Pathology Influences Blood Pressure Change following Vagal Stimulation in an Animal Intubation Model

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Introduction

The intubation of children during critical care illness can provoke alterations in both heart rate [1,2,3] and blood pressure [4,5]. Heart rate generally falls during intubation due to the activation of the Vagus nerve, either by hypoxia in the aortic bodies or mechanical stimulation of the superior laryngeal nerve [3,6]. Certain induction [7,8] and muscle relaxant drugs [9] can also contribute to falls in heart rate.

Changes in blood pressure during intubation in critical care illness are more complex. In adults there is a tendency for the mean blood pressure to fall by 30 mmHg [10]. In contrast, an increased blood pressure has been reported during neonatal intubation despite significant decreases in heart rate [4,5]. A possible explanation for these discordant observations is that different diseases may have different effects on haemodynamics during intubation. For example, sepsis reduces peripheral vaso-motor tone [11] whereas hypovolaemia is associated with vasoconstriction [12]. Certain anaesthetic induction agents also modify vascular tone [13].

Our objective was to determine whether the blood pressure response to vagal stimulation was modified by endotoxaemia or hypovolaemia.

Abstract

Purpose: The haemodynamic response to critical care intubation is influenced by the use of sedation and relaxant drugs and the activation of the vagal reflex. It has been hypothesized that different disease states may have a contrasting effect on the cardiovascular response to vagal stimulation. Our objective was to determine whether the blood pressure response to vagal stimulation was modified by endotoxaemia or hypovolaemia.

Methods: New Zealand White rabbits were anaesthetised with urethane before tracheotomy. The exposed left Vagus nerve of randomised groups of control (n = 11), endotoxin (n = 11, 1 mg/kg), hypovolaemia 40% (n = 8) and hypovolaemia 20% (n = 8) rabbits were subjected to 10 Hz pulsed electrical stimulations of 25 s duration every 15 min. Haemodynamic parameters were recorded from a catheter in the right carotid artery connected to an iWorx monitor. Serum catecholamines were measured every 30 min using reverse-phase ion-pairing liquid chromatography. The change in blood pressure after vagal stimulation was compared to controls for one hour after the first death in the experimental groups.

Results: 29% of the rabbits died in the hypovolaemia 40% group and 27% in the endotoxin group. One rabbit died in the hypovolaemia 40% group before vagal stimulation and was excluded. Following electrical stimulation of the Vagus nerve there was a fall in blood pressure in control rabbits. Blood pressure was conserved in the hypovolaemic rabbits compared to controls (p<0.01). For the endotoxoaemic rabbits, there was a non-significant trend for the mean blood pressure to decrease more than the controls. Serum catecholamines were significantly raised in both the hypovolaemic and endotoxiaemic rabbits.

Conclusions: Pathology may contribute to modifications in blood pressure when vagal activation occurs. Patients who are either already vasoconstricted, or not vasoplegic, may be less at risk from intubation-related vagally mediated reductions in blood pressure than those with vasodilatory pathologies.
were euthanised with a lethal dose of anaesthetic (5 g/kg
urethane). Male New Zealand White (NZW) rabbits were obtained
from Hypharm, La Corbière, 49450, Roussay, France. In line with
recommendations, a maximum of 15 rabbits at a time were
maintained in an aird pen 3 m by 4 m and 3 m high with a dense
layer of straw and hay [14]. Food and water was provided ad
libitum.

Preparation of the rabbits

The rabbits were anaesthetised by the injection urethane in a
marginal vein of an ear. 1.0 g/kg was used for rabbits weighing
≤1.8 kg and 2.0 g/kg for rabbits weighing >1.8 kg. The level of
anaesthesia was monitored using paw and corneal reflexes.
Urethane was chosen as an anaesthetic because of its single dose
regimen and because it has previously been established to have
minimal effect on haemodynamic parameters in anaesthetised rats
[15]. Another advantage of using urethane was that the animals
remained spontaneously breathing.

