Nestlé) in phosphate buffer saline (PBS) 0.1 M containing and Tween 0.2%, followed by two rinses in PBS, the membrane was incubated with an antibody against LTC₄ synthase diluted 1:1000 in PBS buffer containing Tween (0.05%), normal goat serum (1%) and defatted dried milk (5%). The antibody generated in rabbit was kindly donated by Masayoshi Abe (Fukuoka University, Fukuoka, Japan). After incubation for 16 h at 4 °C, the membrane was incubated with a secondary antibody (anti-rabbit immunoglobulin G) conjugated with horseradish peroxidase (Dako North America, Inc., Carpinteria, CA, USA, diluted 1:4000 in PBS containing 1% dried milk). A chemiluminescence reaction kit (enhanced chemiluminescence [ECL], Amersham Biosciences, Pittsburgh, PA, USA) followed by exposure to X-ray film was used for detection of the LTC₄ synthase. The X-ray films were scanned and the ECL detected protein bands were quantified by ImageJ (National Institutes of Health [NIH], public domain program). The results were transformed into arbitrary units.

**Blood pressure determination**

The animals were anesthetized with TBE 2.5% and had a polyethylene catheter inserted into the femoral artery and filled with 0.3% heparin in sterile saline. The PE-10 (Becton Dickinson, Franklin Lakes, NJ, USA) segment was introduced into the femoral artery until its tip reached the abdominal aorta. The free end of the catheter was guided under the skin to be exteriorized on the back between the scapulae. Starting after the next day, basal mean arterial blood pressure (MAP) was continuously measured using a Biopac System (BIOPAC Systems, Inc., Goleta, CA, USA) connected to a disposable pressure transducer (Maxim Medical, Athens, TX, USA). Subsequently, the animals were submitted to CLP surgery. The MAP was again measured for up to 6 h (early phase of sepsis) and in the next day between 22 and 24 h (late phase of sepsis) after experimental procedure.

**Drugs**

MK-886 (Merck Frosst Canada Ltd., Quebec, Canada) is a FLAP and LTC₄ synthase inhibitor (Lam et al., 1994). It was dissolved in DMSO 5% in saline for i.c.v. injection.

**Data analysis**

Survival rate was expressed as percentage, and a Log-rank (Mantel-Cox) Test was used to determine differences in survival curves. The other data are reported as mean±SEM. Statistical analyses were performed by two-way analysis of variance (ANOVA) and a post hoc Tukey test. Values of *P*<0.05 were considered statistically significant. RIA data were analyzed by logit transformation of the raw data.

**RESULTS**

**Effect of central administration of MK-886 on the survival rate in CLP septic rats**

Animals submitted to CLP presented around 80% mortality on the third day after surgery. In contrast, animals submitted to CLP and that received previously an i.c.v. injection of MK-886 presented at this time only around 10% mortality at this time point. This difference between the groups was statistically significant (*P*<0.05). In the sham operated group (Sham+DMSO 5% or Sham+MK-886), all rats survived for 3 days after surgery (Fig. 1).

**Effect of central administration of MK-886 on plasma and neurohypophysis AVP levels and hypothalamus LTC₄ content**

Sepsis induced by CLP produced a significant increase (*P*<0.05) in plasma AVP levels at 4 and 6 h after surgery with a peak at 4 h. At 24 h the levels had dropped to basal levels. Pretreatment with MK-886 (1.0 μg/kg) blocked the increase (*P*<0.05) in plasma AVP levels observed in consequence of CLP. In an attempt to correlate changes in plasma AVP levels with those found in the brain, we also determined the AVP content in the neurohypophysis. The changes in the neurohypophyseal AVP content in CLP rats

![Fig. 1. Effect of central administration of MK-886 on the survival rate.](image-url)

* *P*<0.05 compared to CLP+DMSO 5%; ** *P*<0.05 compared to Sham+DMSO 5% (Log-rank test). n=Number of animals.
were in the opposite direction compared to those observed in plasma AVP levels, showing a marked reduction \((P<0.05)\) at 4, 6 and 18 h and a return to control levels at 24 h after CLP surgery (Fig. 2). Pretreatment with MK-886 partly blocked the decrease \((P<0.05)\) in neurohypophysial AVP content.

When analyzing hypothalamic LTC\(_4\) synthase content in CLP septic rats we noted an increase \((P<0.05)\) at 4 h after surgery and a return to control levels at 24 h after CLP surgery (Fig. 2). Pretreatment with MK-886 partly blocked the decrease \((P<0.05)\) in neurohypophysial AVP content.

