Norepinephrine and vasopressin counteract anti-inflammatory effects of isoflurane in endotoxemic rats

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Abstract. Volatile anesthetics such as isoflurane have been shown to offer anti-inflammatory effects during experimental endotoxemia whereas the α-adrenergic vasopressor norepinephrine exhibits proinflammatory properties on systemic cytokine release under the same conditions. However, during major surgery and in patients with systemic inflammatory response syndrome or sepsis both agents are frequently administered concurrently. We therefore aimed to investigate the influence of preexisting i.v. administration of noradrenaline or vasopressin on the anti-inflammatory effects of isoflurane during experimental endotoxemia. Anesthetized, ventilated Sprague-Dawley rats (n=7 per group) were randomly treated. In the LPS-only group, animals received lipopolysaccharide (LPS, 5 mg/kg, i.v.) with no further specific treatment. In the LPS-isoflurane group, isoflurane inhalation at 1 MAC was initiated simultaneously with induction of endotoxemia (LPS 5 mg/kg, i.v.). Animals in the LPS-isoflurane-norepinephrine group received norepinephrine infusion at 50 μg/kg/h 10 min prior to injection of LPS and inhalation of isoflurane. In the LPS-isoflurane-vasopressin group, vasopressin was administered at 0.5 IE/kg/h 10 min prior to LPS and isoflurane. In the LPS-norepinephrine and the LPS-vasopressin groups the infusion of each vasopressor was started prior to LPS injection without any application of isoflurane. A Sham group served as the control. After 4 h of endotoxemia, plasma levels of TNFα, IL-1ß and IL-10 were measured. Alveolar macrophages (AM) were cultured *ex vivo* for nitrite assay. Induction of endotoxemia resulted in a significant rise in measured plasma cytokines and nitrite production from cultured AM. Inhalation of isoflurane significantly attenuated plasma levels of TNFα (-65%) and IL-1ß (-53%) compared to the LPS-only group whereas it had no effect on nitrite production from cultured AM. Preexisting infusions of norepinephrine or vasopressin abolished the anti-inflammatory effects of isoflurane. The data demonstrate that the administration of norepinephrine or vasopressin both counteracted the anti-inflammatory effects of inhaled isoflurane on proinflammatory cytokine release during experimental endotoxemia in rats.

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Introduction

The systemic inflammatory response syndrome (SIRS) and clinical sepsis are significant clinical problems following major surgery and in intensive care medicine. Despite considerable therapeutic improvements the associated mortality rates are still high. The pathophysiology of SIRS and sepsis include a cascade of cytokine release from immunocompetent cell populations like mononuclear cells and tissue macrophages. Proinflammatory cytokines are important mediators and promoters of the pathophysiologic sequences of any kind of systemic inflammation. Specifically, IL-1ß and TNFα act as key mediators in the early phase of the immune response, mediating adverse effects of inflammation such as systemic vasodilation, organ injury and shock (1).

Basic therapeutical approaches to restore hemodynamic stability and organ perfusion include fluid rescuscitation and infusion of vasopressors. Norepinephrine has been favored due to its efficiency to restore and maintain adequate systemic blood pressure in septic patients (2). Over time, there is a vascular hyporeactivity to catecholamines most likely due to excessive nitric formation and an activation of adenosine-triphosphate-sensitive K^+ channels and a reduction in Ca^{2+} influx (3). Here, vasopressin has been shown effective in reversing catecholamine-resistant hypotension in septic shock (4,5).

There is evidence that catecholamines exhibit pro-inflammatory properties (6) whereas the impact of vasopressin on systemic inflammation thus far has not been investigated in detail.

The use of volatile anesthetics for long-term sedation in intensive care medicine is of growing interest (7,8). In addition to their intended narcotic effects, volatile anesthetics modulate the inflammatory response through decreasing the release of

proinflammatory cytokines from stimulated mononuclear cells *in vitro* and during experimental endotoxemia *in vivo*, respectively (9-12). More recently, we found that even a brief exposure (50 sec) to isoflurane markedly attenuated levels of proinflammatory cytokines in the plasma of endotoxemic rats (13).