Sterile surgical conditions were used to make a midline incision
in the anterior aspect of the neck. The trachea was opened before
being intubated with a 7 cm plastic tube of with an internal
diameter of 4.0 mm and 5.6 mm external diameter (Saint Gobain
Verneret, La Mothe-aux-Aulnaires, F89120 Charny, France). The
right carotid artery was cannulated with a 22 gauge cannula, and the
left Vagus nerve was exposed. Continuous mean blood pressure
readings were made using an iWorx® 214 monitor connected to
the carotid artery catheter (iWorx Systems Inc, 62 Littleworth
Raod, Dover NH 03820, USA). Arterial blood gas measurements
were made using an i-STAT® system (Abbott Point of Care Inc.,
400 College Road East, Princeton, NJ 08540, USA). The rabbits
were entered into the experiment when baseline blood pressure
had been stable for at least 15 minutes and arterial pH was
between ≥7.35 and ≤7.45 (normal limits of rabbit pH 7.2–7.5
[16]).

Conduct of the experiment

The rabbits were randomly assigned into four groups; control
(11 rabbits), endotoxemia 1 mg/kg (11 rabbits), hypovolaemia
20% reduction in blood volume (HV20%, 8 rabbits) and
hypovolaemia 40% reduction in blood volume (HV40%, 8 rabbits).

Rabbits in the hypovolaemia groups were bled (estimated
blood volume of 5.5 ml of blood per 100 g of body weight [17])
over a period of five minutes from the carotid artery catheter
20 minutes after baseline. The rabbits assigned to the endotox-
aemia group were injected with 1 mg/kg of endotoxin (LPS
from E.Coli B:055, Sigma-Aldrich, France) diluted at 1 mg/ml in
0.9% saline solution at a rate of 2 mg/5 minutes in the
marginal vein of an ear.

Blood Sampling and Biochemical Analysis

Blood sampling was performed at the following times for all
rabbits; baseline, then 30 minute intervals until 150 minutes. At
150 minutes the surviving hypovolaemic rabbits were euthanised.

The control and endotoxaemic rabbits were then sampled at
180 minutes and then 60 minute intervals until 300 minutes,
when the survivors were euthanised. The samples taken from the
endotoxaemic rabbits at 30, 90 and 150 minutes were not
analysed so as to ensure identical experimental conditions for all
groups, see Figure 1.

Samples of 1.3 ml of blood were drawn from the carotid artery.
Seventy microlitres were separated for blood gas (pH, PCO2, base
excess, bicarbonates) and serum lactate (normal range 0.5–
1.5 mmol/l [18]) analysis. A haematocrit (NZW normal range
31.3–43.3%, mean weight of rabbit 3.6 kg [19]) was determined
by placing 0.1 ml of whole blood in a heparin coated plastic tube
(100 µl safeCLINITUBES, Radiometer Medical A/S, Akadevej
21, 2700 Brønshøj, Denmark) which was centrifuged at 2000
revolutions/minutes for four minutes. The remaining 1.1 ml was
transferred into an EDTA tube, shaken gently before centrifuga-
tion at 4000 rpm at a temperature of 4°C for 5 minutes.
Thereafter, the plasma was drawn off and transferred directly
for storage at −40°C in the dark for subsequent catecholamine
analysis.

Stimulation of the Vagus Nerve

The Vagus nerve was intermittently lifted free from other
structures in the neck, laid across two stainless steel electrodes
5 mm apart and electrically stimulated using the iWorx system
with a 3.0 mA current, 250 pulsations of 10 milliseconds at
10 Hz (duration of stimulation 25 seconds). A three-minute
interval was left after taking blood before a vagal stimulation.
Stimulations were made at baseline, then at 15 minute intervals
for all rabbits until 180 minutes and then half-hourly for the
control and endotoxaemic rabbits until 300 minutes, see Figure 1.

Catecholamine analysis

All catecholamine analyses were performed at the Hôpital
Robert Debé, Paris, France in accordance with the reverse-phase
ion-pairing liquid chromatography methodology described by
Candito et al. in 2002 [20]. Fifteen to 25 samples were batched for
analysis. A negative control (acid), internal control (urine
catecholamine) and standard control (quantified plasma catechol-
amine [Chromosystems Instruments and Chemicals GmbH,
Heimburgstrasse 3, 81243 München, Germany]) were analysed
in sequence before each batch.