Effect of central administration of MK-886 on serum nitrate and sodium, hematocrit, plasma osmolality and protein

CLP had no effect on serum sodium levels in any of the periods studied, but we observed a progressive increase \((P<0.05)\) in nitrate levels after 4 h of CLP. Plasma protein levels of the CLP group gradually decreased \((P<0.05)\) 4 h after surgery and remained reduced until 24 h. Osmolality was reduced \((P<0.05)\) at 18 h and the hematocrit showed an increase \((P<0.05)\) at 4 h and returned to basal levels 24 h after surgery. Pretreatment with MK-886 did not affect substantially hematocrit, plasma protein levels, and osmolality and serum sodium (Table 1) but caused slight reduction in nitrate concentrations at every time-point studied (Fig. 4).

Effect of central administration of MK-886 on blood pressure

The administration of MK-886 increased \((P<0.05)\) the blood pressure in the early phase of sepsis compared to the group that received the vehicle only. But this difference disappears in the late phase. The vehicle (DMSO 5%) itself seems to cause fluctuations on the blood pressure decrease that usually occurs during the sepsis (Fig. 5).

DISCUSSION

The present study was designed to examine whether LTs may modulate vasopressin release during experimental sepsis and whether this may affect survival rate. We saw that central injection of the LT synthesis blocker MK-886 prevented the increase in plasma AVP levels that normally occurs as a consequence of CLP-induced experimental sepsis in rats. Similarly, the increase in LTC\(_4\) synthase

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**Fig. 2.** Effect of central administration of MK-886 on plasma and neurohypophysis vasopressin content. The results are expressed as means±SEM. * \(P<0.05\) compared to Sham group. ‡ \(P<0.05\) compared to CLP+DMSO group. \(n=5–6\) each group.

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**Fig. 3.** Effect of central administration of MK-886 on hypothalamus LTC\(_4\) synthase content. The results are expressed as means±SEM for four membranes. * \(P<0.05\) compared to Sham group and other time-points within the group. ‡ \(P<0.05\) compared to CLP+DMSO group. In the upper panel a photo of a representative Western blot.
The results are expressed as means ± SEM. * indicates the i.c.v. injection and the second arrow indicates the induction of stimulus (Sham or CLP). *P < 0.05 compared to the 0 time point.

Table 1. Effect of central administration of MK-886 on hematocrit, serum sodium, osmolality, and plasma protein levels

| Time (h) | 0     | 4     | 6     | 18    | 24    |
|---------|-------|-------|-------|-------|-------|
| Hematocrit (%) |       |       |       |       |       |
| Sham + DMSO | 44.7 ± 0.6 | 45.2 ± 0.8 | 45.6 ± 0.9 | 43.3 ± 0.9 | 44.3 ± 0.7 |
| CLP + DMSO | 45.6 ± 0.6 | 52.7 ± 0.2** | 50.4 ± 1.6** | 48.8 ± 1.5 | 45.6 ± 0.8 |
| Sham + MK-886 | 44.5 ± 0.6 | 45.0 ± 0.6 | 46.3 ± 0.9 | 45.3 ± 0.6 | 46.0 ± 1.2 |
| CLP + MK-886 | 44.2 ± 1.0 | 50.7 ± 0.9** | 49.0 ± 1.5* | 47.4 ± 1.5 | 47.4 ± 0.9 |

Serum sodium (mEq/kg)

| Sham + DMSO | 133.5 ± 1.4 | 138.4 ± 3.1 | 140.8 ± 2.1 | 140.0 ± 3.6 | 133.4 ± 1.4 |
| CLP + DMSO | 132.0 ± 2.8 | 135.5 ± 1.5 | 137.8 ± 2.1 | 135.4 ± 2.9 | 131.3 ± 2.5 |
| Sham + MK-886 | 134.7 ± 0.8 | 139.4 ± 0.2 | 141.6 ± 1.6 | 141.6 ± 2.1 | 136.0 ± 1.2 |
| CLP + MK-886 | 134.4 ± 1.8 | 137.6 ± 1.0 | 137.8 ± 3.2 | 137.3 ± 4.2 | 135.8 ± 2.4 |

