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Renal interstitial concentration of adenosine during endotoxin shock

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Abstract

The present experiments were designed to measure the renal interstitial concentration of adenosine in an attempt to determine whether adenosine participates in the regulation of renal hemodynamics during endotoxin shock. The renal concentration of adenosine in response to lipopolysaccharide (LPS) administration was measured in anesthetized dogs using a microdialysis method. Renal hemodynamic responses to LPS were also determined with and without the adenosine A_1 receptor antagonist, (*E*)-(*R*)-1-[3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)acryloyl]pyperidin-2-ylacetic acid (FK352). Intravenous administration of LPS (0.5 mg/kg) significantly decreased renal blood flow and mean arterial pressure. These parameters reached the minimum level at 5–10 min after the LPS administration and then returned to their respective preinjection levels. The renal interstitial concentration of adenosine increased from 118 ± 18 to 381 ± 46 nM. During treatment with FK352, LPS decreased renal blood flow and mean arterial pressure, however, these reductions were significantly attenuated. LPS also increased adenosine concentration, but its rise was reduced along with the attenuation of LPS-induced renal blood flow reduction. These results suggest that adenosine was involved in LPS-induced renal hemodynamic changes and that FK352 has a protective effect against renal dysfunction during endotoxin shock. Since the adenosine concentration was inversely proportional to renal blood flow levels, it can be assumed that adenosine plays an important role as a mediator, but not as an initiator of renal hemodynamic changes during endotoxin shock.

Keywords: Adenosine; Lipopolysaccharide; Endotoxin shock; Renal hemodynamics; Microdialysis; Adenosine A_1 receptor antagonist

1. Introduction

Acute renal failure is a serious and common complication of endotoxin shock and produces a high mortality rate (Groeneveld et al., 1991; Levy et al., 1996). The mechanisms of lipopolysaccharide (LPS)-induced renal dysfunction have been studied extensively but remain elusive. The administration of a lethal dose of LPS results in an immediate decrease in renal blood flow and glomerular filtration rate along with a fall in cardiac output and mean arterial pressure during the initial phase of experimental endotoxin shock (Hinshaw et al., 1961; Petrucco et al., 1972; Gullichsen, 1991). Various vasoactive mediators such as the sympathetic nervous system, catecholamine, thromboxane, and the renin–angiotensin system have been implicated in the renal hemodynamic changes in the initial stage of endotoxin shock (Nykiel and Glaviano, 1961; Isakson et al., 1977; Keeler, 1981; Henrich et al., 1982; Badr et al., 1986), however, the mechanism by which this occurs is poorly understood.

A possible candidate mediator of LPS-induced renal dysfunction is adenosine, which has been shown to play an important role in the regulation of renal hemodynamic changes during various forms of acute renal failure (Churchill and Bidani, 1987). In fact, Churchill et al. (1987) have proposed that adenosine mediates renal hemodynamic changes during endotoxin shock. This hypothesis is supported by the finding that treatment with a non-selective antagonist prevented LPS-induced renal vasoconstriction. Moreover, Knight et al. (1993) have reported that blocking A_1 adenosine receptors prevents LPS-induced reduction in renal blood flow and reduces the severity of acute renal failure during endotoxin shock. This indicates that adenosine induces renal vasoconstriction via the

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adenosine A_1 receptor. These observations suggest that adenosine production and release in the kidney might be involved in LPS-induced renal vasoconstriction. However, to date, no direct measurements of intrarenal adenosine have ever been carried out.

Several groups have recently measured the renal interstitial concentration of adenosine using a microdialysis method (Baranowski and Westenfelder, 1994; Siragy and Linden, 1996; Nishiyama et al., 1997). This method is well-suited for studies of intrarenal adenosine under various conditions because the adenosine receptors are located on the surface of the cell membrane (Spielman and Thompson, 1982). The present experiments examined the effect of LPS on renal hemodynamics and on the renal interstitial concentration of adenosine in anesthetized dogs using an in vivo microdialysis method. We also assessed to what extent the administration of (*E*)-(*R*)-1-[3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)acryloyl]pyperidin-2-ylacetic acid (FK352), a highly selective A_1 adenosine receptor antagonist (Kusunoki et al., 1995) would modulate the LPS-induced renal hemodynamic changes.