Patients suffering from SIRS or sepsis often receive respiratory and vasopressor therapy. In addition, they require sedation and are frequently scheduled for surgical interventions. In both cases, volatile anesthetics are potentially used. Thus far, the influence of vasopressor therapy on the inflammatory response during endotoxemia and its impact on coexisting administration of a volatile anesthetic has not been investigated.

We therefore aimed to evaluate the effects of norepinephrine and vasopressin in experimental endotoxemia with and without coexisting inhalation of isoflurane in a randomized animal study.

Materials and methods

After permission of the governmental review board for the care of animal subjects (Regierungspraesidium Darmstadt, Germany), the study was performed with 49 male Sprague-Dawley rats (Janvier Le Genest-St-Isle, France; mean body weight 550±40 g). Animals received care in compliance with the 'Guide for the Care and Use of Laboratory Animals', National Academic Press, Washington D.C., 1996, which is enforced by local regulations.

Initially, all rats were anesthetized with pentobarbital (Pentobarbital, Merial, Hallbergmoos, Germany; 50 mg/kg, i.p.) and fentanyl (Fentanyl, Jannssen-Cilag GmbH, Neuss, Germany; 0.05 mg/kg, i.p.). Animals were placed supine on a heating pad, tracheotomized, endotracheally intubated with a 13-G canula (Abbott, Wiesbaden, Germany) and ventilated with an infant ventilator (Stephanie®, Stephan, Gackenbach, Germany) [p_{max},1.6 kPa; PEEP, 0.4 kPa; respiratory rate (RR), 30 per min; and inspiratory oxygen fraction (F_IO_2), 0.21]. Respiratory settings were adjusted to maintain normocapnia according to hourly performed arterial blood gas analyses. The respirator allowed the administration of volatile anesthetics by a standard vapor (Draeger, Luebeck, Germany).

Fluid-filled polythene catheters were inserted into the right inguinal vein and artery (ID, 0.58 mm; OD, 0.96 mm; SIMS Portex, Hythe, UK) for infusion of fluids (saline 0.9% 12 ml/kg/h) and anesthetics and for measurement of arterial blood gases, blood pressure and heart rate, respectively. Body temperature was monitored with a rectal probe and kept stable throughout the protocol at 37-38˚C. Following a 30-min stabilization period, seven groups of rats (n=7, each group) were randomly treated as follows. In the Sham group, the LPS-only group, the LPS-norepinephrine (LPS-nor) and the LPS-vasopressin (LPS-vaso) groups, general anesthesia was maintained by continuous i.v. infusion of pentobarbital (5-10 mg/kg/h) and fentanyl (2.5-5 μg/kg/h) throughout the experiment. For those animals receiving isoflurane, anesthesia was maintained by the administration of isoflurane at 1.0 MAC (1.1 vol%, human minimum alveolar concentration) supplemented by fentanyl (2.5-5 μg/kg/h, i.v.). The expiratory concentration of isoflurane was measured continuously by a monitor for volatile anesthetics (Vamos, Draeger, Luebeck, Germany).

With the exception of the Sham group, all animals received endotoxin (LPS from *E. coli* 055:B5, Sigma-Aldrich, Deisenhofen, Germany; 5 mg/kg, i.v.).

In the LPS-isoflurane (LPS-iso) group, isoflurane (Forane, Abbott, Wiesbaden, Germany) was administered at 1 MAC simultaneously with injection of LPS (5 mg/kg, i.v.). In the LPS-isoflurane-norepinephrine (LPS-iso-nor) group administration of isoflurane was combined with the continuous infusion of norepinephrine (Arterenol, Merck, Darmstadt, Germany) at a dosage of 50 μg/kg/h whereas the LPS-isoflurane-vasopressin (LPS-iso-vaso) group received a continuous infusion of vasopressin (Pitressin, Pfizer, Karlsruhe, Germany) at 0.5 IE/kg/h. Infusion of both vasopressors were started immediately before injection of LPS. Likewise, animals of the LPS-norepinephrine and the LPS-vasopressin groups received the respective vasopressor (norepineprine, 50 μg/kg/h and vasopressin, 0.5 IE/kg/h) with the LPS injection, but without concurrent administration of isoflurane.