Osmolality (mosM/kg)

| Sham + DMSO | 277.8 ± 3.8 | 276.2 ± 1.5 | 275.8 ± 1.4 | 269.3 ± 3.1 | 275.0 ± 4.4 |
| CLP + DMSO | 282.4 ± 2.3 | 279.2 ± 1.8 | 273.4 ± 2.2 | 263.6 ± 2.8* | 265.0 ± 2.6* |
| Sham + MK-886 | 276.0 ± 2.3 | 277.3 ± 2.2 | 274.8 ± 2.6 | 271.2 ± 5.1 | 268.2 ± 2.3 |
| CLP + MK-886 | 280.2 ± 2.9 | 280.5 ± 1.4 | 276.4 ± 3.8 | 262.3 ± 2.1* | 265.0 ± 2.6* |

Plasma protein (g/dL)

| Sham + DMSO | 5.2 ± 0.1 | 4.7 ± 0.1 | 4.9 ± 0.1 | 5.1 ± 0.2 | 4.9 ± 0.1 |
| CLP + DMSO | 5.1 ± 0.2 | 4.2 ± 0.1** | 4.3 ± 0.1** | 4.5 ± 0.2** | 4.4 ± 0.2** |
| Sham + MK-886 | 5.5 ± 0.1 | 4.8 ± 0.2 | 4.8 ± 0.2 | 4.9 ± 0.1 | 5.3 ± 0.4 |
| CLP + MK-886 | 5.4 ± 0.1 | 4.3 ± 0.2** | 4.2 ± 0.1** | 4.3 ± 0.2** | 4.8 ± 0.1* |

Measurements are expressed as mean ± SEM for each group comprising five to six animals.

* P < 0.05 compared to the sham groups.
** P < 0.05 compared to 0 and 24 h within the group.
* P < 0.05 compared to the other time-point within the group.
+ P < 0.05 compared to 0 h within the group.

content in the hypothalamus that coincides with the increase in plasma AVP levels in CLP rats was blocked by previous treatment with the MK-886. These data suggest that blocking an enzyme crucial to the cys-LTs biosynthesis prevents the AVP secretion normally observed during experimental sepsis.

It is still unclear whether the prevention of AVP secretion may be due to an effect of the drug on synthesis or release of the hormone from its stocks in the neurohypophysis. A reduction in AVP synthesis could, however explain the discrepancy between plasma and neurohypophyseal AVP contents, especially at 6 h.

To our knowledge this is first study analyzing hypothalamic LTC₄ synthase content after an experimental challenge that induces AVP release. The increase in enzyme levels found in the early phase of sepsis suggests that cys-LTs are produced at a higher rate and that this may contribute to the increase in AVP release from the neurohypophysis. We confirmed this by analyzing AVP neurohypophyseal content which decreased after CLP and was partially reversed with i.c.v. injection of MK-886. A neu-

![Fig. 4. Effect of central administration of MK-886 on serum nitrate. The results are expressed as means ± SEM. * P < 0.05 compared to the Sham group. † P < 0.05 compared to the 0 time point. n = 5–6 each group.](image)

![Fig. 5. Effect of central administration of MK-886 in the blood pressure. The results are expressed as means ± SEM. The first arrow indicates the i.c.v. injection and the second arrow indicates the induction of stimulus (Sham or CLP). * P < 0.05 compared to the vehicle group. n = 4 each group.](image)
The neuroendocrine role of LTs was first suggested by demonstration of colocalization of gonadotropin-releasing hormone (GnRH) and LTC₄ in neurons of the median eminence in rats (Hulting et al., 1985). Subsequent in vitro studies showed that the release of luteinizing hormone (LH) from pituitary cells in response to GnRH was partly mediated by LTs. The response appears to be LTC₄ specific since LTB₄ did not alter the release of LH (Hulting et al., 1985). LTC₄ synthase was also detected as being selectively localized in neurons of the paraventricular and supraoptic nuclei of the hypothalamus and in the suprachiasmatic and retrochiasmatic nuclei in the mouse. This LTC₄ synthase localization seems to be specific for vasopressinergic neurons since oxytocinergic neurons did not show LTC₄ synthase immunoreactivity suggesting that the enzyme is specially involved in vasopressin functions in the neuroendocrine system (Shimada et al., 2005).

Increases in serum sodium levels and osmolality are strong stimuli for vasopressin release (Cunningham and Sawchenko, 1991). In recent experiments we saw that Cy-Lts seem to affect AVP secretion during osmotic challenge (data not shown) since under this condition MK-886 increased even more the osmotically induced AVP secretion. In the present experiment representing, sepsis conditions, however, we could not see any increase in serum sodium and osmolality. Instead, we saw a decrease in osmolality at 18 and 24 h after the CLP. Furthermore pretreatment with MK-886 did not alter any of these parameters.