2. Materials and methods

2.1. General procedure

Experiments were carried out on adult male mongrel dogs weighing from 10 to 15 kg, which had been maintained on standard laboratory chow for 1 week. All surgical and experimental procedures were performed according to the guidelines for the care and use of animals as established by the Kagawa Medical University. The animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and given additional doses as required. After tracheotomy, the animals were mechanically ventilated with room air. A polyethylene catheter was inserted into the right brachial vein for infusion of isotonic saline or drug solution. Isotonic saline or drug solution was infused at a rate of 0.2 ml/kg/min throughout the experiment. Another catheter was placed in the abdominal aorta via the right femoral artery, and mean arterial pressure was continuously measured and recorded with a pressure transducer (NEC-San-ei Model NO. 361, Japan) and a polygraph (NEC-San-ei Model NO. 361). The left kidney was exposed through a retroperitoneal flank incision. The kidney was carefully denervated by dissecting all visible nerve fibers as well as the tissue connecting the renal hilum cephalic to the renal artery. An electromagnetic flowmeter (MFV-1200, Nihon Kohden, Tokyo, Japan) was positioned around the renal artery, and renal blood flow was continuously monitored. The renal capsule was carefully opened along the convexity, and the microdialysis probes were gently implanted into the renal cortex as shown in the previous paper (Nishiyama et al., 1999). The probes were connected to a CMA/100 microinfusion pump (Carnergie Medicine, Stockholm, Sweden) and were perfused with isotonic saline solution with heparin (30 units/ml) containing iodotubercidine (10 μM), an inhibitor of adenosine kinase, and *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA: 100 μM), an inhibitor of adenosine deaminase, at a rate of 10 μl/min. Since the degradation of adenosine was very rapid, the above two inhibitors of adenosine metabolism were used to detect the changes of intrarenal production of adenosine in the present study. The dialysate was collected in a chilled tube over a 5–10-min sample period and was analyzed for adenosine concentration. Samples were stored at −70°C prior to analysis. After surgery, the animal was left alone for 90 min to allow for the stabilization of mean arterial pressure, renal blood flow and renal interstitial adenosine concentration. This equilibration time was sufficient for the tissue to recover from the trauma produced by insertion of the probe as described below. At the end of each experiment, the exact position of the probe was verified by inspection. If the probe was situated in the outer medulla at least in part, we omitted the data of these dialysates.

2.2. Experimental protocols

A series of preliminary experiments were performed to determine the recovery time necessary following the implantation of the microdialysis probe. The results show that the dialysate concentration of adenosine was elevated immediately after the implantation of the microdialysis probe. The adenosine concentration dropped rapidly within the first 30 min and remained stable after 60 min. Therefore, the following experiments were started 90 min after the implantation of the microdialysis probe.

2.2.1. Effects of LPS on renal hemodynamics and the renal interstitial concentration of adenosine

Following two 10-min control periods, LPS (*Escherichia coli* 055: B5, Sigma) at 0.5 mg/kg, dissolved in a 2 mg/ml solution with isotonic saline, was injected intravenously in anesthetized dogs ($n = 7$). The dialysate was collected for 10 min at 5-min intervals. Ten minutes after the administration of LPS, additional samples were collected for 110 min at 10-min intervals. Thus, the interstitial concentration of adenosine, mean arterial pressure and renal blood flow were monitored for 120 min after the LPS administration.

We performed a time control experiment of this protocol in five dogs in which samples were collected for 150 min following the administration of isotonic saline solution (2 ml).

