Chapter 7
Effects of endotoxin on tryptophan metabolism in pigs

S. Russo¹ MD, I.P. Kema² PhD, F.A.M. Muskiet² PhD, M.R. Fokkema² Ms, S.J. Koopmans³ PhD, E.G.E. de Vries⁴ MD, PhD, J. Korf¹ PhD

Departments of Psychiatry¹ and Laboratory Medicine², University Hospital Groningen, Department of Nutrition and Food, Animal Sciences Group Lelystad³ and Medical Oncology⁴, the Netherlands

Abstract

Background Inflammatory processes are associated with depletion of the essential amino acid tryptophan, the precursor of serotonin (5-HT). We evaluated the peripheral responses of tryptophan and both peripheral and brain metabolism of 5-HT to intravenous lipopolysaccharide (LPS) administration in pigs.

Methods In 6 crossbread barrows Escherichia Coli LPS was infused. Six pigs served as controls. Arterial and cerebrovenous levels of plasma tryptophan, 5-hydroxyindoleacetic acid (5-HIAA) and platelet 5-HT were assessed 4, 24 and 120 h after infusion.

Results In LPS treated pigs plasma tryptophan levels were depleted 4 and 24 h after infusion with no arterial-venous differences. 5-HT levels were enhanced in LPS exposed pigs with again no arteriovenous differences. Venous 5-HIAA levels were enhanced compared to arterial levels 120 h after LPS infusion. Plasma tryptophan levels correlated positively with with 5-HIAA levels 4 h after infusion in the LPS group.

Discussion Following LPS there is an increased metabolism of peripheral and brain 5-HT.
Introduction

Administration of endotoxin in the form of lipopolysaccharide (LPS) has often been used to induce inflammatory reactions in animals. This paradigm has been used successfully to elucidate many aspects of inflammatory responses (9). In men, infusion of LPS causes the release of cytokines resulting in short lasting sickness behavior (10). Long-term inflammation induces enzymes such as indoleamine dioxygenase (IDO) in peripheral macrophages and cerebral microglia and liver tryptophan pyrroloase, that ultimately cause chronically decreased plasma tryptophan levels (6). A consequence of low plasma tryptophan is low brain serotonin (5-HT) synthesis, as the latter depends highly on the uptake of the precursor amino-acid in the brain (1). In the present study we investigated the responses of 5-HT and tryptophan in LPS exposed animals. It is questionable whether the acute effects of LPS mimic the inflammatory response of the tryptophan-5-HT metabolism seen in chronic inflammation. In rats, enhanced production of 5-HT lasting about 24 h has been observed following LPS administration (8). In contrast, during chronic inflammation attenuated 5-HT levels have been described caused by decreased availability of its precursor tryptophan (4). It is not yet clear at which time point the enhanced production of 5-HT ceases because of exhaustion of the precursor. More insight into the metabolism of 5-HT in relation to the time course of an acute immune response might elucidate the physiological role of 5-HT.

In order to assess peripheral and brain tryptophan metabolites during an inflammatory challenge we performed experiments in pigs exposed to intravenous LPS. We took simultaneous samples of both arterial and cerebrovenous blood. Appropriate surgery was impossible in smaller animals. The difference between arterial and cerebrovenous levels was used as a measure of brain excretion of each compound. In these samples tryptophan, 5-hydroxyindole acetic acid (5-HIAA), the main metabolite of 5-HT, and platelet 5-HT were determined. The present experiment shows that an increased metabolism of both peripheral and brain serotonin was observed following infusion of LPS.
Materials and Methods

Animals and housing
Experimental protocols describing the management, surgical procedures, and animal care were reviewed and approved by the ID-Lelystad Animal Care and Use Committee (Lelystad, the Netherlands). The study was performed in 12 crossbred Yorkshire barrows with an average body weight of 35±2 kg (mean ± SEM).

