Ketamine and Etomidate Down-regulate the Hypothalamic–Pituitary–Adrenal Axis in an Endotoxemic Mouse Model

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ABSTRACT

Background: We compared the effects of etomidate and ketamine on the hypothalamic–pituitary–adrenal axis during sepsis.

Methods: Mice (n = 5/group) were injected intraperitoneally with lipopolysaccharide (10 mg/kg) and 6 h later randomized to receive ketamine (100 mg/kg), etomidate (30 mg/kg), or saline. At two time points (12 and 48 h), messenger RNA levels of hypothalamic corticotropin-releasing hormone, pituitary proopiomelanocortin, and four adrenal enzymes (P450 side-chain cleavage, 3β-hydroxysteroid deshydrogenase, 21-hydroxylase, and 11β-hydroxylase) were measured by *in situ* hybridization (results are presented as optical density), and plasma levels of corticosterone and adrenocorticotropin hormones were measured by enzyme-linked immunosorbent assay (mean ± SD).

Results: At 12 h, lipopolysaccharide induced an overexpression of corticotropin-releasing hormone (32 ± 5 *vs.* 18 ± 6, $P < 0.01$), proopiomelanocortin (21 ± 3 *vs.* 8 ± 0.9, $P < 0.0001$), P450 side-chain cleavage (32 ± 4 *vs.* 23 ± 10, $P < 0.05$), 21-hydroxylase (17 ± 5 *vs.* 12 ± 2, $P < 0.05$), and 11β-hydroxylase (11 ± 4 *vs.* 6 ± 0.5, $P = 0.001$), and an elevation of corticosterone (642 ± 165 *vs.* 98.3 ± 63 ng/ml, $P < 0.0001$). Etomidate and ketamine reduced P450 side-chain cleavage (19 ± 7 and 19 ± 3 *vs.* 32 ± 4, $P < 0.01$), 21-hydroxylase (8 ± 0.8 and 8 ± 1 *vs.* 17 ± 5, $P < 0.001$), 11β-hydroxylase (4 ± 0.5 and 7 ± 1 *vs.* 11 ± 4, $P < 0.001$ and $P < 0.05$), and corticosterone (413 ± 189 and 260 ± 161 *vs.* 642 ± 165 ng/ml, $P < 0.05$ and $P < 0.01$). Ketamine also inhibited adrenocorticotropin hormone production (2.5 ± 3.6 *vs.* 36 ± 15 pg/ml, $P < 0.05$). At 48 h, all four adrenal enzymes were down-regulated by lipopolysaccharide administration with corticosterone levels similar to the control group. Ketamine and etomidate did not modify corticosterone plasma levels.

Conclusions : Our endotoxemic model induces an initial activation of the hypothalamic–pituitary–adrenal axis, followed by a secondary inhibition of adrenal steroidogenesis processes. Ketamine and etomidate inhibit the enzyme expression and activity of the adrenal gland at the early stage. **(Anesthesiology 2017; 127:347-54)**

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SEPSIS is a major public health concern, involving 20% of patients admitted to intensive care units and leading to significant mortality rates ranging between 20 and 45%.[1] Septic process may cause relative adrenal insufficiency (RAI), a dysfunction responsible for the inadequate production of corticosteroids related to inflammatory condition.[2,3] Annane *et al.*[3] showed increased mortality in patients with septic shock and higher cortisol levels but insufficient response to stimulation with tetracosactide, an analog of the adrenocorticotropin hormone (ACTH). The mechanisms associated with RAI are poorly understood and should be addressed because they are critical for patient care. RAI may involve lack of cortisol and/or ACTH synthesis, a decrease in the transport of cortisol, and alteration in the clearance of cortisol.[4–6]

The use of specific anesthetic drugs during sepsis is the focus of current intense debate. Indeed, etomidate has been reported to be responsible for RAI after a single injection, either in a sepsis context or not.[7,8] This adrenal insufficiency is related to direct inhibition of the adrenocortical enzymes involved in the biosynthesis of cortisol and especially of 11β-hydroxylase, the final enzyme of the cascade. Several studies have identified the administration of a single bolus of etomidate as being

