Acute phase response in reindeer after challenge with Escherichia coli endotoxin

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Abstract

The serum concentrations of two acute phase proteins (APPs), haptoglobin (Hp) and serum amyloid-A (SAA), were monitored in reindeer after challenge with endotoxin. Four adult female reindeer received either 0.1 mg/kg Escherichia coli 0111:B4 lipopolysaccharide B or saline solution intravenously. At the second challenge, the treatments were reversed. In addition to the APPs, changes in blood chemistry and rectal temperature were monitored. The endotoxin challenge caused a significant increase in SAA (peak 48 h) and a sharp decrease (8–12 h) of serum iron concentrations in all animals. The mean Hp concentration increased at 8 h and remained elevated until 48 h, but no statistically significant differences were found. This investigation demonstrates that challenge with a single-bolus dose of E. coli endotoxin can activate the acute phase response (APR) and SAA appears to be a more sensitive indicator of the APR than Hp during bacterial infection in reindeer.

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Résumé

Les concentrations de deux protéines de l’inflammation, l’haptoglobine (Hp) et l’amyloïde A sérique (SAA), la température rectale et les paramètres biochimiques sanguins ont été suivis chez quatre rennes adultes femelles ayant reçu lors d’une première inoculation intraveineuse soit 0.1 μg/kg de lipopolysaccharide B d’Escherichia coli O111:B4, soit du sérum physiologique, puis lors d’une seconde inoculation l’inverse.

L’injection d’endotoxine a entraîné une augmentation significative de la concentration de SAA (pic à 48 h) et une diminution brutale (8–12 h) du fer sérique chez tous les animaux. La concentration

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1. Introduction

The inflammatory reaction is a series of complex physiological events occurring in the host after tissue injury or infection. The purposes of these events are to eliminate the infecting agent, prevent further tissue damage and restore the homeostasis of the host organism. The early sets of reactions that occur immediately after tissue damage are known as the acute phase response (APR). It is characterized by the presence of an inflammatory reaction at the site of injury or infection and systemically by multiple changes throughout the organism such as fever, depression, leucocytosis, increased permeability of blood vessels, stimulation of adrenocorticotropic hormone (ACTH) production, redistribution of trace elements, etc. One of the main changes occurring during the APR is hepatic production of various plasma proteins [1]. These so-called acute phase proteins (APPs) play a very important role in the defense response of the host. For example, some APPs are involved in nonspecific protection against infections caused by gram-negative bacteria [2], but in many cases their exact physiological function is not clear.

Monitoring the plasma concentrations of APPs can provide information on progression of the APR. Their potential use in veterinary medicine has been extensively investigated and they are already widely used as markers of disease in veterinary and medical science. Some of the many applications for APPs include evaluation of collective health of herds by detection of subclinical diseases and identification of animals with inflammatory lesions at slaughter. Thus, APPs may also serve as valuable tools in reindeer husbandry as well as in giving diagnostic information on the detection, prognosis and monitoring of diseases in reindeer.

Although the APR is highly conserved in nature, APP profiles show significant variability between different species [3]. Haptoglobin ( Hp) has been the APP most commonly monitored as a marker for inflammation [4–8] in cattle and other domestic ruminants. The plasma Hp concentration is very low in healthy bovines [7] but during the APR it can increase over 100-fold. Serum amyloid-A (SAA) is another major APP in many animal species, including cattle, and has been shown to be a good diagnostic marker for determining the presence of infections and inflammatory conditions [7,9–11]. Evaluation of the concentrations of different APPs allows better estimates of the complex systemic effects of inflammatory mediators during various inflammatory conditions, e.g. in distinguishing between acute and chronic inflammation and for evaluation of disease severity.

The main objective here was to investigate the host response of reindeer (Rangifer tarandus tarandus) after bacterial endotoxin challenge as an acute phase stimulant and to evaluate the changes occurring in serum concentrations of SAA and Hp as potential
markers of infections. In addition to the APPs, other changes occurring in blood chemistry and in some clinical parameters were also monitored.

