Renal function and oxygen consumption during bacteraemia and endotoxaemia in rats

A. E. J. Heemskerk, E. Huisman, A. A. van Lambalgen, G. C. van den Bos, M. Hennekes, L. G. Thijs and G. J. Tangelder

1Laboratory for Physiology, 2Department of Clinical Chemistry and 3Medical Intensive Care Unit, Vrije Universiteit, Amsterdam, The Netherlands

Abstract

Background. The hypothesis that renal failure during septic shock may occur as a result of hypoxia-related cell dysfunction was investigated in two rat models of distributive shock.

Methods. Pentobarbitone-anaesthetized rats received either a bolus (1 ml) of living Escherichia coli bacteria (hospital-acquired strain, $1 \times 10^9$ CFU/ml; BA-group, $n=7$), or a 1-h infusion of endotoxin ($E. coli$ O127:B8: 8 mg/kg; ET-group, $n=7$), or saline to serve as time matched controls (C-group, $n=7$).

Results. Urine flow in the BA- and ET-group reached a nadir at 1 h, but thereafter increased and reached values higher than control at 3 h. At this time point, renal oxygen delivery had decreased, in the BA-group mainly due to a fall in arterial oxygen content and in the ET-group to a fall in renal plasma flow (clearance of $^{131}$I-hippurate). However, renal oxygen extraction had significantly increased, by 31% in the BA and by 59% in the ET group, while renal oxygen consumption remained the same. Net tubular sodium reabsorption had decreased by 55% in the BA and by 25% in the ET group, due to a fall in glomerular filtration rate (clearance of creatinine). Hence, an excess oxygen consumption was found which was caused neither by an increased renal glucose release nor by the presence of an increased number of leukocytes stuck in the glomeruli. Renal tubular cells showed normal morphology. An indication that proximal tubular function in the BA and ET group remained largely intact were normal ATP levels, absence of urinary glucose, and a normal fractional excretion of sodium. However, since urine flow had increased in shocked rats at 3 h, water appeared selectively lost.

Conclusions. Our data indicate that in rat models of septic shock renal failure is not caused by cortical hypoxia or a shortage of cellular energy supply.

Key words: $E. coli$ bacteria; $E. coli$ endotoxin; oxygen consumption; renal blood flow; sodium reabsorption; urine flow

Introduction

Acute renal failure is a complication in septic patients associated with a high mortality rate [1]. An important characteristic is a decrease in glomerular filtration rate, which is even present in situations where renal blood flow has been maintained [2]. Prevailing theories on the pathogenesis of acute renal failure suggest an inappropriate and uncontrolled release of various inflammatory mediators [1]. These may be directly cytotoxic, and/or cause maldistribution of blood flow and oxygen supply within the cortical microcirculation. The latter may cause ineffective oxygen extraction and hypoxia-related cell dysfunction. Although studies in some animal models of sepsis suggest an inability of the kidney to extract enough oxygen [3,4], the role of oxygen remains controversial.

In previous studies we have described two rat models of distributive shock, i.e. one induced by endotoxin infusion [5,6] and the other by injection of live bacteria [7]. In the present study, we have used both sepsis models to investigate renal oxygen consumption in relation to changes in renal function, such as glomerular filtration rate, urine output and tubular sodium reabsorption. The latter is considered to be the major energy-consuming process in the kidney [8,9]. In addition, energy status and leukocyte adherence in the cortex, as well as possible tissue damage in cortex and medulla were evaluated. Activated leukocytes may increase their oxygen consumption considerably, the so-called oxygen burst [10]. Since in isolated, perfused rat kidney no direct effect of endotoxin was found on glomerular filtration rate and sodium reabsorption [11] and given the contribution of extrarenally released mediators to renal dysfunction in sepsis [1,2], the present study was performed in whole animals.
Subjects and methods

Animals

All experiments in this study were performed in accordance with the guiding principles for research with experimental animals (Helsinki Declaration).

Experiments were performed on male Wistar rats (250–350 g, Harlan Sprague–Dawley, Zeist, the Netherlands) which had been fasted overnight. Anaesthesia was induced by two intraperitoneal injections of pentobarbitone (Nembutal®, 25 mg/kg) 20 min apart; additional doses were given when necessary, guided by the presence of interdigital reflexes. Lidocaine HCl was injected locally during instrumentation (see below). The rats were in supine position on a heating pad which maintained rectal temperature at 38 °C.