What We Already Know about This Topic

- Septic processes may cause relative adrenal insufficiency
- Etomidate may cause relative adrenal insufficiency after a single injection in both nonseptic and septic subjects
- Ketamine may be an alternative to etomidate in septic patients, although its effects on the hypothalamic–pituitary–adrenal axis and steroidogenesis are not well understood

What This Article Tells Us That Is New

- Induction of endotoxemia in mice by injection of lipopolysaccharide induced the relative failure of the hypothalamic–pituitary–adrenal axis function by down-regulation of the expression of major adrenocortical enzymes and likely relative adrenal insufficiency
- Both etomidate and ketamine produced an early deficit of adrenal function in the endotoxemic model that was reversible within 48 h

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responsible for mortality during serious septic conditions.[8–10] Thus, an alternative to etomidate may be ketamine, although its effects on the hypothalamic–pituitary–adrenal (HPA) axis and steroidogenesis are understudied.

The precise mechanisms of RAI and the inhibition of steroid production related to anesthetic administration are poorly understood, in particular concerning the gene expression of the different components of the HPA axis during sepsis. The objective of our experimental work was to evaluate the modulation of the functional and molecular pathways involved in the HPA axis after administration of two anesthetics, etomidate and ketamine, during sepsis.

Materials and Methods

Animal Procedures

Our objective was to evaluate modulation of the HPA axis at early (12 h) and later (48 h) time points after administration of lipopolysaccharide. Based on a previous experiment carried out in our laboratory (unpublished data), five mice per group for each of the two time points were considered sufficient to explore the effects of hypnotics on the HPA axis. No formal *a priori* statistical power analysis was conducted. Thus, 40 C57Bl/6 male mice were obtained from Janvier (France) at 8 to 12 weeks of age and 25 to 35 g of body weight, housed at a constant temperature (21°C) with 14-h/10-h light/dark cycle (lights on at 6 AM). All animals had free access to standard chow and drinking tap water. All experiments were approved by the Regional Ethics Committee for Animal Research (Comité Normandie d'Ethique en Matière d'Expérimentation Animale [CENOMEXA] under National Committee on Animal Experimentation, approval No. N/02-05-09/12/05-12). All procedures were performed in accordance with the French Ethics Committee as well as the guidelines of European Parliament directive No. 2010/63/EU and the Council for the Protection of Animals Used for Scientific Purposes under the supervision of an authorized investigator. The elaboration of this manuscript adheres to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

Inflammation was induced by intraperitoneal injection of bacterial lipopolysaccharide (10 mg/kg from *Escherichia coli* O127: B8; Sigma-Aldrich, France). Six hours after lipopolysaccharide injection, mice were randomized to receive an intraperitoneal injection of etomidate (30 mg/kg; Janssen–Cilag, France), ketamine (100 mg/kg; Panpharma, France), or sodium chloride 0.9% (lipopolysaccharide-NaCl group) corresponding to an administered volume of 4 ml/kg. These dosages have been previously described as preventing movement during nonsurgical procedures.[11] Nevertheless, we tested the effect of the drugs at three different dosages to confirm this choice. Five mice per drug and per dosage were injected to determine optimal effective dose, for a total of 30 mice (etomidate 15, 22, and 30 mg/kg; ketamine 50, 75, and 100 mg/kg). The potency was explored as described previously[12]: loss of righting reflex, presence of spontaneous movement, and presence of a reaction to a painful stimulation (pinching hind limb with nails) were observed. The recovery from anesthesia was evaluated by the time to revival of righting reflex and walking. Only etomidate 30 mg/kg and ketamine 100 mg/kg achieved an absence of reaction to a painful stimulation in more than 50% of cases (etomidate, 3 of 5 mice and ketamine, 4 of 5 mice). Moreover, no significant difference was observed concerning time to loss of righting reflex (127 ± 15 *vs.* 111 ± 17 s, $P > 0.99$) and to recovery of righting reflex (2,773 ± 1,566 *vs.* 2,102 ± 480 s, $P > 0.99$) between etomidate 30 mg/kg and ketamine 100 mg/kg. Etomidate induced a longer disruption in walking behavior for all dosages. The complete results are presented in Supplemental Digital Content 1 (http://links.lww.com/ALN/B463).