2. Materials and methods

2.1. Animals and experimental procedures

This experiment was carried out at the Zoological Gardens of the Department of Biology, University of Oulu, Finland. The first experiment was initiated on February 18, 2002 and the second on April 8, 2002. Eight adult female reindeer were randomly divided into two equal groups. The mean weight of the animals was 76.7 kg (range 67.5–90.5 kg) and age was 5 years (3–6 years). The groups were kept in two separate corrals (about 650 m² each) in which they underwent a long adaptation period before the experiment was initiated. The animals were fed with lichen (Cladina spp.), commercial reindeer pellets and fresh water ad libitum. The first group received 0.1 mg/kg E. coli 0111:B4 lipopolysaccharide B ((LPS); 1 μg/ml Bacto™, Difco Laboratories, Inc., Detroit, MI, USA) into the jugular vein. The second group received the same volume of physiological sodium chloride solution (0.1 ml/kg). After 7 weeks, the same procedure was repeated vice versa. The dose was chosen based on published studies in small domestic ruminants such as goat [12–14]. In these experiments, this endotoxin dose resulted in clear activation of the APR, as shown by significant decrease in serum iron concentrations and increase in rectal temperature and serum cortisol concentrations. This dose caused only modest and short-acting general signs of disease, e.g. depression and alteration of rumen contractility. The rectal temperature was recorded at the same time that blood was taken during the first day of the experiments. Clinical observations without manual handling were done every hour during the first 12 h. After the experiments, the animals were killed and autopsies performed.

2.2. Blood samples

The group of reindeer was herded into the small pen near the corrals and then caught and manually restrained. Blood samples were taken from the jugular vein before the endotoxin or physiological saline solution injections and after 1, 4, 8, 12, 24, 48, 96 and 168 h into plain tubes. The serum was separated, frozen in portions and stored at −20 °C for further analysis.

2.3. Serum analysis

Serum Hp was determined using the hemoglobin-binding assay described by Makimura and Suzuki [15] with slight modifications, in which tetramethylbenzidine (0.06 mg/ml) was used as substrate [7]. SAA was measured with a commercially available ELISA kit (Phase SAA kit, Tridelta Ltd, Ireland), according to the manufacturer’s instructions for cattle.

The activities of serum aspartate aminotransferase (ASAT) and creatine kinase (CK) were determined following the recommendations of the Scandinavian Society for Clinical
Chemistry and Clinical Physiology [16,17]. Spectrophotometric methods were used for the determination of serum total protein [18], urea [19], sorbitol dehydrogenase (SDH) [20], albumin [21] and γ-glutamyl transferase (GGT) according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Expert Panel on Enzymes [22]. Serum iron was determined with a colorimetric method [23]. The analyses were performed with an automatic chemistry analyzer (KONE Pro, Konelab, Thermo Clinical Labsystems Oy, Vantaa, Finland). Cortisol was measured in 25-mL duplicates using radioimmunoassay with Coat-A-Count RIA kits obtained from Diagnostic Products Corporation (Los Angeles, CA, USA). All results are expressed as mean ± standard deviation (SD).

2.4. Statistical analyses

Carry-over and period effects were tested using the t-test procedures of Jones and Kenward [24]. These tests were run for selected time points, in cases of insignificant carry-over effects in the examined parameters, the data from both challenges were combined. Differences between the *E. coli* LPS treatment and control groups were analyzed using repeated measures analyses of variance with treatment and time after challenge as within factors. Greenhouse–Geiser adjusted *p*-values were used for evaluation of results, which were significant at *p* < 0.05.

3. Results

After laboratory analysis, two reindeer were excluded from statistical analysis, one from each group. One reindeer already showed increased Hp (over 3-fold) and SAA (over 10-fold) concentrations in the 0-h sample, which remained high during the first experiment. Clear arthritic changes were found in the left tarsal joint of this animal at autopsy; in all other reindeer no pathological changes were found. The second excluded reindeer reacted very strongly to the restraint procedures used at the time of blood sampling; its serum CK and ASAT activities increased markedly during the first 24 h of experiments, indicating muscle injury.

