At early phase of endotoxemic shock the increased $\beta$-adrenergic contractility is dependent on the $\beta_1$-adrenoceptor

David ROUL

l’Institut du thorax

No conflicts of interest
Septic shock is a subset of severe sepsis and is defined as sepsis-induced hypotension, persisting despite adequate fluid resuscitation, along with the presence of hypoperfusion abnormalities or organ dysfunction.
Septic Shock

Septic shock is a subset of severe sepsis and is defined as sepsis-induced hypotension, persisting despite adequate fluid resuscitation, along with the presence of hypoperfusion abnormalities or organ dysfunction.

751,000 patients/year in USA with 28.6% of mortality

15% patients admitted in ICU with 42% of mortality at 2 months
Physiopathology

Antigens (LPS, ...)

Blood

TLR

Endothelial Cell
Physiopathology

Antigenes (LPS, ...)

Blood

TLR

Endothelial Cell

Transcription Factors (NFκB, ...)

l'institut du thorax
Physiopathology

Antigens (LPS, ...)

Transcription Factors (NFκB, ...)

TLR

Endothelial Cell

Blood

TNF-α, IL-1, IL-6, ...

ICAM-1, VCAM-1, E-selectin, ...

COX, iNOS, Endothelin, ...
Physiopathology

Antigenes (LPS, ...)

Transcription Factors (NFκB, ...)

TLR

Endothelial Cell

TNF-α, IL-1, IL-6, ...

ICAM-1, VCAM-1, E-selectin, ...

COX, iNOS, Endothelin, ...

Myocardial Dysfunction

Endothelial Dysfunction

Vascular Hyporeactivity

Coagulation
Physiopathology

Antigens (LPS, ...)

TLR

Transcription Factors (NFκB, ...)

TNF-α, IL-1, IL-6, ... ICAM-1, VCAM-1, E-selectin, ...

COX, iNOS, Endothelin, ...

Myocardial Dysfunction

Endothelial Dysfunction

Vascular Hyporeactivity

Coagulation

Hypoperfusion

Multiple Organe Dysfunction
Physiopathology

Antigenes (LPS, ...)

Transcription Factors (NFκB, ...)

TLR

Blood

Endothelial Cell

- TNF-α, IL-1, IL-6, ...
- ICAM-1, VCAM-1, E-selectin, ...
- COX, iNOS, Endothelin, ...

Myocardial Dysfunction

Endothelial Dysfunction

Vascular Hyporeactivity

Coagulation

Hypoperfusion

Multiple Organe Dysfunction

Death
Physiopathology

Antigenes (LPS, ...)

Transcription Factors (NFκB, ...)

TNF-α, IL-1, IL-6, ...

ICAM-1, VCAM-1, E-selectin, ...

COX, iNOS, Endothelin, ...

Myocardial Dysfunction

Endothelial Dysfunction

Vascular Hyporeactivity

Coagulation

Hypoperfusion

Multiple Organe Dysfunction

Death
Physiopathology

Antigenes (LPS, ...)

Transcription Factors (NFκB, ...)

TLR

Blood

Antibiotherapy

TNF-α, IL-1, IL-6, ...

ICAM-1, VCAM-1, E-selectin, ...

COX, iNOS, Endothelin, ...

Myocardial Dysfunction

Endothelial Dysfunction

Vascular Hyporeactivity

Coagulation

Hypoperfusion

Multiple Organe Dysfunction

Death

Resuscitation
Hypoperfusion
Multiple Organe Dysfunction
Physiopathology

Antigénes (LPS, ...)
TLR
Transcription Factors (NFκB, ...)

Antibiothérapie

TNF-α, IL-1, IL-6, ...
ICAM-1, VCAM-1, E-selectin, ...
COX, iNOS, Endothelin, ...

Inotropes ???
Myocardial Dysfunction
Endothelial Dysfunction
Vascular Hyporeactivity
Coagulation

Hypoperfusion

Death

Resuscitation
Vasopressors ???
Cardiac β-adrenergic system and septic shock

Involvement of the cardiac β-AR at the early shock

→ Contradictory studies

No studies evaluating simultaneously the 3 cardiac β-AR subtypes

One study demonstrate an increased cardiac β_3-AR in myocardium from patients deceased after a septic shock (Moniotte et al., 2007).
Involvement of the cardiac β-AR at the early shock

→ Contradictory studies

Cardiac β-AR remodeling at the early shock

the 3 cardiac β-AR subtypes

One study demonstrate an increased cardiac β₃-AR in myocardium from patients deceased after a septic shock (Moniotte et al., 2007).
The endotoxemic rat

1h 2h 3h

- Tachycardia and hypotension
- Altered diastolic and systolic functions
- Metabolic acidosis and hyperventilation
- Kidney Failure
- Smooth muscle and endothelial dysfunction