Surgery
Under general anaesthesia, polyethylene catheters (Tygon, i.d. 1.02 mm, o.d. 1.78 mm, length 1 m) were placed into the right carotid artery and the right external jugular vein according to a modified procedure as previously described (3,4). The catheters were filled and sealed with physiological NaCl containing 50 IU heparin and 150,000 IU penicillin (Procopen; AUV, Cuijk, the Netherlands) per mL and kept in and protected by a back pack which was glued to the skin of the pig's back. After 1 week of postsurgical recovery, the first series of blood sampling was performed. In the period between surgery and the first series of measurements, the pigs were habituated to the blood sampling procedure.

Endotoxin administration
Escherichia coli LPS was dissolved in 0.9% NaCl (0.1 mg/mL). This was administered via the portal vein at a dose of 5 mg/kg bodyweight for 2 h and subsequently a dose of 2 mg/kg for the following 7 h (7). In control pigs 5 ml 0.9% NaCl was administered.

Blood sampling and plasma analyses
Blood samples of 5 mL were collected in heparinized tubes. Arterial and venous blood was sampled at 4, 24 and 120 h after the start of LPS infusion out of the carotid artery and jugular vein simultaneously.
Heparinized blood samples were collected in vacutainer tubes containing 0.12 mL (0.34 mmol/L) ethylene diamine tetraacetic acid solution. Plasma samples, after centrifugation, were stored at -20°C until analysis. The quantification of total plasma tryptophan, plasma 5-HIAA and platelet rich plasma 5-HT was performed with methods...
based on high performance liquid chromatography with fluorometric detection (5).

**Statistical analysis**
Venous plasma tryptophan in mmol/L, plasma 5-HT in ng/L and 5-HIAA levels in mmol/L 4, 24 and 120 h after administration were compared between the LPS and NaCl group using analysis of variance. Arterio-venous differences for plasma tryptophan, 5-HIAA and 5-HT levels were performed separately in both groups using analysis of variance. Correlations were calculated between levels of 5-HIAA and plasma tryptophan at corresponding moments using the Spearman rank test. For analyses SPSS version 10.0 was used. Only p-values smaller or equal 0.05 were considered significant.
Results

In tabel 1 all results are summarized separately for LPS and NaCl treated pigs in arterial and venous blood. For each measurement, the number of pigs studied is indicated. Venous plasma tryptophan levels were attenuated in LPS treated pigs 4 (F=15.6, p<0.01), 24 (F=7.4, p= 0.022) and trend-wise 120 h (F=4.9, p= 0.052) after LPS administration compared to NaCl treated animals. This is shown in figure 1. Plasma tryptophan levels showed no arterial-venous differences in all cases. The quantification of 5-HT and 5-HIAA was possible in three pigs of each group. Plasma 5-HT levels were enhanced 24 h after infusion in LPS exposed pigs (F= 8.40, p= 0.022). Arterial-venous 5-HT differences were not present in both groups. Plasma 5-HIAA levels did not differ between groups. In the LPS, but not in the saline group, arterial-venous differences in 5-HIAA were present due to enhanced venous levels 120 h after LPS infusion (F= 8.49, p= 0.044). Plasma tryptophan levels correlated positively with plasma 5-HIAA levels 4 h after LPS administration (p< 0.01).
Table 1: Summary of results in LPS and NaCl treated pigs