Measurements were performed at baseline (prior to pharmacologic interventions and induction of endotoxemia, respectively) and then hourly after LPS injection until the end of the protocol, respectively. Specifically, baseline values were compared with the measurement at 1 h following LPS (Mea 1) and at the end of the protocol (Mea 4, 4 h following LPS).

After 4 h of endotoxemia, animals were exsanguinated, and plasma samples were obtained. A bronchoalveolar lavage (BAL) was performed with 8 aliquots of 10 ml phosphate-buffered saline (PBS, Serva, Heidelberg, Germany). BAL-fluid was centrifugated twice at 1500 rpm for 10 min. BAL-derived cells were re-suspended in RPMI-1640 culture medium supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, and 10% fetal bovine serum (Gibco-BRL, Eggenstein, Germany).

Cell viability was assessed by trypan blue exclusion. Cells were plated in 24-well culture plates ($0.2x10^6$ vital cells/well) and cultured for 2 h (37˚C, 5% CO_2, and 21% O_2) to adhere. Subsequently, cells were washed. Adherent cells were regarded as alveolar macrophages (AM), covered with the aforementioned culture medium and cultured for 24 h.

Nitric oxide (NO) release from AM was determined by evaluating the accumulation of its oxidation product nitrite in supernatants with the Griess reaction. Absorbance at 540/595 nm and comparison with a nitrite standard provided nitrite concentrations.

Plasma levels of TNFα, IL-1ß and IL-10 were determined by enzyme-linked immunosorbent assay (ELISA, R&D Systems, Wiesbaden, Germany) according to the manufacturer's instructions.

Since pentobarbital was administered in two different modalities (i.p. single shot and i.p. with subsequent i.v. infusion), we performed an additional study to exclude the relevant influence of these different modes of pentobarbital administration on the immune response in the present model. In 6 rats, (pentobarbital-induction and continuous infusion group, PICIG), anesthesia was induced by i.p. injection of pentobarbital and fentanyl (50 mg/kg and 0.05 mg/kg,

Table I. Heart rate [HR (1/min)] as determined before (baseline) and 4 h following induction of endotoxemia (Mea 4), respectively.[a]

	Sham	LPS-only	LPS-iso	LPS-iso-nor	LPS-iso-vaso	LPS-nor	LPS-vaso
Baseline	370 (15)	403 (11)	391 (12)	386 (13)	372 (19)	398 (8)	418 (13)
Mea 4	356 (12)	394 (13)	369 (5)	377 (6)	336 (11)	418 (5)	360 (14)

[a]Data are represented as the mean (± SEM).

Table II. Mean arterial blood pressure [MAP (mmHg)] as determined before (baseline) and 4 h following (Mea 4) the induction of endotoxemia, respectively.[a]

	Sham	LPS-only	LPS-iso	LPS-iso-nor	LPS-iso-vaso	LPS-nor	LPS-vaso
Baseline	122 (3)	131 (5)	123 (4)	126 (6)	134 (5)	127 (5)	123 (5)
Mea 4	110 (3)	98 (5)	98 (5)	101 (5)	110 (6)	96 (5)	127 (5)

[a]Data are represented as the mean (± SEM).