Relevant model
Cardiac β-adrenergic remodeling
Cardiac β-adrenergic remodeling

Western Blotting experiments

β₁,₂ and ₃-AR Left Ventricle protein levels
Cardiac $\beta$-adrenergic remodeling

Western Blotting experiments
$\beta_{1,2}$ and $\beta_3$-AR Left Ventricle protein levels

Papillary Muscle Contractility
$\beta$ globale, $\beta_{1,2}$ and $\beta_3$-AR contractility
Cardiac β-adrenergic remodeling

Global β-AR contractility

Cardiomyocyte

β₁-AR

β₂-AR

β₃-AR

Inotropism

ACm

Gs

Gs

Global β-AR contractility

CTRL (n=7)

Peak tension (%)

[isoproterenol] (M)

Basal -10 -9 -8 -7 -6 -5 -4
Cardiac β-adrenergic remodeling

Global β-AR contractility

- β1-AR
- β3-AR
- β2-AR

Cardiomyocyte

Inotropism

Cardinal β-adrenergic remodeling

β1-AR

Inotropism

Global β-AR contractility

- Basal
- LPS (n=7)
- CTRL (n=7)

Log [isoproterenol] (M)

Peak tension (%)

0 100 200 300 400

-10 -9 -8 -7 -6 -5 -4
Cardiac $\beta_1$-adrenergic remodeling

Cardiomyocyte

$\beta_1$-AR relative expression to GAPDH1 (% of CTRL)

Inotropism

Peak tension (%)

log [isoproterenol] (M) + L 748,337 and ICI 118,551 at $10^{-6}$ M

CTRL (n=6)
Cardiac $\beta_1$-adrenergic remodeling

Cardiomyocyte

$\beta_1$-AR
$\beta_2$-AR

Inotropism

$\beta_1$-AR (72 kD)

GAPDH

$\beta_1$-AR relative expression to GAPDH (% of CTRL)

CTRL

LPS

Peak tension (%)

log [isoproterenol] (M)

+ L 748,337 and ICI 118,551 at $10^{-6}$ M

CTRL (n=6)
Cardiac $\beta_1$-adrenergic remodeling

$\beta_1$-AR relative expression to GAPDH1 (% of CTRL)

Inotropism

Cardiomyocyte

$\beta_1$-AR

$\beta_2$-AR

$\beta_3$-AR

Gs

ACm

Baseline

Log [isoproterenol] (M)

+ L 748,337 and ICI 118,551 at $10^{-6}$ M

Peak tension (%)

CTRL (n=6)

LPS (n=5)

$\beta_1$-AR (72 kD)

GAPDH

$\beta_1$-AR relative expression to GAPDH1 (% of CTRL)

CTRL

LPS

*
Cardiac $\beta_2$-adrenergic remodeling

$\beta_2$-AR relative expression to GAPDH1 (% of CTRL)

Log [isoproterenol] (M)

$+ L 748,337 and CGP 20712 at 10^{-6} M$
Cardiac $\beta_2$-adrenergic remodeling

$\beta_2$-AR relative expression to GAPDH1 (% of CTRL)

Inotropism

Cardiomyocyte

$\beta_2$-AR

$\beta_1$-AR

Gs

ACm

Log [isoproterenol] (M) + L 748,337 and CGP 20712 at $10^{-6}$ M

Peak tension (%)

CTRL (n=6)

CTRL (n=6)

Basal

$\log [\text{isoproterenol}] (\text{M})$

+ $L$ 748,337 and CGP 20712 at $10^{-6}$ M

$\beta_2$-AR (55 kD)

GAPDH

$\beta_2$-AR relative expression to GAPDH1 (% of CTRL)

CTRL

LPS

*
Cardiac $\beta_2$-adrenergic remodeling

**β$_2$-AR**

$\beta_1$-AR

Cardiomyocyte

**Inotropism**

$\beta_2$-AR relative expression to GAPDH1 (% of CTRL)

[Graph showing peak tension (%)]

log [isoproterenol] (M) + L 748,337 and CGP 20712 at $10^{-6}$M

[β$_2$-AR (55 kD) and GAPDH Western blot]

[Bar graph showing β$_2$-AR relative expression (% of CTRL)]
Cardiac $\beta_3$-adrenergic remodeling

![Diagram of cardiac β3-adrenergic remodeling](image)

- **β2-AR**, **β3-AR**
- Cardiomyocyte
- Inotropism
- Cardiac β1-AR
- Cardiac β2-AR
- Cardiac β3-AR
- Gs
- ACm