A control group free of any injection was also constituted (five mice at each time point). The mice were sacrificed 12 and 48 h after administration of anesthetics or NaCl, allowing analysis of five mice per group for each time point. *In situ* hybridization and plasma measurements were performed at each time point as described in the following sections. Operators performing *in vivo* experiments and analysis of hybridization tissue slices and plasma measurements were blind to each other's results. Because of the severity of the model, the occurrence of death before the scheduled time was considered possible. In this case, a new set of experiments was performed to complete the study.

Sacrifice was achieved using a lethal dose of intraperitoneal pentobarbital 6% (60 mg/kg; Hospira, France) followed by a transcardiac perfusion of NaCl using an osmotic pump. The brain and pituitary and adrenal glands were removed, postfixed during 24 h in 4% paraformaldehyde, embedded in paraffin, and then cut (10 μm) with a cryostat before mounting sections on glass slides.

The primary endpoint of the study was the variation in messenger RNA (mRNA) levels of the hormones and enzymes of the HPA axis over time between groups. The secondary endpoint was the variation in plasma levels of corticosterone and ACTH over time.

Plasma Measurements

A blood sample was taken transcardially after the sacrifice of each animal and before the infusion of NaCl. The samples were centrifuged at 3,000*g* for 15 min, and plasmas were frozen at −20°C before analysis. Corticosterone and ACTH

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levels were measured using specific enzyme-linked immunosorbent assay kits (Corticosterone, Arbor Assays, USA and ACTH, Cloud-Clone, USA). The operator was blinded to the allocated groups.

In Situ *Hybridization*

In situ hybridization was performed as described previously.[13] A specific antisense oligonucleotide probe was used to measure the mRNA levels of hypothalamic corticotropin-releasing hormone, pituitary proopiomelanocortin, as well as adrenal cholesterol side-chain cleavage enzyme (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), 21-hydroxylase, and 11β-hydroxylase (Supplemental Digital Content 2, http://links.lww.com/ALN/B464). Probes were labeled with[35] deoxyadenosine 5'-(a-thio) triphosphate using terminal deoxynucleotidyl transferase. After hybridization, the sections were dehydrated, coated with liquid photographic emulsion (Kodak NTB-2, USA), and processed after 7 days of exposure.

Quantification of mean optical density was achieved by macroautoradiograph (Kaiser Prolite Basic 2 PRIMACEN platform; Kaiser, France) coupled with image analysis software (v4.0 Autoradiographe, Samba technology, PRIMACEN platform; Normandy University, France). The averaged results for each animal were analyzed. The operator was blinded to the allocated groups.

Statistical Analysis and Presentation of the Data

All data are presented as mean ± SD. The results are expressed as optical density for *in situ* hybridization and as ng/ml for corticosterone and pg/ml for ACTH. Comparisons between groups were made at each time point using a two-way ANOVA test including the four groups and the two different time points as the two factors studied. This test was followed by a Bonferroni *post hoc* test for multiple comparisons with a correction of the *P* value applied for all the comparisons. All result values are presented in Supplemental Digital Content 3 (http://links.lww.com/ALN/B465). The analyses were carried out using Xlstat software for Windows (version 2016; Addinsoft, France). A *P* value less than 0.05 was considered statistically significant.

Results

Impact of Lipopolysaccharide on the HPA Axis

The effects of lipopolysaccharide on the HPA axis over time are presented in figure 1 and in Supplemental Digital Content 3 (http://links.lww.com/ALN/B465). One supplementary mouse was included because of premature death.

During the first stage of endotoxemia, *i.e.*, 12 h after lipopolysaccharide injection, HPA activity was enhanced in comparison with control mice. mRNA expression of hypothalamic corticotropin-releasing hormone (CRH; 1.7 ± 4.7 *vs.* 17.9 ± 5.8, +77%, $P = 0.006$) and pituitary proopiomelanocortin (20.5 ± 3.1 *vs.* 8.4 ± 0.9, +144%, $P < 0.0001$) was increased. Circulating levels of ACTH in comparison with control mice were not statistically significant (36.0 ± 15.2 *vs.* 9.0 ± 5.2 pg/ml, $P = 0.1$). Concomitantly, mRNA expression of adrenal enzymes P450scc, 21-hydroxylase, and 11β-hydroxylase was also increased in lipopolysaccharide-injected mice (32.3 ± 4.4 *vs.* 22.5 ± 9.5, +44%, $P = 0.027$; 16.8 ± 5.3 *vs.* 11.5 ± 2.0, +47%, $P = 0.012$; and 11.0 ± 4.1 *vs.* 5.7 ± 0.5, +92%, $P = 0.001$, respectively), with a 6.5-fold increase in corticosterone production in comparison with control mice (642.4 ± 165.0 *vs.* 98.3 ± 62.6 ng/ml, $P < 0.0001$).

