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Origin of Endotoxemia Influences the Metabolic Response to Endotoxin in Dogs

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INTRODUCTION

Endotoxin is often used to mimic the inflammatory and metabolic responses during sepsis. In the literature various routes of administration have been used. In these experiments endotoxin was given intraperitoneally (rats), intravenously (dogs, rats, humans), or into the portal vein (dogs, swine) [1–10]. Endotoxin is readily cleared from the circulation by macrophages with concomitant macrophage activation, resulting in the release of inflammatory mediators, including tumor necrosis factor (TNF) and interleukin-6 (IL-6) [11]. As the liver contains a major proportion of the total body macrophage content, it is possible that endotoxin delivery by portal and systemic routes result in different cytokine responses. Concomitantly, this difference in cytokine response may lead to a difference in hepatic glucose metabolism.

The objective of this study was to compare the inflammatory, endocrine, and metabolic responses to administration of a bolus of endotoxin into a peripheral versus the portal vein in anesthetized dogs. High-dose endotoxin causes hemodynamic instability and decreased organ perfusion [1, 6, 8, 10, 12]. Because circulatory shock itself may induce metabolic changes, we injected that dose of endotoxin (1.0 µg/kg) that induces significant secretion of TNF and IL-6 in anesthetized dogs without the induction of circulatory shock [13]. To control for the effects of surgery and anesthesia, we also studied the effects of peripheral endotoxin administration in awake dogs.

MATERIALS AND METHODS

Animals

Twenty-two male mongrel dogs (weight: 28 ± 2 kg, mean ± SEM) were studied. Sixteen were studied under general anesthesia (portal...
endotoxin administration \( n = 8 \); peripheral administration, \( n = 8 \). Six awake dogs served as the control group. Prior to the study all dogs were observed for 2 weeks. Only dogs with normal stools, no febrile disease, and normal physical examination and laboratory results were included. The dogs were fed a standard diet once a day consisting of 64% carbohydrate, 7% fat, 26% protein, 3% fiber, based on dry weight (D. B. Brok, Hope Farms, Woerden, The Netherlands). The study was approved by the Ethical Committee for Animal Experiments and performed according to the guidelines of the Dutch Law for Animal Experiments.

**Operative Procedure**

**Awake dogs.** To get used to the experimental procedure, these dogs were trained daily in the experiment room to lie quiet for a few hours. Four days before each experiment a femoral artery catheter was inserted during general anesthesia (1% isoflurane [Forene, Abbott Laboratories, Queensbourough, Kent, United Kingdom] and \( N_2O/O_2 (1:1) \) ventilation). After insertion, the catheter was filled with heparin (200 U/ml), closed, and placed in a subcutaneous pocket.

**Anesthetized dogs.** After an overnight fast (18 h) the dogs were anesthetized, intubated, and ventilated artificially. General anesthesia was induced by intramuscular injection of xylazine (0.15 ml/kg; Romans, BAYER, Germany), ketamine hydrochloride (20 mg/kg; Aesculet, Aesculaap BV, The Netherlands), and atropine (0.05 mg/kg; Centrafarma Services BV, Etten-Leur, The Netherlands) and maintained by intravenous sufentanil (1.5 \( \mu g/kg \cdot h \); Janssen-Cilag BV, Turnhout, Belgium). After an overnight fast (18 h) the dogs were anesthetized, intubated, and ventilated artificially. General anesthesia was induced by intramuscular injection of xylazine (0.15 ml/kg; Romans, BAYER, Germany), ketamine hydrochloride (20 mg/kg; Aesculet, Aesculaap BV, The Netherlands), and atropine (0.05 mg/kg; Centrafarma Services BV, Etten-Leur, The Netherlands) and maintained by intravenous sufentanil (1.5 \( \mu g/kg \cdot h \); Janssen-Cilag BV, Turnhout, Belgium).

**Endotoxin Administration**

Endotoxin derived from *E. coli* (0111:B4, Lot 31H4000, phenol extracted; Sigma Chemical Co., St. Louis, MO) was suspended in sterile pyrogen-free saline. A stock solution of 100 \( \mu g/ml \) was made, divided into several plastic tubes (Costar, Cambridge, MA), and kept frozen at \(-20°C\). Before use the endotoxin solution was thawed at \( 37°C\), vortexed for 3 min, diluted, and vortexed again for 10 min.