Catheters (PE50) were inserted into the jugular vein and carotid artery. Via the latter mean arterial pressure (MAP) was measured using a Statham P23Db pressure transducer placed at sternal level. Heart rate (HR) was derived from the pressure signal. A trachea catheter was inserted via the femoral artery into the abdominal aorta. The thermistor was connected to an Edwards 9520A cardiac output computer; measurements were made by injecting 0.2 ml saline at room temperature into the jugular vein. In whole blood collected from the carotid artery, lactate and glucose were measured using a LM3 lactate-glucose analyser (Analox Instruments), the oxygen content was determined with an OSM3 hemoximeter (Radiometer Copenhagen), and the haematocrit using microcentrifugation.

After an abdominal midline incision, the ureter of the left kidney was exposed and catheterized (PE10) to collect urine. Animals were allowed to stabilize for 30 min after instrumentation, then blood was collected from the left renal vein (see below) and the abdomen was closed around the ureteric catheter. At the end of the experiment the abdomen was reopened for another renal vein puncture and kidney biopsies.

Experimental groups and protocol

The animals were randomly divided into three groups, n = 7 each:

1. A group receiving 1 × 10⁹ colony-forming units (CFU) of living Escherichia coli bacteria, given as a bolus of 1 ml within 5 min (BA group, see below);
2. An endotoxin-treated group (ET group), receiving E. coli endotoxin O127:B8 (Difco, 8 mg/kg) at an infusion rate of 0.2 ml/100 g during 1 h (see below);
3. A control group (C group), receiving a saline infusion of 0.2 ml/100 g during 1 h (see below).

Escherichia coli bacteria were cultured from a hospital-acquired strain, and were characterized by O serogroup 18 and the presence of a capsule (O18K1). This strain is capable of growing in rat serum and causing septic shock in vivo [7]. An overnight culture, grown in brain–heart infusion broth (Difco), was diluted 1:200 and grown again at 37 °C until by multiplying the respective arteriovenous concentration difference by the RBF.

After instrumentation and stabilization, the following protocol was applied, with time (t) designated in minutes:

- At t = 0, end of endotoxin infusion; start bolus of live E. coli (BA group);
- At t = 5, end bolus of live E. coli (BA group);
- At t = 60, end of endotoxin infusion (ET group) or saline (C group);
- At t = 140, start of hippurate infusion;
- At t = 180, end of hippurate infusion; blood collection from renal vein and renal biopsies.

Renal function and oxygen consumption

Renal plasma flow (RPF) and glomerular filtration rate (GFR) were obtained from the clearance of 131I-hippurate (OIH; Amersham International, UK) and creatinine respectively, according to the formula: clearance (ml/min) = (U × V) / P, where U is the concentration of OIH or creatinine in urine, V is the urine flow and P is the concentration of OIH or creatinine in blood plasma. In a previous study we found that changes in GFR can be reliably obtained by the clearance of creatinine [6]; creatinine clearance was consistently lower than inulin clearance by a constant factor in control as well as in shocked rats. Hence, to obtain the actual GFR, we multiplied creatinine clearance by this factor, being 1.56. OIH was infused at a rate of 0.2 ml/100 g.h (2 μCi/h) after a priming dose of 0.5 ml (5 μCi). With this method we established a constant plasma level of OIH; blood samples were taken every 10 min during the OIH infusion to verify this. Blood samples for measuring creatinine were taken at t = −40 and t = 180. Urine was continuously collected in periods of 30 min during the entire experiment; in the urine collected from t = −30 to t = 0 and from t = 150 to t = 180 min, OIH and creatinine were measured. OIH in urine and plasma samples was measured by a γ-counter (1282 Compugamma CS, LKB Wallac), and creatinine concentration was determined with a creatinine kit (Merckotest, Merck) and a spectrophotometer (527 nm, Spektraphotometer PM6, Zeiss). RBF was calculated according to: RBF (ml/min) = RPF (ml/100) / haematocrit.