At the later time, *i.e.*, 48 h after lipopolysaccharide injection, a persistent increase in proopiomelanocortin expression was still observed in lipopolysaccharide-injected animals (9.9 ± 3.0 *vs.* 6.1 ± 1.3, +62%, $P = 0.013$), whereas CRH expression and circulating levels of ACTH were not significantly different from controls. Conversely, the four adrenal enzymes showed a decreased level of mRNA expression (P450scc: 4.0 ± 0.9 *vs.* 17.1 ± 6.4, −77%, $P = 0.0002$; 3β-HSD: 13.1 ± 3.0 *vs.* 31.0 ± 3.3, −58%, $P < 0.0001$; 21-hydroxylase: 4.7 ± 1.0 *vs.* 15.3 ± 1.5, −69%, $P < 0.0001$; 11β-hydroxylase: 3.1 ± 0.6 *vs.* 9.7 ± 2.5, −68%, $P < 0.0001$). Corticosterone plasma levels were not statistically different from controls (176.6 ± 76.8 *vs.* 73.0 ± 15.0 ng/ml, $P = 0.327$).

Effects of Etomidate on the HPA Axis during Endotoxemia

The effects of etomidate on the HPA axis in endotoxemic mice are presented in figure 1 and the values in Supplemental Digital Content 3 (http://links.lww.com/ALN/B465). Three supplementary mice were included because of premature death. Etomidate administration did not induce significant variations in mRNA levels of CRH or proopiomelanocortin either at 12 or 48 h after lipopolysaccharide injection. However, at 12 h, ACTH level was significantly higher in lipopolysaccharide-etomidate compared with lipopolysaccharide–NaCl (89.9 ± 35.7 *vs.* 36 ± 15.2 pg/ml, +150%, $P = 0.001$).

At 12 h, we observed an inhibition of the mRNA expression of three of the four adrenal enzymes studied, *i.e.*, P450scc (19.0 ± 6.7 *vs.* 32.3 ± 4.4, −41%, $P = 0.005$), 21-hydroxylase (8.2 ± 0.8 *vs.* 16.8 ± 2.7, −51%, $P = 0.0002$), and 11β-hydroxylase (4.3 ± 0.5 *vs.* 11.0 ± 4.1, −61%, $P = 0.0002$), and a significant reduction in corticosterone plasma levels compared with the lipopolysaccharide–NaCl group (412.7 ± 189.0 *vs.* 642.4 ± 165.0 ng/ml, −36%, $P = 0.032$). At 48 h, etomidate increased the levels of mRNA encoding 3β-HSD (25.8 ± 2.8 *vs.* 13.1 ± 3.0, +96%, $P < 0.0001$) and 21-hydroxylase (7.1 ± 0.9 *vs.* 4.7 ± 1.0, +50%, $P = 0.009$). No significant modulation in P450scc, 11β-hydroxylase mRNA, or corticosterone circulating levels was observed in etomidate-administered animals.

Effects of Ketamine on the HPA Axis during Endotoxemia

The effects of ketamine on the HPA axis in endotoxemic mice are presented in figure 1 and the values in Supplemental Digital Content 3 (http://links.lww.com/ALN/