2.2.2. Effects of LPS on renal hemodynamics and the renal interstitial concentration of adenosine during treatment with the adenosine A_1 receptor antagonist

We used FK352, a highly selective adenosine A_1 receptor antagonist (Kusunoki et al., 1995), to investigate

whether selective blocking of the adenosine A_1 receptors would modify renal hemodynamics and/or the renal interstitial concentration of adenosine during endotoxin shock. In radioligand binding studies, FK352 displaced [^{3}H]-cyclohexyladenosine binding to rat cortical membranes (A_1 receptor) with IC_{50} of 0.14 μM and [*N*-^{3}H]-ethylcarboxamidoadenosine binding to rat striatal membranes (A_2 receptor) with IC_{50} of 18.0 μM (Kusunoki et al., 1995). Thus, in vitro, FK352 displayed a 130-fold selectivity for the A_1 receptor. In preliminary experiments, we have found that intravenous administration of FK352 (priming dose, 3 mg/kg; sustaining dose, 50 μg/kg/min) inhibited the renal vasoconstrictor responses to the intrarenal injection of exogenous adenosine (1 and 10 μg) and slightly augmented the renal vasodilatory responses to adenosine. After two 10-min sampling periods, FK352 (priming dose, 3 mg/kg; sustaining dose, 50 μg/kg/min) was administered intravenously in anesthetized dogs (n = 5). Following one 5-min sampling period, two additional sampling periods were performed at 10-min intervals. After 25 min of FK352 administration, LPS was injected intravenously at 0.5 mg/kg and samples were collected in the same manner as described above.

We performed a time control experiment of this protocol in five dogs. FK352 was administered intravenously at the same dose, and isotonic saline (2 ml), in place of LPS, was administered 25 min after the administration of FK352. The samples were collected for 160 min following the administration of FK352.

2.2.3. Effects of pressure reduction on the renal interstitial concentration of adenosine

In six dogs, we examined whether the reduction of arterial pressure would have an effect on the renal interstitial concentration of adenosine. Renal arterial pressure was reduced to two steps : from the basal pressure to 75 mm Hg, which is the lower limit of the autoregulatory range (step 1) and from 75 to 50 mm Hg, which is below than the autoregulatory range (step 2), using an adjustable aortic clamp. In each step, samples were collected for 10 min.

2.3. Microdialysis probe

In this study, a newly developed microdialysis probe constructed in our laboratory was used (He et al., 1995; Nishiyama et al., 1999). The dialysis membrane is made from cuprophan fiber, measuring 15 mm in length with a 0.22-mm outer diameter and with a 5500-Da transmembrane diffusion cut-off. Steel needles were inserted into both sides of the cuprophan fiber. The efficiency of the microdialysis probe was determined in vitro and in vivo as follows. (1) In vitro: probes were placed into beakers containing an isotonic saline solution with different quantities of adenosine. Each probe was perfused with isotonic saline solution with heparin (30 units/ml) containing iodotubercidine (10 μM), and EHNA (100 μM) at a rate of 10 μl/min. The dialysate was collected, and the recovery rate of adenosine was calculated by dividing the concentration in the dialysate by the concentration in the medium. At a perfusion rate of 10 μl/min, the recovery rate of adenosine was 16 ± 2%. These recovery rates were higher than those obtained with a commercially available microdialysis probes which measures 2 mm in length and 0.65 mm in diameter, with a 10-kDa transmembrane diffusion cut-off. Based on these results, we considered that a perfusion rate of 10 μl/min was suitable for this experiment. (2) In vivo: in a separate set of animal experiments (n = 5), an in vivo recovery rate for this microdialysis probe was determined employing a gradient dialysis technique (Baranowski and Westenfelder, 1994). After a 90-min stabilization period, probe was perfused in vivo with three different concentrations of adenosine (0, 300 and 600 nM) containing iodotubercidine (10 μM) and EHNA (100 μM) at a rate of 10 μl/min. As shown in Fig. 1, a linear relationship was observed between the different adenosine concentrations in the perfusate and the net change of adenosine concentration obtained in the dialysate. The recovery rate that is given by the slope of the regression line, was 28.7%, and the interstitial concentration, the zero intercept on the perfusate axis, was 124 nM. The in vitro and in vivo recovery rates for adenosine were quite different. At present, we cannot explain the reasons for these differences. However, we used the in vivo recovery rate for the estimation of the interstitial concentration of adenosine in the present experiments.