Through a puncture of the left renal vein, 150 μl renal blood was collected in a heparinized syringe; the bleeding was stopped within 30 s with gauze. In whole blood collected from this vein, oxygen content (OSM3 hemoximeter, Radiometer Copenhagen), glucose and lactate (LM3 lactate-glucose analyser, Analox Instruments) were measured. Renal oxygen delivery (DO₂) was calculated by multiplying the arterial oxygen content with the RBF; renal oxygen consumption (VO₂) was determined by multiplying the arteriovenous oxygen content difference (Cv-VO₂) with the RBF. Renal release of glucose and lactate were obtained by multiplying the respective arteriovenous concentration differences by the RBF.

All urine and plasma samples were analysed for sodium and potassium by flame photometry (FLM3, Radiometer Copenhagen) using internal lithium standardization. The urine sodium (U Na⁺; mM) and potassium concentration...
(\(U_\text{Na}^2\) mmol/min) were multiplied by the urine flow (\(V\)) to obtain sodium and potassium excretion respectively (\(U_{\text{Na}}V\) and \(U_\text{K}V\), mmol/min). Tubular sodium reabsorption (\(T_{\text{Na}}\), mmol/min) was determined according to:

\[(GFR \times P_{\text{Na}}) - U_{\text{Na}}V\]

where \(P_{\text{Na}}\) is the plasma sodium concentration. Urinary excretion of sodium as a percentage of the filtered load, i.e. fractional excretion (\(\text{FE}_{\text{Na}}\)), was calculated according to:

\[\text{FE}_{\text{Na}} = 100 \times (U_{\text{Na}}V)/(P_{\text{Na}} \times GFR).\]

Since tubular sodium reabsorption is the major energy-consuming process in the kidney, we divided for the control situation (\(t=0\)) \(V\) by \(T_{\text{Na}}\), yielding the amount of oxygen needed to reabsorb 1 unit (i.e. 1 mmol) of sodium for the control situation (\(t=0\)). Multiplication of this amount of oxygen needed per unit \(T_{\text{Na}}\) under control condition by the actual \(T_{\text{Na}}\) at \(t=180\) yields the expected amount of oxygen needed for sodium reabsorption at \(t=180\). Subtraction of this expected amount from the actually measured \(VO_2\) at \(t=180\), yields what we define as the excess (or deficit) oxygen consumption at \(t=180\), designated excess-\(VO_2\).

**Renal biopsies for metabolic variables and histology**

Biopsies were taken from both poles of the left kidney, i.e. mainly cortical material, and immediately frozen in liquid nitrogen. The samples were homogenized in 2.0 ml of ice-cold 0.66 M perchloric acid with an OMNI 2000 homogenizer (OMNI International, Waferbury, Conn., USA). After centrifugation, 1.0 ml of the supernatant was neutralized by addition of 0.4 ml of a 1.6 M KOH solution and 0.2 ml of 1.0 M acetic acid. Precipitated K⁺-perchlorate was removed by centrifugation. Samples were kept on ice between manipulations. Aliquots of the supernatants were used immediately for the enzymatic determination of glucose (Gluc-DH kit; Behringwerke, Marburg, FRG). The remainder of the supernatant was stored at −20°C for subsequent analysis by HPLC. Gradient ion-pair reversed-phase HPLC methods were used to determine creatine phosphate (CP [13]) and energy-rich phosphates, such as ATP [14].

After these biopsies had been taken, a slice of the midpart of the left kidney, i.e. both cortical and medullary, was immersion fixed in Telly-fixative, which consists of 38 ml tridest, 12.6 ml 0.05 M phosphate buffer, 22 ml formalin (37%), 147.4 ml alcohol (96%), and 1 ml acetic acid (96%). After dehydration (alcohol) the samples were embedded in JB4 plastic (Polysciences Inc.) and 1.5 µm sections were cut (ultramicrotome, Reichert–Jung). These sections were stained with PAS (periodic acid–Schiff) and examined under a light-microscope for tubular damage in terms of increased vacuolization, brush border injury, or debris in the tubular lumen. The number of polymorphonuclear neutrophils (PMNs) was counted in 50 glomeruli at a magnification of 400×.

**Statistical analysis**

Differences between groups at a certain time point were first tested with the non-parametric Kruskal–Wallis one-way analysis of variance. If the level of significance, i.e. 5%, was reached, we subsequently determined whether differences between control and bacteraemic, and between control and endotoxaemic rats existed, and the Mann–Whitney U test was applied. To determine whether differences within a group (compared to the value at \(t=0\)) were significant, the Wilcoxon matched-paired signed-rank test was used. Correlations were done using linear regression. Data are presented as means ± standard error of the mean (SEM).