In the awake dogs, mean arterial blood pressure and heart rate were monitored continuously and recorded every 15 min. In the anesthetized dogs, mean arterial blood pressure, heart rate, electrocardiogram, central venous pressure, pulmonary artery pressure, and pulmonary artery wedge pressure were monitored continuously and recorded at intervals of 15 min. Cardiac output was determined by thermal dilution technique using a cardiac output computer (Edward Labs, Santa Ana, CA). Blood flow in the hepatic artery and portal vein was monitored continuously with Doppler flow probes, connected to a flowmeter (three-channel ultrasonic flow-dimension system; G. J. Hartley, Baylor College of Medicine) and recorded every 10 min. Total hepatic blood flow was the sum of the blood flow through the hepatic artery and portal vein.

Blood samples for the determination of glucose enrichment and plasma concentrations of insulin, glucagon, and cortisol were collected hourly from the femoral artery. Blood samples for the determination of plasma concentration of TNF and IL-6 were collected hourly and also 30 and 90 min after endotoxin administration from the femoral artery and (in anesthetized dogs) from the portal vein and a hepatic vein.

The observation period after endotoxin administration lasted 240 min. At the end of each experiment the correct position of the catheters was confirmed radiographically. Subsequently, the dogs were sacrificed by intravenous injection of sodium pentobarbital.

**Sample Processing**

Blood was collected in prechilled tubes and stored on ice. Immediately after sampling the blood samples were centrifuged (3000g, 4°C, 10 min) and plasma was stored at \(-20°C\) until determination. Blood for analysis of insulin, cortisol, TNF, IL-6, and \([6,6-2H_2]\)glucose was collected in heparinized tubes. Whole blood was added to reduced glutathione—BSA buffer and trasylof for the determination of catecholamines and glucagon, respectively.

**Biochemical Analysis**

All measurements were performed in duplicate. All samples of each animal were analyzed in the same run. Plasma insulin concentration was measured by radioimmunoassay (RIA) (Insulin RIA 100, Pharmacia Diagnostics AB, Uppsala, Sweden; detection limit, 2 mU/liter), as was plasma glucagon (Daiichi Radioisotope Laboratories, Tokyo, Japan; detection limit, 15 ng/liter; glucagon antiserum elicited in guinea pigs against pancreatic specific glucagon; cross-reactivity with glucagon-like substances of intestinal origin less than 1%). Plasma catecholamine concentrations were measured by HPLC and electrochemical detection, after purification on Bio-Rex 70 and concentration by solvent extraction [15], and cortisol by fluorescence polarization immunomassay on Technical Device X (Abbott Laboratories, Chicago, IL; detection limit, 50 nmol/liter).

TNF bioactivity in plasma was measured with a WEHI 164 subclone 13 line (kindly provided by W. A. Buurman, Academic Hospital, Maastricht, The Netherlands). The WEHI assay is based on the cytotoxic action of TNF on this fibroblast cell line [16]. TNF standard contained recombinant human TNF (Ernst-Boehringer, Vienna, Austria), which was serially diluted [16]. The detection limit was 10 pg/ml. IL-6 bioactivity was measured with an IL-6-dependent B-9 hybridoma cell line (kindly provided by I. A. Aarden, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) [17]. The detection limit of the IL-6 assay was 1 pg/ml. IL-6 standard contained human recombinant IL-6 (HECS; Costar, Bad Niederbrunn, Thet Netherlands), which was serially diluted.

Glucose concentrations and enrichments were determined by gas chromatography—mass spectrometry (Gas Chromatograph Model 5890 II, Mass Spectrometer Model 5989 A, Hewlett-Packard, Fullerton, CA). Results were compared to the mean baseline levels. The probe was placed 1 mm distal to the portal vein Doppler flow probe. The other catheter was inserted during general anesthesia (1% isoflurane [Forene, Abbott Laboratories, Queensbourough, Kent, United Kingdom] and \( N_2O/O_2 (1:1) \) ventilation). After insertion, the catheter was filled with heparin (200 U/ml), closed, and placed in a subcutaneous pocket. The study was approved by the Ethical Committee for Animal Experiments and performed according to the guidelines of the Dutch Law for Animal Experiments.
ton, CA; column Heliflex AT-1, 30 cm x 0.25 mm x 0.2 μm, Alltech, Deerfield, IL). β-Phenyl-glucose was used as internal standard [18] (intraassay coefficient of variation of 1 to 4%, interassay coefficient of variation 1.5 to 5%; detection limit, 1.5 mM (0.5% enriched)).