Briefly, the plasma was removed from storage and thawed at
room temperature. Six hundred microlitres was drawn from the
plasma samples and 20 mg of alumina (aluminium oxide,
Al2O3) and 100 µl of the internal control were added to the
600 µl of plasma. The alumina was washed three times before
separation of the catecholamines from the alumina. Fifty
microlitres of the supernatant were removed for injection
through a 717 Waters auto-sampler onto a C18 Purospher
(Merck) column (150×4 mm, 4 mm) equipped with a guard
column. The aqueous isocratic mobile phase adjusted to pH 3.9
consisted of citric acid 20 mM, sodium acetate 50 mM, sodium
heptane sulfonate 0.4 mM in methanol 10%. Mobile phase was
pumped at a flow rate of 1 ml/min by a Waters Model 550
pump. Quantification of the catecholamines was carried out
using an amperometric 2465 Waters detector set at 0.5 V.
Millennium Waters Empower Chromatography software was
used for all calculations.

Presentation of results

The first death in each group was used post hoc as a measure of
severity of pathology. Results are presented for one hour of
monitoring from the vagal stimulation prior to the first death.

Statistical analysis

Qualitative variables are described as numbers and percentages
and quantitative variables as median [quartiles] or mean (standard
development) according to their Gaussian distribution. An unpaired t-
test, or a Mann-Whitney test, were used for continuous data
according to their distribution. All statistical tests were 2-sided and
the probability of a type 1 error (α) was determined at <0.05. All
statistical tests were carried out using SPSS (version 19).
Results

Baseline Characteristics

Baseline characteristics of animals in each group were similar with the exception of adrenaline and noradrenaline which were significantly higher in the endotoxin group versus the control group (Table 1).

Mortality and Presentation of the Results

One rabbit from the 40% hypovolaemia group died after bleeding and before vagal stimulation and was excluded from subsequent analysis. There were two deaths in the HV40% group (2/7, 29%) and three in the endotoxin group (3/11, 27%). No rabbits died in the HV20% group. The first death after vagal stimulation in the HV40% group occurred between +15 and

![Gantt chart showing schema of blood sampling and electrical stimulation.](image)

Control rabbits and the experimental groups all received the same number of stimulations and blood samples. Endotoxaemic rabbits had five stimulations and three blood samples that were not analysed, as according to protocol, so as to ensure identical experimental conditions for all groups.

Table 1. Baseline characteristics of the rabbits.

|                         | Control t = 0 (n = 11) | Endotoxin t = 0 (n = 11) | Hypovolaemia 20% t = 0 (n = 8) | Hypovolaemia 40% t = 0 (n = 7) |
|-------------------------|------------------------|--------------------------|-------------------------------|-------------------------------|
| Weight (kg)             | 1.96 (1.79–2.13)       | 1.93 (1.76–2.10)         | 1.91 (1.71–2.11)              | 1.87 (1.70–2.04)              |
| pH                      | 7.39 (7.37–7.42)       | 7.39 (7.37–7.40)         | 7.39 (7.37–7.41)              | 7.38 (7.36–7.42)              |
| Lactate (mmol/l)        | 2.9 (1.8–5.5)          | 2.3 (1.7–3.3)            | 3.6 (1.7–5.5)                 | 3.0 (2.6–4.5)                 |
| Bicarbonate (mmol/l)    | 25.4 (22.6–28.0)       | 24.0 (21.7–24.5)         | 24.8 (24.5–27.3)              | 23.4 (22.5–24.3)              |
| pCO2 (kPa)              | 5.5 (5.2–6.2)          | 5.1 (5.0–5.4)            | 5.5 (5.1–6.0)                 | 5.4 (5.0–5.7)                 |
| Haemocrit (%)           | 42 (37–45)             | 45 (42–46)               | 43 (40–48)                    | 42 (39–47)                    |
| Adrenaline (nM)         | 2.0 (0–5.2)            | 7.9 (4.3–9.0) *          | 1.8 (0.6–5.8)                 | 0.7 (0.5–2.6)                 |
| Noradrenaline (nM)      | 0.3 (0–1.6)            | 1.5 (0.6–2.4) *          | 0.4 (0–1.4)                   | 0.4 (0–0.5)                   |
| Heart rate (bpm)        | 310 (278–323)          | 279 (267–303)            | 289 (270–297)                 | 294 (264–311)                 |
| Pulse pressure (mmHg)   | 27 (20–31)             | 27 (23–29)               | 24 (19–28)                    | 29 (19–32)                    |
| Blood pressure (mmHg)   | 77 (58–92)             | 72 (64–79)               | 63 (54–80)                    | 73 (64–80)                    |
| Change in blood pressure| 28 (21–37)             | 28 (18–31)               | 23 (14–29)                    | 22 (17–30)                    |

Interquartile ranges [IQR] and standard deviations are shown (SD) according to the distribution of the variables.