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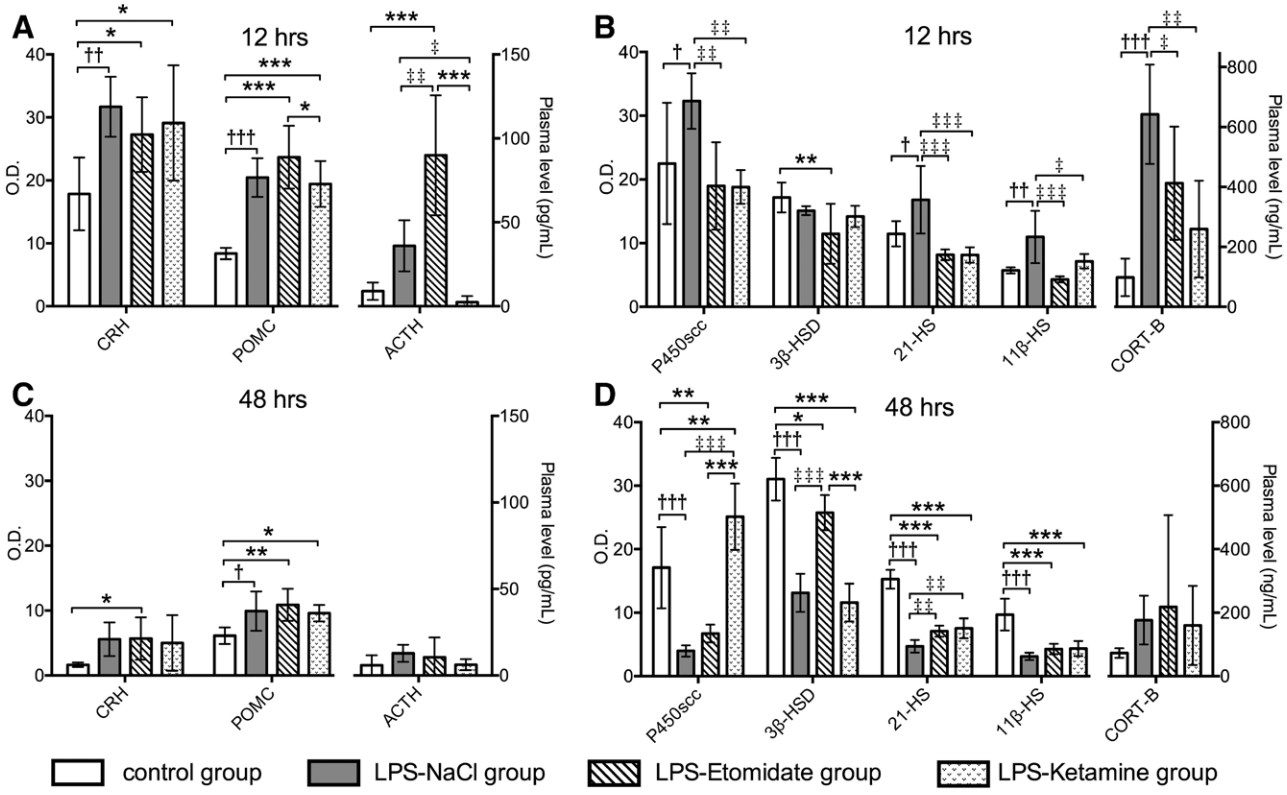

Fig. 1. Effects of lipopolysaccharide, etomidate, and ketamine on hypothalamic–pituitary–adrenal axis in mice. (*A*, *C*) Messenger RNA levels of corticotropin-releasing hormone (CRH), proopiomelanocortin (POMC), and plasma adrenocorticotropin hormone (ACTH) 12 and 48 h after injection. (*B*, *D*) Messenger RNA levels of cholesterol side-chain cleavage enzyme P450 (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), 21-hydroxylase (21-HS), 11β-hydroxylase (11β-HS), and plasma level of corticosterone (CORT-B) 12 and 48 h after lipopolysaccharide (LPS) injection. The values are presented as mean ± SD of optical density (O.D.) and plasma levels. n = 5 per group at each time. †Comparison between control and lipopolysaccharide–NaCl groups. ‡Comparison between lipopolysaccharide–NaCl and etomidate or ketamine groups. *All other comparisons. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