2.4. Analytical procedures

Adenosine in the dialysate was measured according to the method developed by Miura et al. (1991). The proce-

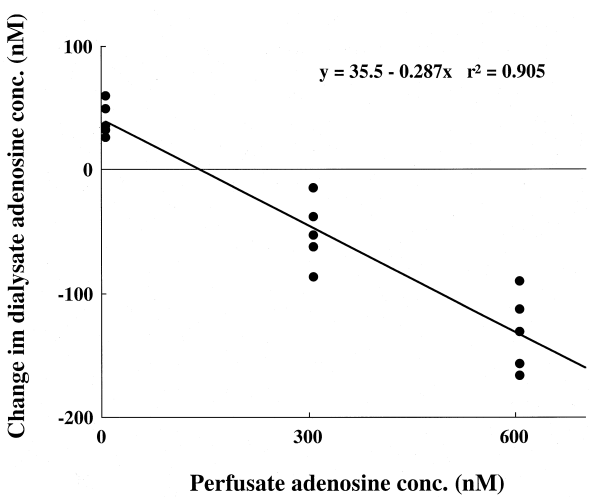

Fig. 1. Linear regression analysis of in vivo adenosine perfusion. The probe was perfused in vivo with three different concentrations of adenosine (0, 300 and 600 nM) at a rate of 10 μl/min (n = 5). The recovery rate that is given by the slope of the regression line, was 28.7%, and the interstitial concentration, the zero intercept on the perfusate axis, was 124 nM.

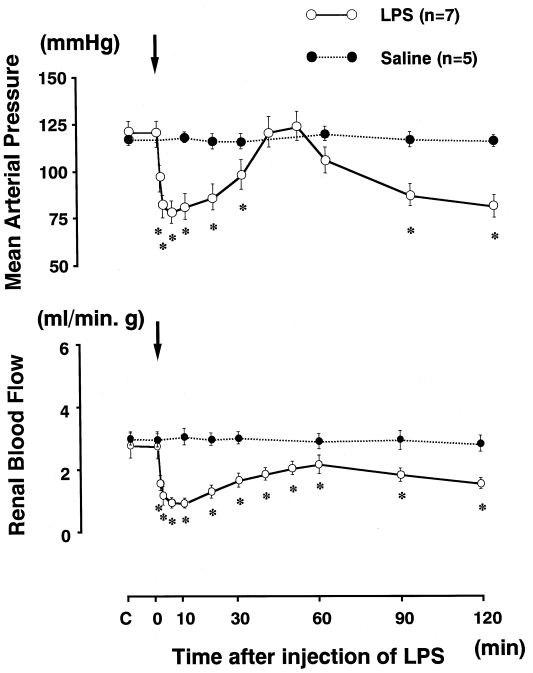

Fig. 2. Changes in the mean arterial pressure and renal blood flow following the intravenous injection of either isotonic saline or LPS. C = Control. * Indicates a significant deviation from the control value ($P < 0.05$).

dure is briefly described as follows: 100 μl of dialysate were transferred into a microcentrifuge tube and 20 μl of 1 M acetate buffer (pH 4.5) and 4.5 μl of 40% chloroacetaldehyde were added. The preparation was incubated at 60°C for 4 h to allow for the conversion of adenosine to a fluorescent 1, N^6-ethenoadenosine. After the incubation, the sample was mixed with a double volume of alamine–freon reagent and was centrifuged at $1000 \times g$ for 3 min and the upper aqueous phase was collected. For high performance liquid chromatography, a reverse-phase column (Nucleosil 7C18, 4.6 mm × 25 cm, Nagel) was maintained at 30°C with a column oven (655A-52, Hitachi). An isocratic elution with 7.5% acetonitrile in 20 mM potassium phosphate buffer (pH 5.7) at a flow rate of 1.32 ml/min was performed with a pump (L-600, Hitachi). Fifty microliters of the sample were injected with an autosampler (655A-40, Hitachi) and the elution was monitored using a fluorescence spectrometer (F-1000, Hitachi) at an excitation wavelength of 280 nm and an emission wavelength of 410 nm. A chromatointegrator (D-2000, Hitachi) was used for recording.

2.5. Statistical analysis

The values are presented as a mean ± SE. Statistical differences of data mean were determined by Student's *t*- and paired *t*-tests. A *p*-value below 0.05 was taken to indicate significant differences between data means.