Statistical Analysis and Calculations

All values are expressed as means ± SEM. The data were analyzed by two-way (i.e., group and time) analysis of variance for repeated measurements and the Newman–Reuls test for post hoc testing. For comparison between the two sampling sites of cytokines within each group the Wilcoxon test for paired samples was used. A P value < 0.05 was considered to be statistically significant.

Systemic vascular resistance (SVR) was calculated using the following formula [12]:

\[
SVR = \frac{(MABP - CVP) \times 80}{CO},
\]

where MABP is mean arterial blood pressure (mm Hg), CVP is central venous pressure (mm Hg), and CO is cardiac output (liters/min). SVR is given as \(\times 10^4\) dyn \cdot s cm\(^{-5}\). Net hepatic TNF and IL-6 production was calculated using the following formula:

\[
\text{net hepatic cytokine production} = (Q_{HV} \times C_{HV}) - [(Q_{PV} \times C_{PV}) + (Q_{PA} \times C_{PA})],
\]

where \(Q_{HV}\), \(Q_{PV}\), and \(Q_{PA}\) are blood flow through the hepatic vein, portal vein, and hepatic artery, and \(C_{HV}\), \(C_{PV}\), and \(C_{PA}\) are cytokine concentrations in the hepatic vein, portal vein, and femoral artery, respectively. Net hepatic cytokine production is given in nanograms per minute. The non-steady-state equations were used to calculate the rate of appearance \((R_a)\) of glucose as adapted for the use of stable isotopes [19].

RESULTS

Temperature

Basal temperature was slightly higher in awake dogs (38.2 ± 0.1°C) versus anesthetized dogs (peripheral, 36.2 ± 0.4°C; portal 35.9 ± 0.4°C; \(P < 0.05\)). Endotoxin increased the temperature in the conscious dogs to a maximum 40.6 ± 0.5°C (\(P < 0.01\)), but did not affect the temperature in anesthetized dogs.

Hemodynamics

Endotoxin induced a hyperdynamic response in the circulation of anesthetized dogs (Fig. 1). Cardiac output increased transiently by 30–35% (\(P < 0.05\) vs basal) in both groups to a peak value at 60 min after endotoxin administration. The route of endotoxin administration did not affect the hemodynamic responses to endotoxin. Systemic vascular resistance decreased after endotoxin to a nadir at 60 min (peripheral group from 0.39 ± 0.04 to 0.27 ± 0.02 \(\times 10^4\) dyn \cdot s cm\(^{-5}\); portal group from 0.44 ± 0.04 to 0.29 ± 0.03 \(\times 10^4\) dyn \cdot s cm\(^{-5}\); \(P < 0.05\) vs basal; NS between both groups). Total hepatic blood flow (basal: 9.0 ± 0.6 ml/kg \cdot min vs 8.1 ± 0.4 ml/ kg \cdot min, respectively) was not affected by portal or systemic endotoxin administration. In awake dogs endotoxin resulted in a transient increase in blood pressure (\(P < 0.05\) vs anesthetized dogs).

Cytokines

In anesthetized dogs peak arterial levels of TNF were reached 60 min after endotoxin administration, with a gradual decline to baseline afterward (portal group, from below detection limit to 5 ± 2 ng/ml, vs peripheral group, from below detection limit to 6 ± 2 ng/ml, both \(P < 0.01\) vs basal) (Fig. 2). Arterial levels of TNF were not affected by the route of endotoxin administration.