### Graphs

#### Peak tension (%)

- **CTRL (n=6)**
- **log [isoproterenol] (M)**
- + Nadolol at $10^{-6}$ M

#### β3-AR relative expression to GAPDH (% of CTRL)

- **CTRL**
- **LPS**
- β3-AR (72 kD)
- GAPDH

---

Inotropism
Cardiac $\beta_3$-adrenergic remodeling

$\beta_2$-AR

$\beta_1$-AR

$\beta_3$-AR

Cardiomyocyte

Inotropism

$\beta_3$-AR (72 kD)

GAPDH

$\beta_3$-AR relative expression to GAPDH1 (% of CTRL)

CTRL

LPS

Inotropism

log [isoproterenol] (M) + Nadolol at $10^{-6}$ M

Peak tension (%)

CTRL (n=6)

-10 -9 -8 -7 -6 -5

Basal

0 100 200 300

0

100

200

300

0

100

200

300

0

100

200

300

CTRL

LPS

*
Cardiac $\beta_3$-adrenergic remodeling

Diagram showing $\beta_2$, $\beta_1$, and $\beta_3$-adrenergic receptors with associated Gs proteins and ACm. Inotropism is depicted with a bar graph showing $\beta_3$-AR relative expression to GAPDH1 (% of CTRL) with a * indicating significance. Bar graphs compare CTRL (n=6) and LPS (n=6) peak tension (%).

Log [isoproterenol] (M) with Nadolol at $10^{-6}$ M.
The β-AR increased contractility in papillary muscle seemed to involve the β₁-AR.
Cardiac β-adrenergic remodeling

<table>
<thead>
<tr>
<th>Proteins (LV)</th>
<th>Function (Papillary muscle)</th>
<th>Global β-AR</th>
<th>β₁-AR</th>
<th>β₂-AR</th>
<th>β₃-AR</th>
</tr>
</thead>
</table>

The β-AR increased contractility in papillary muscle seemed to involve the β₁-AR.

Endothelial dysfunction during the shock

The endocardial endothelium (EE) is known to modulate cardiac contractility.
Cardiac β-adrenergic remodeling

<table>
<thead>
<tr>
<th>Global β-AR</th>
<th>β₁-AR</th>
<th>β₂-AR</th>
<th>β₃-AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins (LV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Function (Papillary muscle)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The β-AR increased contractility in papillary muscle seemed to involve the β₁-AR.

**EE involvement in the β₁-AR increased contractility?**

The endocardial endothelium (EE) is known to modulate cardiac contractility.

Cardiomyocyte

Inotropism

Endocardial Endothelial Cell

β₁-AR

ACm

Gs
Endocardial Endothelium Removal

EE+

3s Triton X-100 at 0.5%

EE-

From Guijarro
Endocardial Endothelium Removal

**Papillary muscle contractility**

- **EE+** (n=7)
- **EE-** (n=13)

*From Guijarro*
Endocardial Endothelium Modulation

Cardiomyocyte

Endocardial Endothelial Cell

Cardiac contractility modulation

Inotropism

$log [\text{isoproterenol}] (M)$

+ L 748,337 and ICI 118,551 at $10^{-6}M$

Peak tension (%)

CTRL EE+ (n=6)

LPS EE+ (n=5)

$\beta_1$-AR
Endocardial Endothelium Modulation

\[ \beta_1-\text{AR} \]

Cardiomyocyte

\[ \text{ACm} \]

Endocardial Endothelial Cell

\[ \text{Gs} \]

Inotropism

Cardiac contractility modulation

Peak tension (%)

Baseline -10 -9 -8 -7 -6 -5 -4

CTRL EE+ (n=6)

LPS EE+ (n=5)

LPS EE- (n=6)

log [isoproterenol] (M) + L 748,337 and ICI 118,551 at \(10^{-6}\)M

**OOO OOO**

*** ***
Endocardiomyocyte

ACm

Gs

β₁-AR

Cardiomyocyte

Endocardial Endothelial Cell

Cardiac contractility modulation

Endothelial pathway involved?

Log [isoproterenol] (M) + L 748,337 and ICI 118,551 at 10⁻⁶M

CTRL EE+ (n=6) LPS EE+ (n=5) LPS EE- (n=6)

Endocardial Endothelium Modulation
Endothelial Pathways Modulation

β₁-AR

Cardiomyocyte

Inotropism

ET-R

ACm
GS

NOS
COX
ET

Endocardial Endothelial Cell

Log [isoproterenol] (M) + L 748,337 and ICI 118,551 at 10⁻⁶ M

Peak tension (%)

LPS EE+ (n=5)
LPS EE- (n=6)