**Results**

**Systemic variables**

Systemic haemodynamic changes between start and end of the experimental period are presented in Table 1. Mean arterial pressure (MAP) and cardiac output (CO) decreased in both bacteraemic and endotoxaemic rats within 30 and 60 min after \(t=0\) respectively, and remained lower in the control group. Heart rate (HR) was significantly elevated in both the BA and ET groups during the whole experiment. Table 2 shows arterial biochemical variables. Haematocrit had at the end decreased in all rats; in endotoxaemic animals, however, after an initial increase with a peak value of about 55% at \(t=60\) min. Arterial lactate increased continuously in both bacteraemic and endotoxaemic rats. Arterial glucose first showed in bacteraemic and endotoxaemic rats a peak at \(t=60\) of about 4 mmol/l, and thereafter decreased. Arterial oxygen content was

| Table 1. Systemic haemodynamic variables at \(t=0\) and \(t=180\) min in control (C, \(n=7\)), bacteraemic (BA, \(n=7\)), and endotoxaemic rats (ET, \(n=7\)) |
|-----------------|-----------------|-----------------|
|                 | C \(t=0\)    | BA \(t=0\)    | ET \(t=0\)    |
| MAP             | 112.1 ± 7.9    | 122.0 ± 5.7    | 123.6 ± 3.0   |
|                 | 93.3 ± 6.2     | 91.3 ± 5.4#    | 85.0 ± 5.6#   |
| CO              | 102.4 ± 5.0    | 98.7 ± 5.2     | 103.5 ± 5.31  |
|                 | 93.3 ± 2.3     | 80.5 ± 5.8     | 66.1 ± 1.98*  |
| HR              | 388.6 ± 11.2   | 407.1 ± 11.3   | 411.4 ± 13.9  |
|                 | 381.4 ± 13.9   | 443.4 ± 9.2#*  | 461.4 ± 17.2#*|

*Values are means ± SEM. MAP, mean arterial pressure (mmHg); CO, cardiac output (ml/min); HR, heart rate (bts/min). #Significant difference (\(P<0.05\)) from concomitant value in the C group.

| Table 2. Arterial biochemical variables at \(t=0\) and \(t=180\) min in control (C, \(n=7\)), bacteraemic (BA, \(n=7\)), and endotoxaemic rats (ET, \(n=7\)) |
|-----------------|-----------------|-----------------|
|                 | C \(t=0\)    | BA \(t=0\)    | ET \(t=0\)    |
| Haematocrit     | 44.4 ± 1.3     | 45.5 ± 1.1     | 46.5 ± 0.7    |
|                 | 44.6 ± 1.2#    | 38.7 ± 1.2#    | 43.4 ± 0.99   |
| Lactate         | 0.88 ± 0.09    | 0.89 ± 0.11    | 0.80 ± 0.08   |
|                 | 0.73 ± 0.05    | 3.09 ± 0.57#*  | 3.55 ± 0.28#* |
| Glucose         | 2.83 ± 0.23    | 3.06 ± 0.19    | 2.85 ± 0.24   |
|                 | 3.00 ± 0.14    | 2.51 ± 0.33    | 1.79 ± 0.26#* |
| Oxygen content  | 17.4 ± 0.83    | 17.2 ± 0.67    | 17.1 ± 0.32   |
|                 | 15.6 ± 0.50    | 14.7 ± 0.39#   | 17.5 ± 0.51*  |

*Values are means ± SEM. Haematocrit in %, lactate and glucose in mmol/l and oxygen content in vol%. #Significant difference (\(P<0.05\)) between values at \(t=0\) and \(t=180\) min. *Significant difference (\(P<0.05\)) from concomitant value in the C group.
Renal function during septic shock in rats