Table III. Arterial oxygen tension, p_aO_2 (mmHg) at baseline and 4 h (Mea 4) following LPS injection.[a]

	Sham	LPS-only	LPS-iso	LPS-iso-nor	LPS-iso-vaso	LPS-nor	LPS-vaso
Baseline	108 (2)	102 (2)	110 (3)	111 (2)	110 (2)	104 (2)	108 (3)
Mea 4	114 (3)[b]	87 (3)[b]	89 (3)[b]	93 (8)[b]	98 (4)[b]	93 (3)[b]	97 (1)[b]

[a]Data are represented as the mean (± SEM). ANOVA on ranks, Student-Newman-Keuls test ($p<0.05$); [b]versus baseline.

respectively). Subsequently, animals received tracheotomy with tracheal intubation, respiratory support and arterial and venous canulation as described above. Anesthesia was maintained by continuous infusion of pentobarbital and fentanyl (7.5 mg/kg/h and 3.75 μg/kg/h). After completion of the surgical preparation, LPS was injected intravenously at 5 mg/kg.

In 6 further rats, (pentobarbital-induction-only group, PIOG), induction of anesthesia was performed by i.p. injection of pentobarbital and fentanyl (50 mg/kg; 0.05 mg/kg, respectively). Following surgical preparation, analgesia was supplemented by continuous infusion of fentanyl (3.75 μg/kg) with no additional pentobarbital. Rats received LPS (5 mg/kg, i.v.) following completion of instrumentation.

Due to the long half-life of pentobarbital, we expected that animals would recover from the narcotic effects 3-4 h after its initial application. Therefore, the observation period was defined as 3 h following induction of endotoxemia. Blood samples were withdrawn prior to the injection of LPS (baseline) and then every 30 min following LPS injection until the end of the protocol (180 min), and plasma levels of TNFα were measured to detect the relevant influence of the mode of application of pentobarbital on the systemic inflammatory reaction.

Statistical analysis. For statistical analysis the SigmaStat® software package (version 2.0, Jandel Scientific, Erkrath, Germany) was used. Data were analyzed with the Kruskal Wallis one way analysis of variance on ranks and the Student-Newman-Keuls *post hoc* test. The α error level was set to 0.5. For the supplementary performed study on the influence of pentobarbital on the inflammatory reaction, groups were compared at each time point using the t-test.

Results

Hemodynamics and oxygenation. If not otherwise specified, *in vivo* parameters were analyzed from measurements at baseline, and at the end of protocol following 4 h of experimental endotoxemia.

At baseline, there were no differences in parameters of hemodynamics and oxygenation among the groups (Tables I-III). Experimental endotoxemia over 4 h neither result in a significant change in heart rate (HR, Table I) nor in mean arterial blood pressure (MAP, Table II) when the LPS-only group was compared to Sham operated animals. Only values of arterial oxygen tension (p_aO_2, Table III) were found significantly reduced after 4 h in all groups receiving LPS with no differences between these endotoxemic groups.