Hypovolemia and hypotension that usually occur during sepsis may also lead to AVP release (Parrillo, 1993). In fact we could see an increase in the hematocrit at 4 h of sepsis concomitant with the increase in plasma vasopressin levels. Hypovolemia can be due to the liberation of inflammatory mediators that can cause damage to endothelial cell, with a consequent capillary leakage and high fluid and protein loss into the interstitial space. We observed a decrease in plasma protein concentration after CLP surgery in this and in a previous study (Corrêa et al., 2007; Oliveira-Pelegrin et al., 2009). This is probably due the shift of protein from plasma to interstitial spaces (Fishel et al., 2003). Interestingly, the central pretreatment with the LTC₄ synthase blocker MK-886 did not substantially affect the plasma protein and hematocrit. Therefore, hypovolemia cannot be taken as the sole factor responsible for the vasopressin release observed during sepsis. Previous work in our laboratory, using the same (Pancoto et al., 2008) or a similar (Corrêa et al., 2007; Oliveira-Pelegrin et al., 2009) experimental model of sepsis showed a progressive hypotension after 3 h of CLP. This hypotension was due to NO derived from inducible nitric oxide synthase (iNOS), since pretreatment with the iNOS inhibitor aminoguanidine, prevented the increase in plasma nitrate levels and the drop in blood pressure (Corrêa et al., 2007). In the present study we showed that there is in fact a progressive increase in the plasma nitrate levels, which are an indicator of NO formation (Krafft-Jacobs et al., 1997) and that the i.c.v. injection of MK-886 caused only a minor reduction in nitrate production. We performed additional experiments and found that there was increase in blood pressure in the early phase of sepsis when using MK-886. Therefore hypotension can be one factor responsible for vasopressin secretion during the early phase of sepsis. Experiments are under way in our laboratory to find out the role of cys-LTs in non-septic hypovolemia and/or hypotension situations.

At 24 h after CLP, as expected, plasma AVP levels were basal, as shown in previous studies (Corrêa et al., 2007; Pancoto et al., 2008; Oliveira-Pelegrin et al., 2009). Such low levels of plasma AVP in the late phase of sepsis are still enigmatic because they occur despite the continuous hypotension reported (Torres-Duenas et al., 2006). These low plasma AVP levels, however, are in conformity with the observation that the pretreatment with MK-886 did not alter the plasma AVP levels at 24 h in spite of a marked increase in hypothalamic LTC₄ synthase content. We propose that this increase may be due to an upregulation of LTC₄ synthase gene activity that was suppressed by MK-886 during the early phase of sepsis. The fact that at 24 h this increase did not reflect an increase in plasma AVP levels may be due to other factors affecting the hormone release in the late phase of sepsis. For example the NO levels were not substantially affected by pretreatment with MK-886, and NO was previously reported to be one of the factors responsible for the decreased AVP release during the late phase of sepsis (Corrêa et al., 2007; Oliveira-Pelegrin et al., 2009). An interesting observation in general terms was that the MK-886 pretreated CLP animals had a much improved survival rate. A similar effect was also seen in CLP-induced sepsis in mice when the drug was administered by gavage and was attributed to the reduction of sepsis-induced hypotension (Benjamim et al., 2005). Since we saw increase of blood pressure and decrease of vasopressin secretion in the early phase of sepsis we can presume that the hypotension or the elevated secretion of vasopressin could be deleterious for the organism. Moreover we used a blocker for V1a receptor vasopressin 1 h before CLP surgery and the results showed that similar to MK-886 the drug increased survival rate (results not shown).

The production of cys-LTs has also been related to neurological inflammation (Ciana et al., 2006) that can worsen the septic conditions. Septic shock induces inflammatory and non-inflammatory processes that affect endothelial cells, glial cells, and neurons and that induce blood-brain barrier breakdown, dysfunction of intracellular metabolism, and cell death (Siami et al., 2008). Although we did not analyze neural cys-LTs content our results suggest it may be reduced in the early phase of sepsis when we pretreated the rats with the LTC₄ synthase blocker MK-886. This decrease could be beneficial in the late phase of septic condition despite the apparently deleterious effects of decreased plasma AVP levels.

CONCLUSION

In conclusion, the results of this study suggest that cys-LTs play a role in vasopressin release and that they affect survival rate during experimental sepsis.

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