Renal function

Urine flow (V), urinary sodium concentration (U\textsubscript{Na}) and sodium excretion (U\textsubscript{Na}V) are presented in Figure 1. V remained unchanged in the C group, whereas at t = 180 min it had significantly increased in the BA as well as in the ET group, in the latter after a severe decrease with a nadir at t = 60 min. U\textsubscript{Na} (middle panel) increased in the C group, but fell and remained low in the bacteraemic and endotoxaemic animals. Urinary potassium concentration (initial mean values 130–165 mmol/l) decreased in all groups within 60 min to a plateau, which in the control group remained at about 65% of the initial value and in the two shocked groups at around 45%. Sodium excretion (U\textsubscript{Na}V; lower panel of Figure 1) had increased in control animals by 169% at t = 180, whereas in bacteraemic and endotoxaemic rats U\textsubscript{Na}V had reached control values at this time point after an initial nadir at t = 60 min. Potassium excretion had transiently decreased in control and diseased animals, whereafter it increased again to reach control values at t = 180 min. No excretion of glucose was found. The urine creatinine concentration was maintained in control animals at a value of ~130 and ~4 mmol/l respectively. The plasma creatinine concentration was maintained in control animals at 0.38 ± 0.03 mg/dl (at t = 180 min), whereas it had increased at this time to 0.51 ± 0.02 mg/dl in bacteraemic and 0.58 ± 0.06 mg/dl in endotoxaemic rats.

Renal oxygen consumption

In Figure 2 renal oxygen delivery (DO\textsubscript{2}), oxygen extraction (C\textsubscript{a-\textsubscript{v}}O\textsubscript{2}), and oxygen consumption (VO\textsubscript{2}) at the start and end of the experiment are shown. DO\textsubscript{2} had significantly decreased in the BA as well as in the ET group, whereas C\textsubscript{a-\textsubscript{v}}O\textsubscript{2} had significantly increased in these groups. VO\textsubscript{2}, on the other hand, was maintained at control values in all groups.

Figure 3 presents one of the two variables used to calculate DO\textsubscript{2}, i.e. renal plasma flow (RPF), as well as the glomerular filtration rate (GFR) and fractional excretion of sodium (FE\textsubscript{Na}). RPF (upper panel) was maintained in control as well as in bacteraemic rats, whereas in endotoxaemic animals it had significantly decreased at t = 180 min. In this latter group renal blood flow (RBF) decreased even more, from 10.0 ± 1.0 ml/min to 7.2 ± 0.7 ml/min, because of the decrease in haematocrit (see Table 2). In control rats RBF was 9.0 ± 0.8 ml/min at t = 0 and 8.9 ± 1.3 ml/min at t = 180. In bacteraemic animals, RBF decreased
from 11.1 ± 0.8 ml/min at t = 0 to 8.0 ± 1.5 ml/min at t = 180, albeit non-significantly. Thus, the decrease in renal DO$_2$ in bacteraemic rats (Figure 2) was caused more by the significant decrease in arterial oxygen content (Table 2) than by the slight decrease in RBF. In endotoxaemic animals the decrease in DO$_2$ was caused by the decreased RBF only, which was due mainly to the decrease in RPF. Note in this respect...
the significant decrease in MAP and CO in the endotoxaemic animals (Table 1). GFR (middle panel) had fallen in both shocked groups at t=180 min, i.e. by 45% in bacteraemic and by 30% in endotoxaemic rats. Fractional excretion of sodium (FE\textsubscript{Na}, lower panel), i.e. the percentage sodium filtered in the glomeruli which is eventually excreted in the urine, remained below 0.8% in all groups with no significant increases in the diseased groups.

In Figure 4, relationships between the effective or net tubular sodium reabsorption (net-T\textsubscript{Na}) and VO\textsubscript{2} are presented, since T\textsubscript{Na} is the major energy-consuming process in the kidney. Linear regression equations are shown if significant. In the control group the relationship had not changed, but in the two shock groups it was shifted to the left, indicating a higher VO\textsubscript{2} per millimole sodium effectively reabsorbed. This excess oxygen consumption might be due to less efficient net sodium transport or to other processes in the kidney which consume oxygen.

In Figure 5 the number of polymorphonuclear neutrophils (PMNs) present in 50 glomeruli is plotted against the excess oxygen consumption (excess VO\textsubscript{2}) at t=180 min. PMNs may show an oxygen burst when activated, and hence may then contribute to the excess VO\textsubscript{2}. However, no significant relationship was found between the excess oxygen consumption in bacteraemic as well as endotoxaemic rats and PMNs. In both shock groups the level of excess VO\textsubscript{2} was similar. The figure also shows that the number of adherent leukocytes was greater in endotoxaemic than in bacteraemic rats, and in both groups significantly larger than in controls. No correlation was found between the number of PMNs and the percentage decrease in GFR.