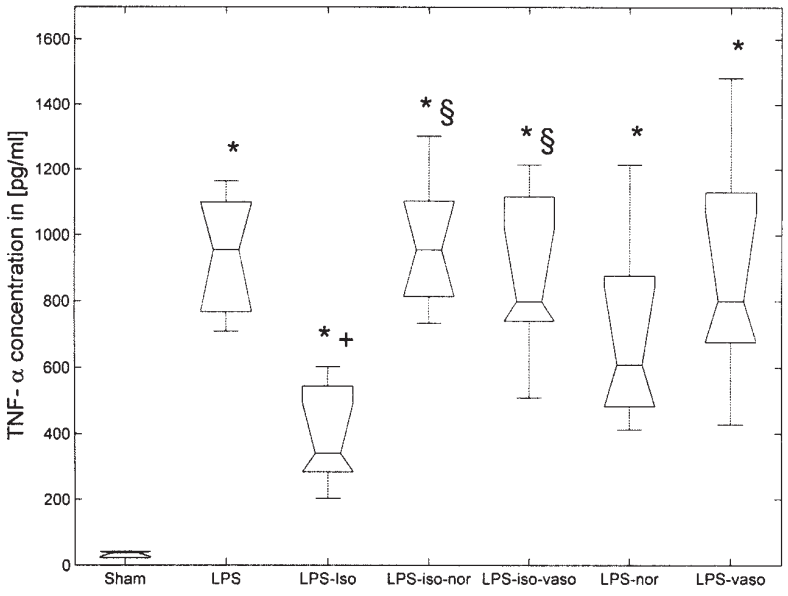

Figure 1. Levels of immunoreactive TNFα in plasma (pg/ml) after 4 h of endotoxemia as determined by ELISA. Data displayed as box plots (median, 1st-3rd quartile). ANOVA on ranks, Student-Newman-Keuls test ($p<0.05$); (*) versus Sham, (§) versus LPS-iso, (+) versus LPS-only.

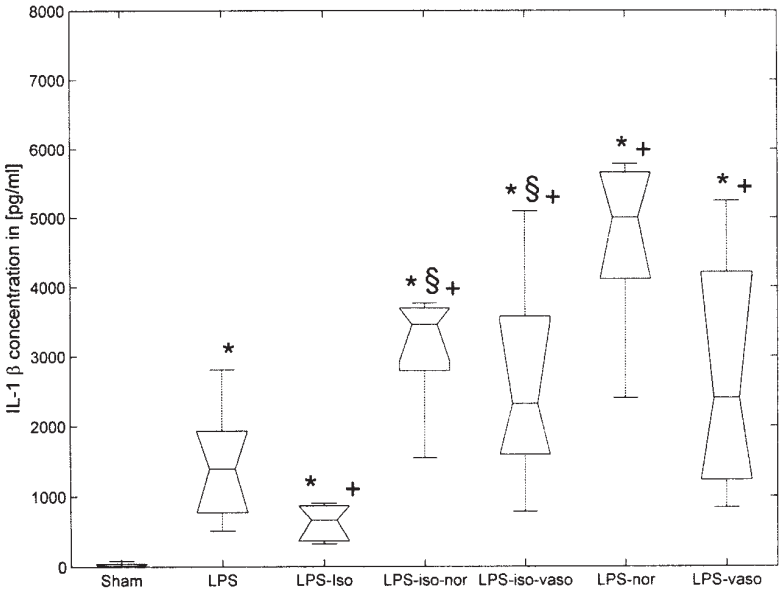

Figure 2. Levels of immunoreactive IL-1ß in plasma (pg/ml) after 4 h of endotoxemia as determined by ELISA. Data displayed as box plots (median, 1st-3rd quartile). ANOVA on ranks, Student-Newman-Keuls test ($p<0.05$); (*) versus Sham, (§) versus LPS-iso, (+) versus LPS-only.

The MAP of endotoxemic rats receiving only vasopressin (LPS-vaso) was found significantly elevated as compared to all other groups except the LPS-nor group at Mea 4. In an additionally performed statistical analysis, MAP values of the LPS-vaso group did not differ at baseline and Mea 4 (Student's t-test, $p=0.15$). At the end of the protocol, the heart rate in the LPS-nor group was found significantly increased compared to all other groups except the LPS-only group.

Concentration of isoflurane. The expiratory isoflurane concentration was constant at 1.1±0.1 vol% throughout the protocol. The detection limit of the applied monitoring system (Vamos) is ±0.1 vol% according to the manufacturer.

Plasma cytokine concentrations. Induction of endotoxemia resulted in a significant increase in proinflammatory TNFα and IL-1ß and of the anti-inflammatory IL-10 (for LPS-only vs. Sham), respectively. The concomitant inhalation of isoflurane in endotoxemic animals (LPS-iso) resulted in a significant reduction of plasma TNFα and IL-1ß as compared to the LPS-only group. Plasma IL-10 was not influenced by inhaled isoflurane (Figs. 1-3).