3. Results

3.1. Effects of LPS on renal hemodynamics and the renal interstitial concentration of adenosine

Intravenous injection of LPS (0.5 mg/kg) resulted in triphasic changes in renal blood flow and mean arterial pressure. Renal blood flow and mean arterial pressure first decreased from 2.80 ± 0.40 and 125 ± 6 to 1.01 ± 0.16 ml/min g and 82 ± 6 mm Hg, respectively, at 5 min after the LPS injection (Fig. 2). The calculated renal vascular resistance that increased significantly from 45 ± 8 to 85 ± 11 mm Hg/ml/min/g, indicated a renal vasoconstriction. Following this, renal blood flow and mean arterial pressure reached the minimum level at 5 min after the LPS administration, and then both parameters gradually increased. At 40–60 min after the administration of LPS, mean arterial pressure had returned to preinjection levels and renal blood flow had returned to 80% of the preinjection levels. The levels then decreased again, however, whereby minimum levels were reached at 90–120 min after the administration of LPS and remained at the same level throughout the experiment (Fig. 2). The basal adenosine concentration in the renal interstitial space, which was measured at 90 min after the implantation of the microdialysis probe, was 118 ± 18 nM (Fig. 3). Twenty minutes after LPS injection, the adenosine concentration increased to 381 ± 46 nM and then gradually decreased. At 90–120 min after the administration of LPS, the concentration of adenosine showed a tendency to increase again and this was inversely proportional to the reduction observed in renal blood flow (Fig. 3).

In five dogs, we performed a time control experiment of this protocol. Intravenous administration of isotonic saline solution (2 ml) in place of LPS had no effect on renal blood flow or mean arterial pressure (Fig. 2). At 90–120 min after the administration of isotonic saline solution, the renal interstitial concentration of adenosine showed a ten-

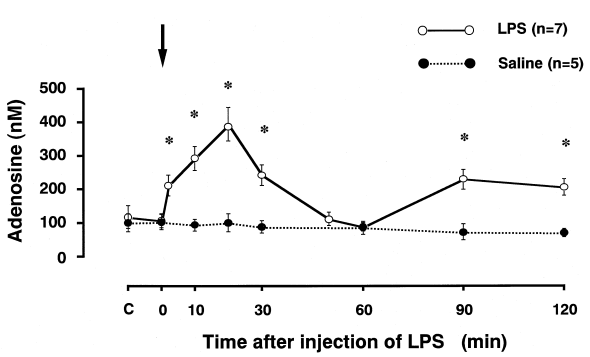

Fig. 3. Changes in the renal interstitial concentration of adenosine following the intravenous injection of either isotonic saline or LPS. C = Control. Values are mean ± SE. * Indicates a significant deviation from the control value ($P < 0.05$).

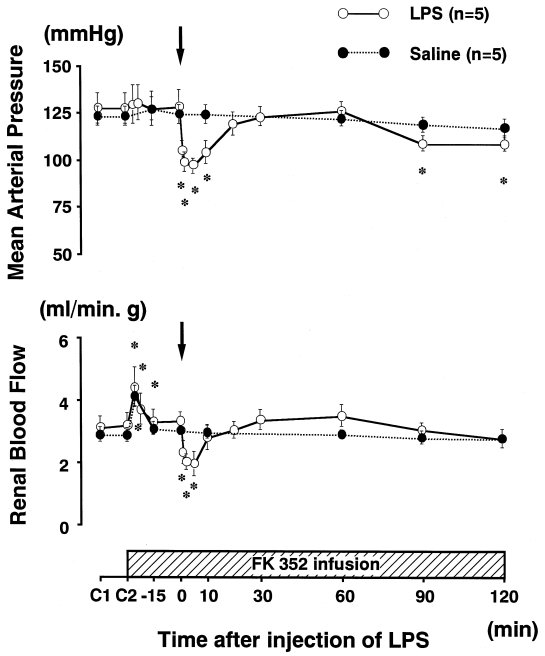

Fig. 4. Changes in the mean arterial pressure and renal blood flow following the intravenous injection of either isotonic saline or LPS during treatment with FK352. C = Control. * Indicates a significant deviation from the C2 ($P < 0.05$).

dency to decrease, however, this change was not statistically significant (Fig. 3).