There were no net hepatic TNF production prior to endotoxin administration. However, net hepatic TNF production increased significantly after endotoxin administration in both groups (NS between groups) (Table 1). Data on plasma TNF levels were not obtained in the conscious dogs, because these plasma samples were
Peripheral group

Portal group

Peripheral group

Portal group

FIG. 2. Plasma TNF levels (ng/ml) in the femoral artery (circles) and hepatic vein (triangles) before and after administration of 1.0 μg/kg endotoxin into a cephalic vein or into the portal vein. All values are given as means ± SEM. There was no difference between the groups. Plasma TNF levels were increased in both groups (P < 0.01 vs basal). Plasma TNF levels were higher in the hepatic veins than in the femoral artery after portal endotoxin administration but not after peripheral endotoxin administration.

Endotoxin administration increased arterial plasma IL-6 concentrations in all dogs, with peak levels 180 min after endotoxin administration (portal group, from 2 ± 1 to 95 ± 14 ng/ml, vs peripheral group, from 2 ± 1 to 86 ± 15 ng/ml, both P < 0.01 vs basal) (Fig. 3). The route of administration did not influence the arterial IL-6 concentrations in the anesthetized dogs, but the values were about three times higher in the anesthetized dogs compared with the awake dogs (awake thawed by accident and therefore we could not obtain reliable plasma TNF levels.

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FIG. 3. Plasma IL-6 levels (ng/ml) in the femoral artery (circles) and hepatic vein (triangles) before and after administration of 1.0 μg/kg endotoxin into a cephalic vein or into the portal vein. All values are given as means ± SEM. There was no difference between the groups. Plasma IL-6 levels were increased in both groups (P < 0.01 vs basal). Plasma IL-6 levels were higher in the hepatic veins than in the femoral artery after portal endotoxin administration (AUC: P < 0.05), but not after peripheral endotoxin administration.

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<td>C</td>
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<td>927 ± 1195</td>
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<td>587 ± 1220</td>
<td>2622 ± 2973</td>
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*Values are given as means ± SEM.

*P < 0.05 vs basal values. There were no significant differences between groups.
dogs, from below detection limit to 27 ± 3 ng/ml, \( P < 0.01 \) vs basal; \( P < 0.05 \) vs both anesthetized groups).

There was no net hepatic IL-6 production prior to endotoxin administration. Net hepatic IL-6 production was stimulated after portal, but not after peripheral, endotoxin administration (\( P < 0.05 \) vs basal)(ns between groups) (Table 1).

**Glucoregulatory Hormones**

Plasma concentrations of glucoregulatory hormones are shown in Fig. 4. The route of endotoxin did not affect the neuroendocrine response to endotoxin in the anesthetized dogs.

Prior to injection of endotoxin, concentrations of cortisol and glucagon were higher in anesthetized dogs, whereas insulin and norepinephrine levels were lower compared with awake dogs (\( P < 0.05 \)). Endotoxin stimulated insulin secretion only in awake dogs (\( P < 0.05 \)), whereas glucagon secretion was increased in all three groups (NS between groups). There was a variable response of (nor)epinephrine to endotoxin within each group (NS between groups). Cortisol concentrations did not change after endotoxin in anesthetized dogs, whereas cortisol increased to higher values within 3 h of endotoxin in awake dogs compared with anesthetized dogs (\( P < 0.01 \)).

**Glucose Kinetics**

Plasma concentrations and rates of appearance of glucose are shown in Fig. 5. Basal plasma glucose concentrations were not different between the three groups. Basal rate of appearance of glucose was not different in the three groups (conscious dogs: 13.8 ± 1.2 \( \mu \)mole/kg·min; anesthetized dogs: peripheral group, 16.5 ± 1.3 \( \mu \)mole/kg·min, and portal group, 15.8 ± 1.1 \( \mu \)mole/kg·min). In anesthetized dogs, peripheral injection of endotoxin did not influence \( R_a \) glucose (16.8 ± 1.2 \( \mu \)mole/kg·min at 240 min), whereas a significant decrease was found after intraportal injection (13.6 ± 0.9 \( \mu \)mole/kg·min at 240 min; \( P < 0.05 \) vs peripheral endotoxin administration). In contrast, endotoxin induced a considerable rise of ~75% in glucose produc-
tion in the awake dogs ($P < 0.05$ vs basal; $P < 0.05$ vs anesthetized dogs).