*p < 0.05,

**p < 0.01.

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+30 minutes from bleeding and between +240 and +270 minutes in the endotoxin group. According to protocol, the rabbits’ parameters were analysed from +15 minutes in the hypovolaemia groups and +240 minutes for the endotoxin group for a period of one hour.

Changes in Biochemical and Haematological Parameters in Hypovolaemia

The hypovolaemia groups showed decreases in pH, CO2, HCO3, haematocrit and pulse pressure, mean blood pressure and heart rate with a increases in adrenaline and lactates compared to the control group (Tables 1 and 2). These changes were proportional to severity of hypovolaemia.

Changes in Biochemical and Haematological Parameters in Endotoxaemia

In the endotoxin group there were significant decreases in CO2 and bicarbonate with no change in pH, lactates and haematocrit and a significant increase in noradrenaline (Table 3). There were no changes in the haemodynamic parameters between control and endotoxaemic rabbits except a difference in blood pressure at +240 minutes (Figure 2).

Changes in blood pressure following vagal stimulation

During electrical stimulation of the Vagus nerve there was significant, and dose dependent, maintenance of mean blood pressure in the hypovolaemic rabbits compared to controls (Figure 3). For the endotoxaemic rabbits, there was a non-significant trend for the mean blood pressure to decrease more than the controls (Figure 3).

Discussion

We disproved our hypothesis. Following vagal stimulation the blood pressure of the hypovolaemic rabbits was significantly conserved when compared to controls. When compared to controls, endotoxaemic rabbits displayed a non-significant tendency towards a greater fall in blood pressure.

Predicatable effects of hypovolaemia and endotoxaemia on haemodynamics

Classically the effect of hypovolaemic shock on haemodynamic integrity is biphasic and asymmetric. Mean blood pressure is maintained during the early compensatory Phase I by combination of increasing heart rate and vasoconstriction despite a fall in

### Table 2. Changes in biochemistry, catecholamines and haematocrit in the hypovolaemic rabbits.

| Group | Time t =+15 | Time t =+45 | Time t =+75 |
|-------|-------------|-------------|-------------|
| Number of rabbits | | | |
| Control | 11 | 11 | 11 |
| HV 20% | 8 | 8 | 8 |
| HV 40% | 7 | 6 (1 death) | 5 (2 deaths) |
| pH | | | |
| Control (ref) | 7.39 (7.37–7.41) | 7.40 (7.37–7.41) | 7.39 (7.37–7.41) |
| HV 20% | 7.34 (7.29–7.38) | 7.35 (7.28–7.39) | 7.34 (7.29–7.38) |
| HV 40% | 7.40 (7.33–7.43) | 7.25 (7.1–7.3) * | 7.30 (7.12–7.3) * |
| Lactate (mmol/l) | | | |
| Control (ref) | 3.3 (1.8–5.5) | 3.5 (1.5–5.6) | 4.2 (1.5–5.6) |
| HV 20% | 4.3 (1.3–6.7) | 5.5 (2.0–8.8) | 5.9 (2.0–9.8) |
| HV 40% | 5.5 (4.1–5.9) | 9.2 (5.7–11.7) * | 8.7 (6.0–16.1) * |
| Bicarbonate (mmol/l) | | | |
| Control (ref) | 26.6 (23.6–28.1) | 25.3 (24.6–28.4) | 25.4 (24.5–28.1) |
| HV 20% | 24.8 (21.4–26.4) | 22.6 (21.6–23.9) | 22.2 (20.6–26.6) |
| HV 40% | 18.6 (17.9–22.1) * | 16.5 (12.9–19.2) * | 16.2 (12.2–18.4) * |
| pCO2 (kPa) | | | |
| Control (ref) | 6.0 (5.5–6.2) | 5.8 (5.4–6.2) | 5.8 (5.5–6.0) |
| HV 20% | 5.2 (5.0–6.2) | 5.2 (4.9–6.0) | 5.5 (5.1–6.4) |
| HV 40% | 4.5 (4.1–4.6) * | 4.6 (4.4–7.3) * | 4.5 (4.0–5.6) |
| Haematocrit (%) | | | |
| Control (ref) | 41 (37–47) | 41 (37–45) | 41 (36–44) |
| HV 20% | 38 (36–40) | 37 (35–39) | 36 (33–38) * |
| HV 40% | 36 (28–38) * | 32 (28–34) * | 32 (26–34) * |
| Adrenaline (nM) | | | |
| Control (ref) | 0.45 [0–2.4] | 0 [0–0.55] | 0 [0–0.55] |
| HV 20% | 0.83 [0.32–1.2] | 0.14 [0–1.1] | 0.62 [0.7–1.5] |
| HV 40% | 2.45 [0.43–13.9] | 1.3 [0.36–22.4] * | 1.34 [0.3–7.3] |
| Noradrenaline (nM) | | | |
| Control (ref) | 1.00 [0–3.2] | 0.40 [0–2.1] | 0 [0–1.9] |
| HV 20% | 2.42 [0.55–3.8] | 1.50 [0.10–3.9] | 1.79 [0.11–3.2] |
| HV 40% | 0.39 [0–1.9] | 0.56 [0.37–7.7] | 0.88 [0.38–3.6] |