The administration of both vasopressors (norepinephrine or vasopressin) in endotoxemic rats counteracted the anti-inflammatory effects of isoflurane. Both proinflammatory cytokines measured were found significantly increased as compared to the endotoxemic rats which had received

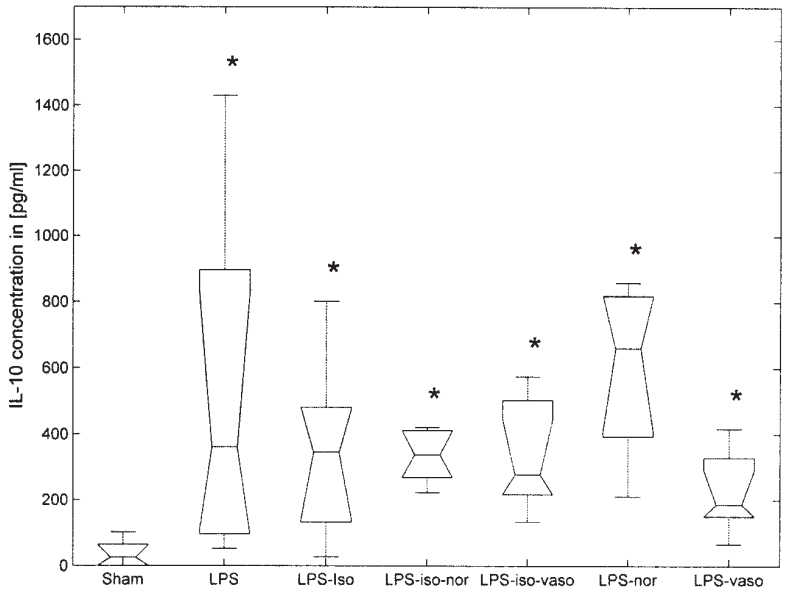

Figure 3. Levels of immunoreactive IL-10 in plasma (pg/ml) after 4 h of endotoxemia as determined by ELISA. Data displayed as box plots (median, 1st-3rd quartile). ANOVA on ranks, Student-Newman-Keuls test ($p<0.05$); (*) versus Sham, (§) versus LPS-iso.

Table IV. Cumulative concentrations of nitrite in supernatants of cultured (24 h) alveolar macrophages (AM).

	Sham	LPS-only	LPS-iso	LPS-iso-nor	LPS-iso-vaso	LPS-nor	LPS-vaso
Nitrite (μM)	3 (2)	24 (9)[a]	32 (11)[a]	27 (9)[a]	17 (7)[a]	27 (10)[a]	18 (9)[a]

[a]Versus Sham.

isoflurane only. Moreover, plasma levels of IL-1ß from endotoxemic animals that received isoflurane together with norepinephrine or vasopressin significantly exceeded the concentrations found in samples from the LPS-only group. The continuous infusion of norepinephrine or vasopressin in endotoxemic animals without concurrent inhalation of isoflurane resulted in significantly increased plasma concentrations of IL-1ß as compared to the LPS-only group. Administration of any of the two vasopressors had no influence on the levels of IL-10 in endotoxemic animals.

Nitrite production from cultured alveolar macrophages. Spontaneous production of nitrite from cultured alveolar macrophages (AM) derived from rats receiving only LPS was significantly increased as compared to the Sham group. The inhalation of isoflurane during endotoxemia had no effect on nitrite concentrations in supernatants of cultured AM as compared to the LPS-only group. The nitrite production of each group of endotoxemic rats with or without isoflurane or each vasopressor was significantly increased as compared to Sham animals but was not altered as compared with the LPS-only group (Table IV).

In the additionally performed study focusing on the duration of pentobarbital administration, there were no differences in plasma levels of TNFα detectable when pentobarbital was administered only i.p. (50 mg/kg) for induction of anesthesia or when pentobarbital was administered intravenously at 7.5 mg/kg/h following its i.p. injection (Table V).

Discussion

In the present study, the effects of exogenous norepinephrine and vasopressin on experimental endotoxemia with and without co-administration of isoflurane were investigated. Four hours after injection of LPS (5 mg/kg, i.v.), plasma levels of TNFα, IL-1ß and IL-10 were determined. After 24 h of *ex vivo* culture, supernatants of alveolar macrophages (AM) were assayed for cumulative nitrite concentration.