Renal metabolic variables

Kidneys of control as well as diseased animals released the same amount of glucose at t=180 as compared to t=0 (between 0 and 10 μmol/min) and also lactate release remained unchanged (between 0 and 5 μmol/min), with no significant differences between the three groups. Hence, the excess-VO\textsubscript{2} consumed at t=180 was not used for extra glucose release. In Table 3 tissue concentrations in the renal cortex of glucose, lactate, creatine phosphate (CP) and ATP are presented. In bacteraemic rats, renal glucose and lactate did not differ significantly from the values in control animals, although lactate tended to be higher. In endotoxaemic rats, however, renal glucose as well as lactate had decreased and increased respectively. Renal lactate content in control and endotoxaemic rats did not correlate with the arterial lactate concentration at t=180 min (see Table 2), whereas a significant correlation was found in bacteraemic animals (R=0.77). This implies that in the latter the tendency of an increased renal lactate content is related to the increased arterial lactate concentration, whereas in endotoxaemic animals the increased renal lactate content also has a different origin. By contrast, as shown in Table 3, renal CP and ATP content did not differ significantly between groups. Furthermore, no tubular damage was found in any group in terms of increased vacuolization, brush border injury, or debris in the tubular lumen.
Discussion

The present study shows that in rats subjected to septic shock as induced by either bacteraemia or endotoxaemia, the kidney was still able to increase its oxygen extraction. This increase kept renal oxygen consumption unchanged, despite a fall in oxygen delivery. On the other hand, the effective tubular sodium reabsorption had diminished due to a fall in glomerular filtration rate. Since sodium reabsorption is the major energy-consuming process in the kidney, an excess oxygen consumption was found. Renal glucose release into the blood as well as cortical ATP content remained the same. In addition, no tubular damage was observed and no glucose was lost in the urine. Urine flow, however, had increased considerably in the diseased rats.

Our finding that in two forms of distributive shock in rats the kidneys are still able to increase their oxygen extraction, in the presence of a decreased renal oxygen delivery, is in contrast to other animal studies. In dogs, oxygen extraction is impaired during endotoxaemia [3]. In sheep treated with a lower dose of endotoxin, oxygen extraction did not increase to compensate for the decreased oxygen delivery [4]. In both studies a decreased oxygen consumption was found. Since in our two sepsis models yielded the same result with regard to changes in renal oxygen extraction, the disagreement with the above-mentioned studies might be related to species differences. A difference among species also might indicate that the findings obtained cannot be easily extrapolated to the clinical situation. In addition, our study was limited to a duration of 3 h, while clinical sepsis usually takes days or even weeks. Hence, it cannot be excluded that in these later stages hypoxia-related cell dysfunction might play a role in the development of renal failure in septic patients.

Kidneys of bacteraemic and endotoxaemic rats reabsorb less sodium, −55 and −25% respectively, at the same oxygen consumption as kidneys of control rats. This indicates a higher or excess oxygen consumption as expected on the basis of the work needed to support the observed level of renal functioning. Weber and co-workers also found in hyperdynamic sheep after 12 h of endotoxaemia a proportionally more pronounced decrease in sodium reabsorption (−70%) than in renal oxygen consumption (−35% [4]). The proximal tubular epithelial cells appeared histologically intact and were able to reabsorb all filtered glucose and maintain their ATP content. Moreover, the fractional sodium excretion of bacteraemic and endotoxaemic rats did not differ from that in control animals. Nevertheless, it is possible that the proximal tubular epithelium has become a more leaky barrier, resulting in more back-flux of sodium from the basolateral epithelial crypts towards the tubular lumen. A possible cause are inflammatory and vasoactive mediators released renally or extrarenally during the septic state [1]. An increased back-flux of sodium would have caused more work and thus an elevated oxygen consumption needed for the same effective or net sodium reabsorption. However, more explanations are possible for the excess oxygen consumption observed, of which some will be discussed below.

Sodium reabsorption is the major energy- and oxygen-consuming process in the kidney, but is not the only one [15]. Another renal oxygen-consuming process is gluconeogenesis, and although we measured glucose release into the blood instead of gluconeogenesis, we found no changes in glucose release between groups or time points. Ardawi et al. [16] showed that sepsis even decreases the rate of renal gluconeogenesis through a fall in the activity of gluconeogenic key enzymes. Another animal study demonstrated that moderate renal hypoperfusion as caused by endotoxin did not influence renal release of glucose [3].