Basal -10 -9 -8 -7 -6 -5 -4

0 100 200 300 400 500 600 700 800
Endothelial Pathways Modulation

Inotropism

- LPS EE+ (n=5)
- LPS EE- (n=6)
- LPS EE+ + L-NMMA 1 μM (n=6)

log [isoproterenol] (M) + L 748,337 and ICI 118,551 at 10^-6 M
Endothelial Pathways Modulation

Inotropism

Cardiomyocyte

β₁-AR

ACm

Gs

ET-R

Endocardial Endothelial Cell

NOS

COX

ET

LPS EE+ (n=5)

LPS EE- (n=6)

LPS EE+ + bosentan 10 μM (n=7)

Peak tension (%)

log [isoproterenol] (M) + L 748,337 and ICI 118,551 at 10⁻⁶ M
Endothelial Pathways Modulation

β₁-AR

Cardiomyocyte

Endocardial Endothelial Cell

Peak tension (%)

Log [isoproterenol] (M) + L 748,337 and ICI 118,551 at 10⁻⁶ M
Endothelial Pathways Modulation

COX1 (SC 560) and COX2 (NS 398) specific inhibitors

- LPS EE+ (n=5)
- LPS EE- (n=6)
- LPS EE+ + indo 10 μM (n=6)

log [isoproterenol] (M) + L 748,337 and ICI 118,551 at 10^{-6}M
Endothelial Pathways Modulation

Cardiomyocyte

β₁-AR

ACm

Gs

Inotropism

COX-2

Endocardial Endothelial Cell

COX-1

Endocardial Endothelial Cell

Peak tension (%)

log [isoproterenol] (M)

+ L 748,337 and ICI 118,551 at 10⁻⁶M

LPS EE+ (n=5)

LPS EE- (n=6)

LPS EE+ + SC560 3μM (n=5)
**Endothelial Pathways Modulation**

**Diagram:**
- **β₁-AR**
- **ACm**
- **Gs**
- **Cardiomyocyte**
- **COX-1**
- **COX-2**
- **Endocardial Endothelial Cell**

**Graph:**
- Log [isoproterenol] (M)
- Basal
- LPS EE+ (n=5)
- LPS EE- (n=6)
- LPS EE+ + NS398 3μM (n=6)

**Legend:**
- LPS EE+ (n=5)
- LPS EE- (n=6)
- LPS EE+ + NS398 3μM (n=6)

**Note:**
- log [isoproterenol] (M) + L 748,337 and ICI 118,551 at 10⁻⁶M

**Institute:**
- Institut du thorax
Conclusions

Endothelial COX1 and COX2 pathways are involved in the increased $\beta_1$-AR contractility in the early endotoxemic shock.
Acknowledgment

Chantal Gauthier  Bertrand Rozec  Jean-Noël Trochu
Benjamin Lauzier  Sophie Talon  Morteza Erfanian
Amandine Grabherr  Nolwenn Merlet  Damien Guijarro
Angélique Erraud  Valentine Prat  Marine Ferron
Thank you for your attention and happy new year
At early phase of endotoxemetic shock
the increased β-adrenergic contractility is dependent on
the β1-adrenoceptor

David ROUL

I’ institut du thorax

No conflicts of interest
# Vascular β-adrenergic remodeling

<table>
<thead>
<tr>
<th></th>
<th>β-AR</th>
<th>β₁-AR</th>
<th>β₂-AR</th>
<th>β₃-AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA (Aorta)</td>
<td></td>
<td>←</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Proteins (Aorte)</td>
<td></td>
<td>←</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Fonction (Aorte Mesenteric artery)</td>
<td>↓</td>
<td>←</td>
<td>↑</td>
<td></td>
</tr>
</tbody>
</table>

Isolated Organ Bath
Vascular β-adrenergic remodeling

- **CTRL (n=9)**

- **log \([\text{Salbutamol}]\) (M)**

- **β₂-AR**

- **Endothelial Cell**

- **NO**

- **GTP**

- **GMPc**

- **RELAXATION**

- **Smooth Muscle Cell**

- **β₂-AR**

- **Gs**

- **AC**

- **GC**

- **β₂-AR**
Vascular β-adrenergic remodeling

Potentiation of β2-AR vasodilation
Vascular β-adrenergic remodeling

Endothelial β₂-AR vasodilation in CTRL

log [Salbutamol] (M)

% relaxation

-9 -8 -7 -6 -5

CTRL (n=9)
LPS (n=10)
CTRL endo - (n=13)

Endothelial Cell

β₂-AR

β₂-AR vasodilation in CTRL

Smooth Muscle Cell

GTP

GMPc

Gs

AC

GC

eNOS

NO

AC

RELAXATION

Vascular β₂-adrenergic remodeling
Vascular β-adrenergic remodeling

Smooth muscle β₂-AR vasodilation in LPS