Interquartile ranges [IQR] and standard deviations are shown (SD) according to the distribution of the variables.

*p<0.05,  *p<0.01.
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cardiac output [12,21]. This is followed by a compensatory Phase II which sees a fall in mean blood pressure and pre-load associated with a fall in vascular resistance despite maintenance of cardiac output [12,21].

In a similarity with Phase I hypovolaemia, sepsis is associated with a reduction in pre-load and left ventricular stroke volume [22]. The difference with Phase I hypovolaemia is that whereas hypovolaemia is associated with vasoconstriction sepsis is associated vasodilatation [23].

**Observed effects of hypovolaemia and endotoxaemia in our model**

In the hypovolaemia model, mean blood pressure fell markedly after bleeding (75 mmHg to 20 mmHg in the HV40% model) before increasing marginally (vasoconstrictive Phase I) to reach a plateau and then falling (vasodilatory Phase II) (Figure 2). Generally, the rabbits showed predictable haemodynamic changes with the exception of a fall in heart rate. This unexpected observation has previously been observed in hypovolaemic humans where reduced pre-load is compensated for by a prolongation of diastole which allows better ventricular filling [24]. Parallel dose-related changes in pH, bicarbonates, CO2 and lactates corroborated the haemodynamic alterations identified.

The effect of the endotoxin in our model is more difficult to interpret. Our rabbits did not demonstrate the decrease in blood pressure as early as 1–2 hr after injection that has been observed in other studies in rabbits (1 mg/kg) [25] and dogs (1.5 mg/kg) [26]. Neither was there a significant change in heart rate. Despite the relative paucity of haemodynamic changes, the mortality of the rabbits was similar to that observed in the HV40% model and the endotoxaemic rabbits displayed a significant compensated metabolic acidosis. It is possible that haemodynamic changes could have been recorded after 300 minutes. However, experimentation was terminated at 6 h because of concerns that the time taken to prepare (approximately 1 h) and monitor (300 min) the rabbits could exceed the anaesthetic effect and due to the uncertainty regarding the derangement of physiological homeostasis by a prolonged anaesthetic exposure. Another possible weakness of our model is perhaps demonstrated by the absence of difference between lactate levels between the endotoxaemic and control rabbits, as has been previously observed by Lobo et al. [25].

**Catecholamines**

Raised levels of noradrenaline and adrenaline have previously been demonstrated in animal models of hypovolaemia [27] and sepsis [28]. The effect of these hormones is to result in vasoconstriction [29]. Despite the fact that both the hypovolaemic and endotoxaemic rabbits displayed raised levels of circulating catecholamines, only the hypovolaemic rabbits exhibited diminished pulse pressure, which is an indirect maker of vasoconstriction (Figure 2). This suggests that there was a relative failure of vasoconstriction in the endotoxaemic rabbits.