3.2. Effects of LPS on renal hemodynamics and the renal interstitial concentration of adenosine during treatment with the FK352

Intravenous administration of FK352 (priming dose, 3 mg/kg; sustaining dose, 50 μg/kg/min) did not affect mean arterial pressure but increased renal blood flow transiently (Fig. 4). At 25 min following FK352 administration, LPS was injected intravenously at the same dose (0.5 mg/kg). In the presence of FK352, LPS decreased renal blood flow and mean arterial pressure from 3.34 ± 0.30 and 129 ± 9 to 1.97 ± 40 ml/min g and 98 ± 4 mm Hg, respectively, but these reductions were significantly attenuated in comparison with those in the absence of FK352. At 30 min after the administration of LPS, these parameters returned to the respective preinjection levels. Although renal blood flow and mean arterial pressure showed a tendency to decrease at 90–120 min after the administration of LPS, these changes were also attenuated in comparison with those in the absence of FK352 (Fig. 4). The FK352 treatment did not affect the renal interstitial concentration of adenosine, but LPS caused an increase in adenosine levels from 114 ± 22 to 184 ± 22 nM. This change was significantly attenuated in addition to that of renal blood flow (Fig. 5).

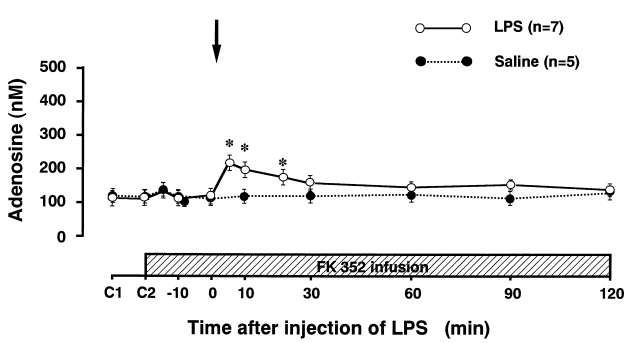

Fig. 5. Changes in the renal interstitial concentration of adenosine following the intravenous injection of either isotonic saline or LPS during treatment with FK352. C = Control. * Indicates a significant deviation from control value ($P < 0.05$).

Table 1
Effects of renal arterial pressure reduction on the renal interstitial concentration of adenosine. All values are mean ± SE ($n = 6$)

Renal arterial pressure (mm Hg)	Renal blood flow (ml/min g)	Interstitial concentration of adenosine (nM)
120 ± 6	3.02 ± 0.41	163 ± 27
76 ± 1	3.15 ± 0.32	176 ± 28
50 ± 1	2.01 ± 0.22*	374 ± 44*

* Indicates significant difference from each value at basal renal arterial pressure.

In five dogs, we performed a time control experiment of this protocol. Intravenous administration of FK352 at the same dose increased renal blood flow transiently, but renal blood flow returned to the control level within 10 min after the FK352 administration and stayed at this level throughout the experiment. FK352 did not affect the renal interstitial concentration of adenosine. The isotonic saline administration of isotonic saline in place of LPS had no effect on mean arterial pressure, renal blood flow or adenosine concentration (Figs. 4 and 5).

3.3. Effects of pressure reduction on the renal interstitial concentration of adenosine

The reduction of renal arterial pressure to the lower limit of autoregulatory range did not affect renal blood flow and the interstitial concentration of adenosine. However, when renal arterial pressure was reduced below the autoregulatory range, the interstitial concentration of adenosine significantly increased along with the reduction of renal blood flow (Table 1).

4. Discussion

An intravenous administration of LPS (0.5 mg/kg) resulted in an immediate decrease in renal blood flow and mean arterial pressure in anesthetized dogs. The renal interstitial concentration of adenosine was also increased significantly. FK352, a highly selective A_1 adenosine re-

ceptor antagonist, significantly attenuated LPS-induced reductions in renal blood flow and mean arterial pressure. The rise in the renal interstitial level of adenosine was also reduced by the administration of FK352 in addition to the attenuation of renal blood flow reduction. These results suggest that intrarenal adenosine was involved in LPS-induced reduction in renal blood flow and that the FK352 had a protective effect against renal dysfunction during endotoxin shock.