The increased number of polymorphonuclear neutrophils (PMNs) in the renal glomeruli of our bacteraemic and endotoxaemic rats might have contributed to the maintained oxygen consumption in the presence of diminished renal work. PMNs when activated increase their oxygen consumption considerably [10], among others for the production of prostaglandins and oxygen free radicals. However, in the present study we did not find a correlation between the number of PMNs and the excess oxygen consumption. Free rad-
icals and enzymes released by activated PMNs can cause tissue damage, and activated PMNs can apparently inhibit Na\(^+\)/K\(^+\) ATPase activity [17]. However, no correlation was found between the number of leukocytes stuck in the glomeruli and the decrease in glomerular filtration rate. The tubules themselves, both cortical and in the medulla, did not show signs of histological damage. To which extent activated PMNs in the glomeruli might have contributed to possible tubular dysfunction, e.g. causing an increase in leakiness of the epithelial barrier (see above), remains to be investigated.

The present study indicates that during bacteraemia and endotoxaemia the rat kidney is able to maintain, during at least 3 h, cortical levels of ATP. This finding adds to the notion that the kidney is able to maintain its cellular ATP content under most conditions [8]. Renal changes in respiration, for example, are usually accomplished with little, if any, changes in ATP content. As discussed previously [5], only ATP accurately expresses the energy status of the kidney. Since the kidney contains little creatine phosphokinase, calculation of the energy charge (i.e. (ATP + 0.5 ADP)/(ATP + ADP + AMP)) is of less relevance in this organ. Nevertheless, if done, this parameter was the same in all groups (means 0.61–0.63).

The bacteraemic as well as endotoxaemic rats showed a significant increase in urine flow. Furthermore, a relative decrease in urinary constituents, such as sodium, was observed. It has been reported in endotoxoaemic rats that a fall in urinary sodium concentration indicates conserved tubular reabsorption capacity, at least for the decreased sodium load [18,19]. Given the increase in urine flow, its colourless appearance, and the finding that the fractional excretion of sodium in diseased animals did not differ from that in controls, it is likely that the diseased animals excreted more water than solutes in the present study. The best way to quantify a net loss of water is calculation of the free water clearance [20,21]. Since the possibility of a net loss of water was not anticipated, urine and plasma osmolality needed to calculate free water clearance were not measured. However, in recent experiments on the influence of fluid resuscitation on renal function in septic rats, we did measure these variables and calculated the free water clearance. As presented in Figure 6, free water clearance steadily decreased in the control group, while it remained significantly higher in both shock groups, indicating an increased water loss due to the disease state. It has been described that an increased free water clearance is an early predictor of acute renal failure in patients [20,21]. The cause of this water loss must lie in the medulla, either at the level of the medullary osmotic gradient or at the level of the collecting ducts. An increased urine flow has also been described in endotoxaemic dogs, where it was related to a spontaneous increase in arterial pressure [22], which, however, was not the case in our septic rats. The precise mechanism for this net water loss in septic rats requires further investigation.

In summary, during bacteraemia as well as endotoxaemia in rats, in spite of a decreased oxygen delivery, the kidney is still able to increase its oxygen extraction. Renal work, on the other hand, had decreased: due to a fall in glomerular filtration rate, net tubular sodium reabsorption had decreased, and renal glucose release remained the same. Since oxygen consumption did not change, more oxygen was consumed than expected. Leukocyte accumulation is not a likely cause of this excess oxygen consumption. Renal cortical tubular cells were also able to maintain in both models their ATP content during at least 3 h. A remarkable finding after 3 h in septic rats was an inappropriate urinary water loss. We conclude that during bacteraemia and endotoxaemia in rats glomerular filtration rate decreases but cortical tubular morphology, metabolism, and function remain largely intact.

Acknowledgements. This study was supported by grant C90.1016 of the Nier Stichting Nederland (Dutch Kidney Foundation). The authors would like to thank Mr. J. P. F. Barends for computer assistance and Mr. W. Gerrissen for his biotechnical help.

References


Received for publication: 3.7.96
Accepted in revised form: 19.3.97