All four experimental groups displayed raised levels of baseline circulating catecholamines after surgical preparation and before vagal stimulations. These were possibly due to the stress of anaesthetic induction and/or preparation. Another possibility is that the rabbits were improperly anaesthetised despite each being tested for paw and corneal reflexes [30]. After the start of the vagal stimulations a decrease in catecholamines was observed in all groups (results not shown) with the exception of the endotoxaemic rabbits. This may be due to the influence of the neuro-inflammatory pathway [31]. Zhang et al. [32] have demonstrated diminished production of catecholamines after repeated vagal stimulation in shocked dogs.

### Table 3. Changes in biochemistry, catecholamines and haematocrit in the endotoxin group.

| Variable                  | Time t = +240     | Time t = +300     |
|---------------------------|-------------------|-------------------|
| Number of rabbits         | 11                | 11                |
| Endotoxin                 | 11                | 8                 |
| pH Control (ref)          | 7.35 (7.34–7.40)  | 7.36 (7.35–7.39)  |
| Endotoxin                 | 7.35 (7.33–7.40)  | 7.33 (7.28–7.36)  |
| Lactate (mmol/l) Control  | 4.2 (2.0–5.7)     | 3.3 (1.8–5.6)     |
| Endotoxin                 | 3.5 (2.1–5.1)     | 4.2 (1.8–5.1)     |
| Bicarbonate (mmol/l) Control | 24.9 (23.9–26.4) | 25.2 (23.5–27.4) |
| Endotoxin                 | 21.7 (18.4–22.4)  | 21.0 (20.1–22.4)  |
| pCO2 (kPa) Control        | 6.1 (5.7–6.5)     | 5.9 (5.6–6.2)     |
| Endotoxin                 | 4.9 (4.6–5.4)     | 5.2 (4.7–6.3)     |
| Haematocrit (%) Control   | 39 (37–43)        | 40 (37–43)        |
| Endotoxin                 | 43 (39–44)        | 38 (38–41)        |
| Adrenaline (nM) Control   | 0.29 [0–0.73]     | 0.31 [0–1.1]      |
| Endotoxin                 | 1.13 [0–3.5]      | 0.66 [0–2.8]      |
| Noradrenaline (nM) Control | 0.75 [0–3.2]     | 0.36 [0–3.7]      |
| Endotoxin                 | 3.45 [2.3–6.0]    | 3.55 [2.4–5.0]    |

Interquartile ranges [IQR] and standard deviations are shown (SD) according to the distribution of the variables.

*p<0.05, †p<0.01.

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**The effect of vagal stimulation on blood pressure**

Vagal stimulations at baseline and in control rabbits reduced blood pressure in the order of 20 to 30 mmHg (Figure 3). After vagal stimulation in the hypovolaemic rabbits there was a surprising maintenance of blood pressure (Figure 3). Indeed, there was an association between lower blood pressure and maintenance of blood pressure after vagal stimulation (Figure 3). Some severely hypovolaemic/hypotensive rabbits increased their mean blood pressure following vagal stimulations when their baseline stimulations in the same rabbits had resulted in decreased blood pressure.

The endotoxaemic rabbits they had similar baseline blood pressure compared to control rabbits (Figure 2) but died at the same rate as the HV40% rabbits. However, following vagal stimulation the endotoxaemic rabbits and were less able to maintain blood pressure than the controls (Figure 3). One possible explanation for these dichotomous results is the different state of vascular reactivity; the hypovolaemic rabbits being vasoconstricted and endotoxaemic rabbits being vasodilated.