The renal hemodynamic changes in the initial phase of endotoxin shock have been well-studied. Administration of a lethal dose of LPS (0.55–2 mg/kg) results in an immediate decrease in renal blood flow and glomerular filtration rate along with a fall in cardiac output and mean arterial pressure in dogs (Hinshaw et al., 1961; Petrucco et al., 1972; Gullichsen 1991). In the present study, intravenous injection of LPS (0.5 mg/kg) resulted in triphasic changes in renal blood flow and mean arterial pressure in anesthetized dogs whereby levels initially fell to a minimum at 5–10 min after the LPS injection. These results are consistent with those of previous publications. In this study, the calculated renal vascular resistance increased significantly, indicating a renal vasoconstriction. Hinshaw and Bradley (1957) reported that the administration of LPS causes renal vasoconstriction prior to a fall in systemic arterial pressure. They suggested that the initial action of LPS on the kidney is probably direct because of the rapid renal vascular response. As shown in micropuncture experiments in rats, LPS causes a constriction of afferent arterioles and a reduction in glomerular hydrostatic pressure (Osswald et al., 1978), suggesting that LPS constricts renal vessels directly. However, the mechanism of LPS-induced renal vasoconstriction is poorly understood.

Adenosine has been suggested to be a possible mediator of renal hemodynamic changes during various forms of acute renal failure (Churchill and Bidani, 1987). Churchill et al. (1987) have demonstrated that the administration of theophylline, a non-selective adenosine receptor antagonist, restores the reduction in the clearance of *para*-aminohippurate and insulin induced by the infusion of LPS (5 mg/kg/h) in rats. They suggested that intrarenal adenosine plays an important role as a mediator of the renal hemodynamic changes associated with the development of LPS-induced acute renal failure. Since it has been demonstrated that adenosine constricts the afferent arterioles via adenosine A_1 receptors (Murray and Churchill, 1985), studies were also carried out using a selective A_1 adenosine receptor antagonist. Knight et al. (1993) reported that the administration of the selective A_1 adenosine receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine, prevents the reductions in renal blood flow and glomerular filtration rate induced by administration of a lethal dose of endotoxin (20 mg/kg), and reduces the severity of acute renal failure during endotoxin shock in anesthetized rats. These results suggest that adenosine production or release in the kidney might be involved in the LPS-induced renal vasoconstriction via activation of adenosine A_1 receptors. However, previously published experiments have been limited to an interpretation of the blockade of the adenosine receptors. Since a direct measurement of intrarenal adenosine has not been carried out, the present experiments were designed to measure the renal interstitial concentration of adenosine using an in vivo microdialysis method. The present data provide the first evidence of the intrarenal dynamics of adenosine during endotoxin shock.

The microdialysis method is well-suited to measure the dynamics of intrarenal adenosine in various conditions because the adenosine receptors are located on the surface of the cell membrane (Murray and Churchill, 1985) and the effects of adenosine on renal hemodynamics and function depend on its concentration in the renal interstitial space (Osswald et al., 1977; Pawlowska et al., 1987). Recently, we were able to minimize tissue injury by making a fiber type probe with a thinner diameter (0.22 mm). In addition, the length of the dialysis membrane is 1.5 cm that is three to four times longer than that of a regular probe. As a result, the dialysis efficiency of the new probe was better than that of a regular probe (He et al., 1995; Nishiyama et al., 1999). We can perfuse our probe at a high perfusion rate (10 μl/min) and shorten the sampling time (5–10 min). Thus, this newly developed microdialysis probe appears to be a useful tool for monitoring the dynamics of adenosine in the renal interstitial space during endotoxin shock.

The basal interstitial concentration of adenosine in the dog's renal cortex was approximately 0.12 μM, which is similar to that observed in previous renal microdialysis studies of the rat kidney (Baranowski and Westenfelder, 1994; Siragy and Linden, 1996; Nishiyama et al., 1997). Twenty minutes after the LPS (0.5 mg/kg) injection, the adenosine concentration increased by about four-fold along with a reduction of renal blood flow. However, since LPS caused a simultaneous fall in arterial pressure and renal blood flow, the reduction of arterial pressure might have stimulated the renal production of adenosine. As tabulated in Table 1, the reduction of renal arterial pressure to the lower limit of the autoregulatory range did not affect renal blood flow or the interstitial concentration of adenosine. However, when renal arterial pressure was reduced below the autoregulatory range, the adenosine concentration increased along with the reduction of renal blood flow. The intrarenal production of adenosine was unrelated to the changes of renal arterial pressure but was stimulated with the reduction of renal blood flow. Thus, the concentration of adenosine was inversely related to the change observed in renal blood flow.