The experiments were performed in a valid animal model that mimics the basic principles of human endotoxemia and systemic inflammation. Endotoxin is found in the blood of surgical patients, patients with pancreatitis, and during bacterial and fungal sepsis, and there is evidence that the degree of trauma is associated with the endotoxin level (14).

The applied model was previously used by other investigators (15) and by our group (12,13,16) with reproducible effects of i.v. LPS at 5 mg/kg in male Sprague-Dawley rats with respect to patterns of inflammatory plasma cytokine levels and nitrite production of cultured AM, respectively. The LPS dosage applied here did not induce a subsequent septic shock with significant mortality rates

Table V. Levels of immunoreactive TNFα in plasma (pg/ml) during experimental endotoxemia as determined by ELISA.[a]

	Baseline	30 min	60 min	90 min	120 min	150 min	180 min
PICIG	3 (1)	430 (130)	670 (240)	1350 (144)	1390 (160)	1300 (200)	1240 (200)
PIOG	3 (1)	360 (103)	683 (292)	1360 (180)	1340 (170)	1300 (190)	1215 (160)

[a]In the PICIG group (pentobarbital-induction and continuous infusion group, n=6), anesthesia was induced by i.p. injection of pentobarbital and fentanyl (50 mg/kg; 0.05 mg/kg, respectively) and then maintained by continuous infusion of pentobarbital and fentanyl (7.5 mg/kg/h and 3.75 μg/kg/h, respectively). In the PIOG group (pentobarbital-induction-only group, n=6) anesthesia was induced by i.p. injection of pentobarbital and fentanyl (50 mg/kg; 0.05 mg/kg, respectively) and then maintained only by infusion of fentanyl (3.75 μg/kg/h) without pentobarbital. Plasma samples were obtained every 30 min following the LPS injection. Data are shown as the mean (± SD), t-test ($p<0.05$).

within the observation period but induced a reproducible and stable cytokine response in these animals. However, the evaluation of a rescue therapy of septic shock was not the aim of the present study.

The clinical relevancy of the present study was based on the fact that patients suffering from SIRS or sepsis often receive vasopressor therapy and frequently require surgical interventions with the use of volatile anesthetics. In addition, there is a growing use of volatile anesthetics for long-term sedation in intensive care medicine. Thus, possible interactions of volatile anesthetics and vasopressors are of particular interest.

Application of volatile anesthetics has been shown to be protective in ischemia-reperfusion injury in various organs (anesthetic preconditioning, APC) and to attenuate the inflammatory response as indicated by suppression of pro-inflammatory cytokine release from immunocompetent cells *in vitro* and *in vivo* (10,11,17,18).

Results of the present study indicated that the co-administration of norepinephrine or vasopressin attenuated the anti-inflammatory effects of isoflurane. Levels of plasma TNFα from endotoxemic rats treated with one of the two vasopressors together with isoflurane were found to be similar to values from animals receiving only LPS without any further specific treatment. In addition, the infusion of each vasopressor in the presence or absence of isoflurane significantly induced endogenous IL-1ß secretion compared to the endotoxemic control group. Based on these findings, a certain proinflammatory profile of both vasopressors may be assumed during experimental endotoxemia.

If these effects are existent in human SIRS or sepsis, potential advantages of the administration of volatile anesthetics in the intraoperative and intensive care scenario with respect to their potentially desired immunomodulative effects might be attenuated or even reverted.