**Limitations of our model**

The most appropriate animal model of human sepsis is subject to conjecture [33]. Our aim by using an intravenous bolus of endotoxin was to obtain maximum harmonisation of experimental conditions. However, in the clinical situation there is rarely an explosive release of cytokines but a more gradual inflammatory response. The understanding of the haemodynamic response to in our endotoxin model would perhaps been helped had we monitored markers of systemic inflammation, such as TNF and IL-1. This was not done due to concerns regarding the extra volume of sampled blood. We did not explore the possible effects of gram positive sepsis.
Figure 2. Changes in haemodynamic parameters for the control; hypovolaemia and endotoxaemia rabbits. The central bar is the mean, the box represents the inter-quartile range and the whiskers the range. Significant differences between the control and experimental groups of $p<0.05$ are noted by an '*' and $p<0.01$ by an '¤'.
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Clearly the haemodynamic response to intubation is a multi-factorial process with operator experience, method of intubation, sedation and relaxant drugs all potentially contributing to change in blood pressure. Inevitably, the more that the associated influences around intubation are controlled for in an experimental environment the less that the model resembles the clinical situation. However, our vagal stimulation model retains some relevance where vagal activation is frequent, such as during the intubation of children who have predominant parasympathetic balance in the early part of life [34]. The use of repeated vagal stimulations in the same animals further distorts the relevance to the clinical situation. The advantage of this methodology, however, is that the changes in blood pressure over time demonstrate the evolution of both disease models and the effect of the vagal stimulations.

**Conclusions**

Pathology may contribute to modifications in blood pressure when vagal activation occurs. Patients who are either already vasoconstricted, or not vasoplegic, may be less at risk from intubation-related vagally mediated reductions in blood pressure than those with vasodilatory pathologies. Our results require confirmation in a model with a larger cohort where vascular resistance, ejection volume and cardiac output are measured.

**Author Contributions**

Performed the experiments: PJ LG CD J-FB. Analyzed the data: PJ J-FB. Contributed reagents/materials/analysis tools: PJ LG CD HC J-FB SD MJP. Wrote the paper: PJ MJP. Designed and carried out the experiments, did the statistical analysis: PJ. Assisted in the preparation of the animals: LG. Made an important contribution to the development of the techniques used: CD. Directed the hormonal analyses and used software to calculate the catecholamines: J-FB. Helped to design the experiments: HC SD MJP.