A carry-over effect was found in cortisol and data analysis was carried out separately in both experiments for that variable. Period differences could be seen in iron (*p* < 0.05) and in the activities of ASAT (*p* < 0.01) and GT (*p* < 0.05). In the first experiment, all animals receiving LPS showed clear signs of general depression (they kept their heads down and moved slowly) and two had tremor of the legs. These clinical signs were observed during the first 4 h after LPS administration. The same reindeer had loose droppings on the following day. The signs of depression were less obvious during the second experiment.

Endotoxin administration caused a significant increase in the concentration of SAA (Fig. 1; *p* < 0.001). The rise in SAA in the LPS group was seen in the 12-h sample and peaked at 48 h (184.7 ± 89.4 mg/l). At this time, the LPS-treated animals displayed a 2 to 29-fold increase of serum SAA from the baseline level. The mean SAA concentrations in the 96-h sample were decreased below the pretreatment levels in both groups.
The mean serum Hp concentrations showed a small tendency to decrease during the first 4-h period in the LPS group, increased slightly in the 8-h sample and attained maximum value at 24 h (0.87 ± 0.25 g/l). The Hp concentrations remained relatively stable in the control group throughout the experiment (Fig. 1); however, these differences between treatments were not statistically significant. Wide individual variation occurred in Hp response to endotoxin administration; only one reindeer showed a near 2-fold increase in the 48-h sample and one showed no increase at any time.

Serum iron concentrations decreased sharply in all endotoxin-treated animals and were significantly lower (p < 0.01) at 8 and 12 h (Fig. 1). The serum cortisol concentrations showed wide variability among animals, but no consistent increases in cortisol in the LPS

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*Fig. 1. Mean (± SD) serum concentrations of SAA (a), Hp (b) and iron (c) in reindeer (n = 6) after IV administration of *E. coli* LPS (○; 0.1 μg/kg) or saline (●) at 0 h. The data are combined from two successive challenges. Statistical differences are given in the text.*
group were detected during the follow-up period (0–96 h) at the time of both sets of the experiment. The only exception was at the time of the first experiment when the LPS group showed values clearly elevated over those of the control animals in the 4-h sample. The reindeer already had showed very high cortisol values in the pretreatment sample (mean 102.9 nmol/l, range 9.8–240 nmol/l; Fig. 2).

Serum urea values began to increase after treatment in both groups simultaneously and after 4 h decreased slowly until 96 h to under the baseline level (Fig. 2; time effect \( p < 0.001 \)). After one week, the mean urea concentration had nearly returned to the pretreatment level. There were no differences between treatment groups at any time point.

![Graphs](image-url)

Fig. 2. Mean (± SD) serum concentrations of urea (a) \( n = 6 \), cortisol from first set (b) \( n = 3 \) and from second set of the experiment (c) \( n = 3 \) in reindeer after IV administration of E. coli LPS (○; 0.1 µg/kg) or saline (●) at 0 h. The urea data are combined from two successive challenges. Statistical differences are given in the text.
Mean rectal temperatures were already higher before treatment in the LPS group and decreased steadily in both groups throughout the 24-h period, which was recorded, but this difference was not significant. The changes in concentrations of serum total protein, albumin, ASAT, CK, GT and SDH recorded during the follow-up period were nonsignificant between the endotoxin and control groups.