B465). One supplementary mouse was included because of premature death. In comparison with controls (lipopolysaccharide–NaCl), ketamine induced a marked reduction in ACTH plasma levels at 12 h (2.5 ± 3.6 *vs.* 36.0 ± 15.2 pg/ml, –93%, $P = 0.02$), without significant modification in the mRNA levels of CRH or proopiomelanocortin. Concomitantly, we observed a reduction in the mRNA levels of P450scc (18.8 ± 2.7 *vs.* 32.3 ± 4.4, –42%, $P = 0.004$), 21-hydroxylase (8.1 ± 1.2 *vs.* 16.8 ± 5.3, –52%, $P < 0.0001$), and 11β-hydroxylase (7.2 ± 1.1 *vs.* 11.0 ± 4.1, –35%, $P = 0.012$) associated with a reduction in corticosterone circulating levels (259.8 ± 161.0 *vs.* 642.4 ± 165.0 ng/ml, –60%, $P = 0.002$). No difference concerning corticosterone plasma levels was observed between the etomidate and ketamine groups ($P = 0.186$). At the 48-h time point, administration of ketamine had no effect on the levels of hypothalamic or pituitary organs. Conversely, a rise in the mRNA levels of P450scc (25.1 ± 5.2 *vs.* 4 ± 0.9, +531%, $P < 0.0001$) and 21-hydroxylase (7.6 ± 1.6 *vs.* 4.7 ± 1.0, +60%, $P = 0.003$) was observed, without any change in 3β-HSD, 11β-hydroxylase mRNAs, or plasma corticosterone levels.

Comparative Effects of Etomidate and Ketamine

The effects of etomidate and ketamine on the HPA axis are represented in figure 1 and the values in Supplemental Digital Content 3 (http://links.lww.com/ALN/B465). At the 12-h time point, both proopiomelanocortin expression and ACTH plasma levels were reduced after ketamine administration in comparison with etomidate-injected mice (19.4 ± 3.6 *vs.* 23.7 ± 5.0, $P = 0.047$, and 2.5 ± 3.6 *vs.* 89.9 ± 35.7 ng/ml, $P < 0.0001$, respectively). No significant difference was observed concerning the expression of adrenal enzymes or the plasma levels of corticosterone at this time. At the 48-h time point, no difference was observed concerning hypothalamic–pituitary function. Regarding adrenal function, a strong increase in P450scc expression was observed in mice injected with ketamine in comparison with etomidate (25.1 ± 5.2 *vs.* 6.7 ± 1.4, $P < 0.0001$), whereas the expression of 3β-HSD was reduced (11.6 ± 3.0 *vs.* 25.8 ± 2.8, $P < 0.0001$). Nevertheless, no difference was observed concerning the plasma levels of corticosterone between these two drugs.

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Discussion

This is the first investigation of the impact of endotoxemia on the transcriptional gene expression of the main components of the HPA axis in a murine model, involving anesthetics commonly used in clinical situations of septic shock. Our objective was twofold: first, to study the mechanisms by which inflammatory conditions affect the transcription of genes involved in corticosteroid synthesis, and second, to evaluate the impact of the administration of the anesthetics etomidate or ketamine on this system.

HPA Axis and Sepsis

Our data highlight an early increase in corticosterone production secondary to inflammatory stress. Several studies have suggested the role of the pituitary and hypothalamic glands. Li *et al.*[14] previously showed that lipopolysaccharide induces a rise in proopiomelanocortin production and its maturation processing by the prohormone convertase-1. ACTH, produced from the cleavage of proopiomelanocortin, induces a rise in cAMP and cytosolic calcium levels in adrenal cells, leading to steroid production.[15] This increase in steroid plasma levels could be secondary to a rise in gene expression. Indeed, Spiga *et al.*[16] observed that early response to ACTH was due to the posttranscriptional regulation of steroidogenic acute regulatory and P450scc proteins, and late response (within 30 min) involved a rise in the gene expression of these two enzymes. Our results are partially in agreement with these observations with a rise in the expression of CRH and proopiomelanocortin. Moreover, we showed that 11β-hydroxylase and 21-hydroxylase were also upregulated. Together, we highlight the activation of the HPA axis at the early stages of sepsis.