**References**

1. Carroll CL, Spinella PC, Corsi JM, Stoltz P, Zucker AR (2011) Emergent endotracheal intubations in children: be careful if it’s late when you intubate. Pediatr Crit Care Med 11: 343–348.
2. Fastle RK, Roback MG (2004) Pediatric rapid sequence intubation: incidence of reflex bradycardia and effects of pretreatment with atropine. Pediatr Emerg Care 20: 651–655.
3. Jones P, Peters MJ, Pinto da Costa N, Kurch T, Alberzi C, et al. (2013) Atropine for critical care intubation in a cohort of 264 children and reduced mortality unrelated to effects on bradycardia. PLoS One 8: e57478.
4. Marshall TA, Deeder R, Pai S, Berkowitz GP, Austin TL (1984) Physiologic changes associated with endotracheal intubation in preterm infants. Crit Care Med 12: 501–503.
5. Oei J, Hari R, Butha T, Lui K (2002) Facilitation of neonatal nasotracheal intubation with premedication: a randomized controlled trial. J Paediatr Child Health 38: 146–150.
6. Jones P, Dauget S, Peters MJ (2011) Bradycardia during critical care intubation: mechanisms, significance and atropine. Arch Dis Child 97: 139–144.
7. Steur RJ, Perez RS, De Lange J (2004) Dosage scheme for propofol in children under 3 years of age. Paediatr Anaesth 14: 462–467.
8. Tirel O, Chanavaz C, Bansard JY, Carre F, Ecolily C, et al. (2005) Effect of remifentanil with and without atropine on heart rate variability and RR interval in children. Anaesthesia 60: 582–589.
9. Leigh MD, Del M, Belton MK, Lewis GB Jr (1957) Bradycardia following intravenous administration of succinylcholine chloride to infants and children. Anesthesiology 18: 698–702.
10. Jaber S, Amraoui J, Leffant JY, Arich C, Gohendy R, et al. (2006) Clinical practice and risk factors for immediate complications of endotracheal intubation in the intensive care unit: a prospective, multiple-center study. Crit Care Med 34: 2355–2361.
11. Feltes TF, Pignatelli R, Kleinert S, Mariscalco MM (1994) Quantitated left ventricular systolic mechanics in children with septic shock utilizing noninvasive wall-stress analysis. Crit Care Med 22: 1647–1658.
12. Riou B (2004) L’hémodynamique au cours du choc hémorragique: implications cliniques. Conseil Scientifique des JEPU. pp. 3–25.
13. Robinson BJ, Ebert TJ, O’Brien TJ, Colino MD, Muzi M (1997) Mechanisms whereby propofol mediates peripheral vasodilation in humans. Sympathoinhibition or direct vascular relaxation? Anesthesiology 86: 64–72.
14. Council NR (1996) Guide pour les soins et l’utilisation des animaux; laboratoires. National Academy Press: 31.
15. Carruba MO, Bondiolotti G, Picotti GB, Catteruccia N, Da Prada M (1987) Effects of diethyl ether, halothane, ketamine and urethane on sympathetic activity in the rat. Eur J Pharmacol 134: 13–24.
16. Fox JG (2002) Laboratory Animal Medicine. 335.
17. Little RA (1970) Changes in the blood volume of the rabbit with age. J Physiol 201: 405–497.
10. Labeurit H, Baron C, Ferran C, Henriet I (1981) [Variations in blood lactic acid and glucose levels in the rabbit after infusion of isoprenaline with or without prior infusion of minaprine]. Agressologie 22: 63–64.
11. Hewitt CD, Innes DJ, Savory J, Wills MR (1989) Normal biochemical and hematological values in New Zealand white rabbits. Clin Chem 35: 1777–1779.
12. Candito M, Billaud E, Chauvettier M, Cotet-Emard JM, Desmoulin D, et al. (2002) [Biochemical diagnosis of pheochromocytoma and neuroblastoma]. Ann Biol Clin (Paris) 60: 15–36.
13. Schacht JC, Lasbrouck J (1993) Hemodynamic and neurohumoral responses to acute hypovolemia in conscious mammals. Am J Physiol 260: H305–318.
14. Jardin F, Fourme T, Page B, Louhiere Y, Vicelliard-Baron A, et al. (1999) Persistent preload defect in severe sepsis despite fluid loading: A longitudinal echocardiographic study in patients with septic shock. Chest 116: 1354–1359.
15. Brierley J, Carcillo JA, Choong K, Cornell T, Decaen A, et al. (2009) Clinical practice parameters for hemodynamic support of pediatric and neonatal septic shock: 2007 update from the American College of Critical Care Medicine. Crit Care Med 37: 666–688.
16. Barriot P, Risu B (1987) Hemorrhagic shock with paradoxical bradycardia. Intensive Care Med 13: 203–207.
17. Lobo SM, Seriano FG, Barbeiro DF, De Backer D, Sun Q, et al. (2009) Effects of dobutamine on gut mucosal nitric oxide production during endotoxic shock in rabbits. Med Sci Monit 15: BR37–42.
18. van Lambalgen AA, Brouveld W, Van den Bos GC, Thijs LG (1984) Distribution of cardiac output, oxygen consumption and lactate production in canine endotoxic shock. Cardiovasc Res 18: 193–205.
19. Ronning G, Busund R, Rekhaug A, Sager G (1993) Effect of haemorrhagic shock and intraosseous resuscitation on plasma and urine catecholamine concentrations and urinary clearance in pigs. Eur J Surg 161: 387–394.
20. Watson JD, Varley JG, Bouloux PM, Tomlin SJ, Rees LH, et al. (1988) Adrenal vein and arterial levels of catecholamines and immunoreactive metenkephalin in canine endotoxic shock and their response to naloxone. Res Exp Med (Berl) 188: 319–328.
21. Rang D (1991) Pharmacology. 2nd Edition: 184.
22. Lake CR, Ziegler MG, Kopin IJ (1976) Use of plasma norepinephrine for evaluation of sympathetic neuronal function in man. Life Sci 18: 1315–1325.
23. Tracey KJ (2002) The inflammatory reflex. Nature 420: 853–859.
24. Zhang Y, Popovic ZB, Bibevski S, Fakhry I, Sica DA, et al. (2009) Chronic vagus nerve stimulation improves autonomic control and attenuates systemic inflammation and heart failure progression in a canine high-rate pacing model. Circ Heart Fail 2: 692–699.
25. Chow LT, Choe SS, Anderson RH, Gosling JA (2001) Autonomic innervation of the human cardiac conduction system: changes from infancy to senility—an immunohistochemical and histochemical analysis. Anat Rec 264: 169–182.