4. Discussion

Bacterial endotoxins, which are LPSs from the cell wall of gram-negative bacteria, are considered to cause most pathophysiological reactions during bacterial infections. The physiological effects of LPS are based predominantly on activation of various molecular mediators [25] such as the cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6, which are released in response to LPS predominantly by monocytes and macrophages [26]. These inflammatory mediators initiate host inflammatory response and the production of hepatic APPs. Since cytokines are the main stimulators of excretion of APPs from the liver [1], LPS administration has been used in APP research as an acute phase stimulant in bovines [5,27,28]. In the present study, injection of E. coli LPS induced activation of cytokine release in reindeer as shown by significant decrease in serum iron concentration and increase in serum SAA concentrations in all LPS-challenged animals. These changes were similar to those previously reported in domestic ruminants [27,29]. A slight tendency for Hp concentrations to increase was seen earlier than SAA following LPS challenge, but the Hp patterns differed widely between individuals and the relative increases were small. A similar, evident but nonsignificant elevation of Hp was reported previously in pigs after a single-bolus LPS dose administration [30], but the Hp response was clearly elevated when repeated injections were used [31]. Hp and SAA differ in their cytokine-controlled induction in the liver, and generally Hp needs stronger cytokine stimulation to be produced than SAA [1,32]. The cytokine response sequence is not affected by LPS dose or administration route, but the amplitude of the response is dose dependent [33] and a single-bolus dose of E. coli LPS may have been insufficient to initiate the cytokine secretion needed for prolonged Hp increase. This is supported by other studies in bovines in which Hp increased after experimental gram-negative infections [34,35], when stronger and prolonged cytokine release occurs, or after higher E. coli LPS dose administration [5].

Variability in APP expression during the APR between animal species can also explain the low Hp response observed. However, the serum Hp concentration can increase manyfold over the baseline level and appears to act as an APP in reindeer. This was observed in the reindeer we had to remove from our study due to the presence of exceptionally high SAA and Hp concentrations probably resulting from arthritis.

Another explanation for the mild APR to endotoxaemia may be the stress reactions of reindeer. Although treatment did not affect the urea concentration, increased concentrations occurring during the first blood samplings and subsequent decrease when animals became used to handling, further confirm the presence of a strong stress response in reindeer. Increased concentrations of serum urea in stressed reindeer have been reported [36,37] and significantly higher urea concentrations have been seen in intensively handled
reindeer versus animals subjected to minimal handling [38]. The presence of a carry-over effect in the cortisol results is very difficult to explain, especially when considering that we had long wash-out period between experiment sets. However, the similarities in mean cortisol concentration patterns within the animal groups during both experiments, regardless of LPS challenge and wide individual variability (Fig. 2), indicate that cortisol was more affected by the experimental procedure than by the presence of endotoxaemia and that the carry-over effect was at least partly the result of stress responses of individual animals to handling. The elevated cortisol levels observed before LPS challenge in this study probably affected the results. High endogenous glucocorticoid concentrations can influence the APR and expression of APPs in many ways; e.g. it can directly stimulate APF production in the liver, but this is not believed to be a very powerful response [1] and administration of dexamethasone failed to stimulate APF in cows [39]. On the other hand, cortisol can inhibit APF increase by decreasing the cytokine response. Endogenous and exogenous glucocorticoids can suppress TNF-α production after endotoxin administration [40], and therefore the stress reaction induced by handling before endotoxin administration may have an inhibiting effect on proinflammatory cytokine release and may be one reason for the mild Hp response to the endotoxin dose used in this study. Further support for the inhibitory effect of stress reactions before LPS administration is given by the fact that no fever response occurred, although the clinical, serum iron and SAA responses were clearly present. Cortisol and fever responses occurring during endotoxaemia to some extent share a common mediating factor, namely prostaglandin, which is mediated by proinflammatory cytokines [14] and the controlling mechanisms for fever and changes in serum iron concentrations are independent [12].

In conclusion, we have demonstrated that single-dose E. coli endotoxin challenge activates the APR and that this model can be used in reindeer for examining the pathophysiology of inflammation and infection, although higher doses may be more appropriate. However, in semidomesticated animals such as reindeer, stress reactions from handling are very strong and their possible effects on the results must be addressed. SAA appears to be a more sensitive indicator of the APR than Hp in reindeer after LPS challenge. Since these proteins have different stimulation patterns, monitoring of both proteins can provide more information on the ongoing APR of the host and thus may provide a useful tool in veterinary medical science in reindeer. Further investigation is needed to increase our understanding of the expression of these proteins in cases of various naturally occurring and experimental infections or inflammations.

References