Corticosteroid production is a key element of the adaptation of an organism to sepsis. Cortisol, or corticosterone in rodents, is the final product of corticosteroid synthesis and displays immunomodulatory effects by increasing the transcription of antiinflammatory cytokines such as interleukin-4 and interleukin-10 and by reducing the production of proinflammatory cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor-α.[6] Furthermore, cortisol exerts additional beneficial functions toward infection, *i.e.*, regulation of blood volume *via* mineralocorticoid-activated pathways, increase in vascular tone, and sensitivity to catecholamines. Thus, insufficient adaptation of the HPA axis may be highly detrimental to patients with sepsis. In our study, circulating levels of corticosterone in mice subjected to lipopolysaccharide were significantly higher than in control mice during the first stage of experiment but became comparable with control mice (free of sepsis) after 48 h. Concomitantly, there was a marked inhibition of the expression of the four tested adrenal enzymes. Taken together, this may suggest the priming of RAI, which occurs in patients with sepsis. A first explanation for this dissociation between low mRNA expression and circulating levels of corticosterone could be, as described by Boonen *et al.*[4] for cortisol, a decrease in corticosterone clearance, maintaining a relatively high plasma level despite a reduction in its production. Second, our data argue for the peripheral inhibition of adrenal enzyme expression together with a hypothalamic–pituitary activity normally adapted to the situation. This may be explained by the shunting of the central regulation and a possible direct inhibitory action of proinflammatory cytokines on corticosteroid production. In support of this hypothesis, it has been shown in fetal adrenal cell cultures that tumor necrosis factor-α can directly inhibit the expression of mRNA encoding P450scc and the production of corticosterone.[17] Moreover, several experimental works not only demonstrated a stimulatory effect of endotoxins on the HPA axis, with an increase in CRH and ACTH, but also a direct role for endotoxins on the production of corticosterone.[18] In addition, in a murine septic model by cecal ligation and puncture (CLP), Koo *et al.*[19] showed that the plasma levels of ACTH were elevated in association with a decreased production of *in situ* corticosterone by the adrenal gland. Our work confirms, in an endotoxemic model, this peripheral inhibition of adrenal function after a primary activation of the HPA axis and highlights the concomitant repression of several adrenal key enzymes likely contributing to the probable initiation of RAI.

HPA Axis and Anesthetics

An intense debate surrounds the choice of drugs used for anesthetic induction during serious septic situations. Etomidate is currently suspected of increasing mortality, thus raising the potential alternative value of ketamine.[8] These two molecules have interesting hypnotic and cardiovascular properties. However, there are few reliable data in support of the choice of ketamine *versus* etomidate.

Etomidate is known to inhibit cortisol production.[20] A single dose of etomidate in nonseptic patients induced a decrease in total plasma cortisol of more than 50%, reversible in 6 h.[7] Although the impact on morbidity and mortality in healthy subjects seems modest, the use of etomidate during major inflammatory situations is the focus of widespread debate. Administration of etomidate in 35 patients during major surgery was associated with RAI for a period of at least 24 h.[21] Similarly, Vinclair *et al.*[22] reported that etomidate impaired HPA function in nonseptic critically ill patients for up to 48 h. Focusing more specifically on sepsis, the CORTICUS (corticosteroid therapy for septic shock) study reported an increased occurrence of RAI from 44 to 61% in the subgroup that received etomidate.[10,23] The reduction in corticosterone observed in our work after etomidate injection seems concordant with these reports. The pathophysiology remains unclear. Published data mostly studied the adrenal blockade by measuring *in vitro* or *in vivo* levels of the different precursors of corticosteroids, accumulating in cases of enzymatic inhibition. Administration of increasing doses of etomidate in cultures of adrenocortical cells induced the blockade of the enzymatic activity of 11β-hydroxylase and, to a lesser extent, of P450scc.[24,25] These data were

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confirmed in minor human surgeries with a decrease in cortisol levels and an accumulation of 11-deoxycortisol 1 h after etomidate injection.[26] The main hypothesis for adrenal inhibition by etomidate is the interaction of its imidazole ring with the heme group of adrenal enzymes belonging to the P450 cytochrome family.[27,28] This is reinforced by the lowered adrenal effect of carboetomidate, a structural analog lacking the imidazole ring.[29] Of note, according to a recent study, etomidate also repressed the expression of 3β-HSD (this latter does not belong to the P450 family) in rat immature Leydig cells, suggesting potential alternative inhibitory mechanisms.[30] Our work, however, has advanced understanding of the mechanisms inducing adrenocortical dysfunction, by identifying, for the first time in the adrenal gland and during sepsis, down-regulation of gene expression of three of the four major enzymes involved in corticosteroid synthesis, soon after etomidate injection. The theory of simple steric inhibition *via* the imidazole ring does not directly respond to this gene repression. Further study will allow a better understanding of the mechanisms involved.