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>n</th>
<th>4 h</th>
<th>24 h</th>
<th>120 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous tryptophan μmol/L LPS (SD)</td>
<td>6</td>
<td>22.6 (5.9)</td>
<td>22.9 (6.6)</td>
<td>31.2 (4.9)</td>
</tr>
<tr>
<td>Arterial tryptophan μmol/L LPS (SD)</td>
<td>6</td>
<td>30.0 (10.2)</td>
<td>23.3 (7.23)</td>
<td>32.6 (6.9)</td>
</tr>
<tr>
<td>Venous tryptophan μmol/L NaCl (SD)</td>
<td>6</td>
<td>38.2 (7.7 )</td>
<td>32.7 (5.8)</td>
<td>37.3 (4.6)</td>
</tr>
<tr>
<td>Arterial tryptophan μmol/L NaCl (SD)</td>
<td>6</td>
<td>37.9 (3.3)</td>
<td>32.6 (5.7)</td>
<td>34.6 (7.5)</td>
</tr>
<tr>
<td>Venous 5-HT ng/L LPS (SD)</td>
<td>3</td>
<td>12.1 (10.5)</td>
<td>19.7 (13.7)</td>
<td>9.7 (5.7)</td>
</tr>
<tr>
<td>Arterial 5-HT ng/L LPS (SD)</td>
<td>3</td>
<td>10.7 (1.8)</td>
<td>17.0 (8.4)</td>
<td>12.0 (4.6)</td>
</tr>
<tr>
<td>Venous 5-HT ng/L NaCl (SD)</td>
<td>3</td>
<td>13 (4.3)</td>
<td>15.2 (6.8)</td>
<td>7.1 (5.0)</td>
</tr>
<tr>
<td>Arterial 5-HT ng/L NaCl (SD)</td>
<td>3</td>
<td>10.1 (2.6)</td>
<td>17.4 (8.3)</td>
<td>7.9 (0.8)</td>
</tr>
<tr>
<td>Venous 5-HIAA mmol/L LPS (SD)</td>
<td>3</td>
<td>321 (134.8)</td>
<td>819 (777.6)</td>
<td>465 (319.0)</td>
</tr>
<tr>
<td>Arterial 5-HIAA mmol/L LPS (SD)</td>
<td>3</td>
<td>246 (45.7)</td>
<td>661 (824.0)</td>
<td>408 (342.4)</td>
</tr>
<tr>
<td>Venous 5-HIAA mmol/L NaCl (SD)</td>
<td>3</td>
<td>192 (71.5)</td>
<td>193 (52.8)</td>
<td>185 (29.7)</td>
</tr>
<tr>
<td>Arterial 5-HIAA mmol/L NaCl (SD)</td>
<td>3</td>
<td>157 (63.5)</td>
<td>208 (82.3)</td>
<td>250 (24.8)</td>
</tr>
</tbody>
</table>
Figure 1: Venous plasma tryptophan levels after LPS (±SD). The interrupted line regards LPS treated pigs. The presence of * indicates significant results.
Discussion

The present study shows that LPS administration to pigs resulted in an attenuation of plasma tryptophan levels 4 and 24 h after administration. Plasma tryptophan depletion has also been detected during chronic inflammation in humans (2). We observed no differences between arterial and cerebrovenous levels. This might be due to the relatively mild fluctuation of plasma tryptophan of about 30% following LPS. One passage through the brain under these circumstances might not be enough to produce a difference. Furthermore, plasma tryptophan depletion is not only due to cerebral uptake. At 24 h peripheral 5-HT, produced out of tryptophan, showed enhanced levels in the LPS group. Therefore, we presume that a fraction of the extra tryptophan that is metabolized in the LPS group has been transformed into 5-HT in the periphery. We additionally observed enhanced 5-HIAA, the metabolite of 5-HT, excretion by the brain 120 h after LPS administration as measured by enhanced cerebrovenous blood content of 5-HIAA. This suggests enhanced brain 5-HT production after LPS administration. This conclusion corresponds with observations of increased 5-HT release in the rat brain after LPS administration (3), suggesting that, at least part of the depletion of tryptophan is due to the transformation to 5-HT both in the periphery and the brain. Enhanced 5-HT production might lead to exhaustion of tryptophan stores so 5-HT production will ultimately attenuate. The studied time period however, does not allow to draw definitive conclusions on this subject, although, venous 5-HIAA levels correlated positively with plasma tryptophan levels only 4 h after LPS administration. The latter suggests that in the early stage of the inflammatory response a direct relation exists between tryptophan stores and 5-HT production. In later stages of an inflammatory response this relation could become distorted due to exhaustion of tryptophan with declining tryptophan levels while 5-HT and consequently 5-HIAA production continues. Taken together, the data of the present study suggest that modification of tryptophan is already apparent in the early stages of the inflammatory response.
References


97