However, it is known that catecholamines not only modify cardiocirculatory physiology, but they can also influence the inflammatory response (6). In clinical SIRS and sepsis this may be of significance since the associated prolonged stress results in enhanced secretion of already raised levels of catecholamines (19). Therapeutic administration of vasopressors may have additive and potentially deleterious effects in this situation. It has been demonstrated that the stimulation of isolated cardiomyocytes with the α-agonist phenylephrine resulted in the translocation of NF-κB as a potent transcription factor for TNFα which induced significant depression in cardiac contractile function (20). In contrast, the administration of the selective α-inhibitor prazosin before induction of acute hemorrhage in rats significantly reduced TNFα expression in heart tissue and restored cardiac contractile (dys-) function to control levels (21). The administration of norepinephrine enhanced the IL-1ß-induced nitrite production from cardiac myocytes, an effect that could be partially reduced by prazosin (22). Moreover, the administration of phenylephrine before induction of experimental murine endotoxemia was associated with significantly increased expression of TNFα mRNA in neutrophils compared with that found after administration of endotoxin alone (23). In addition, the infusion of norepinephrine resulted in significantly increased levels of IL-6 in the plasma and cerebrospinal fluid of rats with acute traumatic brain injury compared to controls without norepinephrine (24). More recently, it was shown that phenylephrine provoked a significant increase of pulmonary neutrophil accumulation in experimental hemorrhage and endotoxemia (25).

In summary, there is a body of evidence that catecholamines have high potential to act as a further proinflammatory force which might be harmful during SIRS or sepsis.

Aside from its advantageous hemodynamic effects in the situation of catecholamine refractory hypotension, vasopressin may be of special interest with respect to its possible inert properties regarding immunologic functions and circulating levels of proinflammatory cytokines.

Vasopressin mediates vasoconstriction by V1-receptors coupled to phospholipase-C, thus increasing the concentration of intracellular Ca^{2+}. Though there is evidence for a down-regulation of V1-receptors during sepsis, their action does not seem to be impaired during sepsis. In addition, vasopressin was effective in septic shock resistant to conventional catecholamine therapy (26). Thus far, the impact of endogenously or exogenously administered vasopressin on the acute inflammatory response has not been elucidated in detail. A long held theory of immunomodulatory effects of vasopressin proposes that vasopressin modulates adrenocorticotropin (ACTH) release thus stimulating the adrenal gland to release glucocorticoids which suppress the immune system including proinflammatory cytokines (27). More recently, Zhao and Brinton revealed that the incubation of a V1-receptor agonist

with stimulated astrocytes significantly suppressed IL-1ß and TNFα expression at the mRNA and secreted protein level. In addition, reduced levels of IL-1ß and TNFα were shown to be neuroprotective against LPS inflammatory insults (28).

The administration of the vasopressin analogue terlipressin inhibited the LPS-induced arterial iNOS expression in rats with liver cirrhosis *in vivo* (29). However, even though an effect of vasopressin on cytokine-induced iNOS induction would be of significance with respect to its anti-inflammatory actions, this observation did not prove existent in septic patients (30).

In the present study the effects of vasopressin were comparable with those of norepinephrine. However, we do not suggest conclusions concerning the anti-inflammatory effects of vasopressin in the present model and study.

Our data indicate that the inhalation of isoflurane during endotoxemia had no significant effect on cumulative nitrite production from *ex vivo* cultured AM from endotoxemic rats. This finding is in contrast to previous results from our group, where we observed suppressive effects on nitrite production from cultured AM following rat endotoxemia using isoflurane in a protocol of short-term pretreatment (13). Nitrite from cultured AM was analyzed as a read out for inducible nitrite-synthase (iNOS) activity in these cells. This differential regulation of iNOS by isoflurane confirms data from a previous *in vitro* study using murine macrophages where isoflurane inhibited iNOS formation through LPS (a condition comparable to short-time exposure). The same study reports a potentiation of iNOS by isoflurane (a condition comparable to long-term exposure) (31).

In conclusion, results of our study demonstrated that the infusion of norepinephrine or vasopressin, respectively, both counteracted the effects of inhaled isoflurane on the release of proinflammatory cytokines during experimental endotoxemia in rats. Our results do not support the concept of anti-inflammatory principles of vasopressin.

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