The main originality of our study was the effect of ketamine, a molecule devoid of imidazole ring. Injection of ketamine also induced down-regulation of P450scc, 21-hydroxylase, and 11β-hydroxylase, accompanied by a significant decrease in corticosterone levels at an early time. Another unexpected result was the drop in ACTH levels despite the absence of reduction in the mRNA levels of proopiomelanocortin compared to the lipopolysaccharide–NaCl group. One possible explanation might be the posttranslational and maturation processes. The proopiomelanocortin polypeptide is cleaved by a prohormone convertase in the corticotropic cells of the anterior pituitary gland, resulting in the production of ACTH,[31] and regulation of this enzyme by ketamine cannot be ruled out. The impact of ketamine on the HPA axis has not been extensively investigated in the literature. This anesthetic has been shown to reduce ACTH response to hypoxia in late gestation ovine fetus[32] and ACTH and corticosterone responses to acute and chronic stress in adult rats.[33,34] Recently, Diaz-Gil *et al.*[35] showed that ketamine administration in nonseptic rats suppresses corticosterone production by almost 50%, suggesting a negative impact of this drug on the HPA axis. Nevertheless, these results in physiologic conditions cannot predict the effects on the HPA axis during sepsis. Effects in humans are little studied. In a prospective multicenter study, Jabre *et al.*[36] randomized 655 patients requiring prehospital intubation (with only 16% of septic patients) to ketamine or etomidate and found no difference in mortality. Although patients administered etomidate presented an excess risk of RAI, patients in the ketamine group also displayed RAI in 48% of cases. Two other clinical trials compared the effects of these two drugs on patient outcomes (and HPA function) for intubation in intensive care units (NCT02105415 and NCT01823328). However, neither of these trials specifically studied the effects in septic patients. Together, data are currently lacking to conclude the harmlessness of ketamine during septic shock. Here, in our endotoxemic model, we observed clear inhibition of the HPA axis early after the administration of ketamine.

After 48 h, both etomidate and ketamine induced overexpression of some adrenal enzymes. The absence of a rise in corticosterone levels could be due to the absence of effect of the anesthetic drugs on the global process and, notably on 11β-hydroxylase, limiting the complete synthesis process, or to a persistent inhibitory effect on adrenal enzymes. Additional long-term experiments are warranted to address this mechanism.

Our work has several limitations. The induction of endotoxemia by injection of lipopolysaccharide, resulting preferentially in activation of Toll-like receptor pathways, was not able to mimic some clinical events occurring during sepsis compared with polymicrobial peritonitis achieved by CLP.[37] Animal models on the study of sepsis are controversial, and none of them meet the criteria allowing direct translation to human pathology. Nevertheless, endotoxemia presents the advantage of ensuring early, powerful, and reproducible stimulation of inflammation, notably regarding its timing. The CLP model probably better mimics clinical features of sepsis but presents variability in the amplitude of stress and inflammatory reaction because of the size of the incision, the width of the puncture site, and the amount of extruded feces.[37] Moreover, the use of murine models is currently hotly debated, notably concerning the poor correlation between genomic responses in mice and humans, raising the question of extrapolating results to clinical practice.[37] However, this preliminary step can provide reassuring data before human trials. Another limitation is that mice mainly produce corticosterone instead of cortisol as a final product because of the lack of 17α-hydroxylase, and we cannot rule out some slight differences in their physiologic effects. Moreover, a more reliable method for determining the precise effect of the inhibition of isolated enzymes would be the measurement of each intermediate product of steroidogenesis. In any case, the main outcome is still the plasma release of the final product, *i.e.*, corticosterone in mice.

In conclusion, this is the first *in vivo* study focusing on the gene expression of the HPA axis in an endotoxemic model in an anesthesia setting. Sepsis induces the relative failure of the HPA axis function *via* down-regulation of the expression of major adrenocortical enzymes and likely RAI. The use of etomidate or ketamine in our model was responsible for the early deficit of adrenal function, which seems reversible within 48 h. Further studies are warranted to fully investigate the safety of ketamine as an anesthetic agent during sepsis.

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Competing Interests

The authors declare no competing interests.

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