**DISCUSSION**

This study indicates that the inflammatory and metabolic effects of endotoxin are in part dependent on the route of endotoxin delivery. Although intraportal injection of endotoxin stimulated hepatic TNF and IL-6 production, and peripheral injection of endotoxin stimulated only hepatic TNF but not hepatic IL-6 production, there were no differences in cytokine responses between both groups. Nonetheless, hepatic glucose production decreased after portal endotoxin administration but not after peripheral delivery of endotoxin. The combination of surgery and anesthesia increased the IL-6 response and modulated the neuroendocrine response to endotoxin, associated with the absence of the normal stimulation of hepatic glucose production to endotoxin. Therefore, the effects of endotoxin, at least with respect to glucose production, are dependent on the site of entry into the circulation. Moreover, the effects of endotoxin are altered by the combination of surgery and anesthetic agents.

TNF and IL-6 levels were considerably increased after endotoxin administration. In accordance with the literature, the increase in plasma IL-6 levels was preceded by a rise in plasma TNF levels [7, 13]. There were no differences in plasma cytokine levels in relation to the origin of endotoxin. However, in accordance with previous observations, there was a large variability in cytokine responses to endotoxin within each group [20]. We cannot exclude the possibility that the detection of differences in cytokine production between both anesthetized groups is precluded by this large within-group variability.

The effects of the route of endotoxin administration were studied in anesthetized dogs. In these dogs a laparotomy was performed on the day of the study for cannulation of the hepatic vessels. Therefore, the inflammatory, neuroendocrine, and metabolic effects in these dogs were affected by the combination of surgery, anesthesia, and endotoxin. However, this combination of factors does not invalidate our conclusions with respect to portal versus peripheral delivery of endotoxin, because the circumstances were identical for both groups of anesthetized dogs except for the route of endotoxin administration.

Hepatic glucose production was stimulated considerably by peripheral endotoxin administration in awake dogs, in contrast to anesthetized, operated dogs. The metabolic changes induced by endotoxin are thought to be mediated at least in part by the induction of TNF and IL-6. Administration of IL-6 and TNF increases glucose production in dogs and humans in association with neuroendocrine activation [21–25]. However, despite the considerable response of TNF and IL-6, the stimulatory effects of endotoxin on hepatic glucose production were absent in anesthetized dogs. There were differences in endotoxin-induced neuroendocrine activation between awake and anesthetized dogs. Although insulin and norepinephrine levels were higher in awake dogs, cortisol and glucagon concentrations were higher in anesthetized dogs. Therefore, we cannot exclude the possibility that the discrepancy in the effects of endotoxin on glucose production between awake and anesthetized dogs are related to these differences in neuroendocrine activation.

Portal administration of endotoxin decreased hepatic glucose production in anesthetized dogs. This effect was not caused by differences in secretion of glucoregulatory hormones, because there were no endocrine differences between both groups of anesthetized dogs. It has been suggested that the hepatic response to endotoxin involves the release of inflammatory mediators, like cytokines from Kupffer cells which interact with hepatocytes to induce nitric oxide production [26]. Recent studies have provided evidence that nitric oxide may be an inhibitory mediator of the effects of endotoxin on hepatic glucose production [27, 28]. Endotoxin exposure and clearance are higher after portal then after peripheral endotoxin delivery [4]. It is possible that in our study the degree of hepatic endotoxin exposure, which is higher after portal then after peripheral endotoxin delivery, altered glucose production by intrahepatic mechanisms like nitric oxide rather than by endocrine mechanisms. Hasibeder et al. studied the effects of systemic versus mesenteric artery infusion of endotoxin in circulatory stable pigs [29]. Interestingly, they found that mucosal microvascular dysfunction is dependent largely on the local microvascular endotoxin concentration, supporting the hypothesis that local mechanisms are important in the reaction to endotoxin.

In summary, there are no major differences in the responses to endotoxin between peripherally treated and portally treated dogs, except for minor differences in glucose production. The glucoregulatory response to endotoxin is altered by the site of entry of endotoxin into the circulation, probably through nonendocrine mechanisms. The inflammatory, neuroendocrine, and metabolic effects of endotoxin are altered by the combination of surgery and anesthesia.

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