In the presence of FK352, LPS (0.5 mg/kg) also decreased renal blood flow and mean arterial pressure, however, these changes were significantly attenuated in comparison with the reductions in the absence of FK352. At 30 min after the administration of LPS, these parame-

ters returned to their respective preinjection levels. The recovery time was also shortened by the administration of FK352. From these results, it can be suggested that adenosine production or release in the kidney play an important role in the LPS-induced renal vasoconstriction via the activation of adenosine A_1 receptors. Since FK352 has a protective effect against the LPS-induced renal vasoconstriction, the adenosine A_1 receptor antagonist may be useful in preventing the acute renal failure associated with endotoxin shock.

FK352 attenuates the LPS-induced rise in the renal interstitial adenosine concentration. If LPS causes an increase in the adenosine concentration directly, FK352 should have no significant effect on the adenosine concentration even if LPS is administered. In this study, however, FK352 reduced the augmentation of adenosine induced by LPS, which was inversely related to the attenuation of renal blood flow. These results suggest that LPS had no direct effect on intrarenal adenosine production or release, and that adenosine played an important role as a mediator, not as an initiator of LPS-induced renal vasoconstriction. It is known that various vasoactive factors such as the sympathetic nervous system, catecholamine, thromboxane, and the renin–angiotensin system are activated during endotoxin shock (Nykiel and Glaviano, 1961; Isakson et al., 1977; Keeler, 1981; Henrich et al., 1982; Badr et al., 1986). We speculated that the initiation of LPS-induced renal vasoconstriction would be mediated by these factors. Since adenosine is known to be produced in renal cells and is released into the interstitium during renal ischemia (Osswald et al., 1977), the reduction in renal blood flow induced by other factors may cause an increase in the concentration of adenosine in the renal interstitial space. Recently, we investigated the role of adenosine in modulating the renal vasoconstrictor action of angiotensin II and norepinephrine in anesthetized dogs (Aki et al., 1997). The angiotensin II or norepinephrine-induced reduction in renal blood flow was enhanced by an intrarenal administration of adenosine, which, in turn, could be diminished by blocking the adenosine A_1 receptors. In addition, angiotensin II or norepinephrine-induced renal vasoconstriction was significantly attenuated during the inhibition of endogenous adenosine with an FK352. These findings indicate that a relationship exists between angiotensin II, norepinephrine and the adenosine A_1 receptor-mediated renal vasoconstriction. During endotoxin shock, various vasoconstrictors, such as angiotensin II and norepinephrine etc., may initially induce renal ischemia, which may then stimulate the production of adenosine in the kidney. Thus, it is possible that the combination of adenosine and angiotensin II or norepinephrine had synergistic effects on renal vasoconstriction during endotoxin shock. Although other factors were not assessed in the present experiments, this is a possible explanation of why adenosine plays an important role in LPS-induced renal hemodynamic change.

In summary, the present experiment demonstrates that intravenous administration of LPS decreased mean arterial pressure and renal blood flow, and significantly increased the renal interstitial concentration of adenosine. Pretreatment with FK352 significantly attenuated the reductions in renal blood flow and mean arterial pressure induced by LPS. The augmentation of adenosine concentration was significantly reduced in addition to an attenuation of renal blood flow reduction. These results suggest that intrarenal adenosine was involved in LPS-induced reduction in renal blood flow and that FK352 had protective effects against the renal dysfunction during endotoxin shock. Since the adenosine concentration was inversely related to renal blood flow, it might be assumed that adenosine played an important role as a mediator, but not as an initiator of renal dysfunction during endotoxin shock.

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References

Aki, Y., Nishiyama, A., Tomohiro, A., Kimura, S., Abe, Y., 1997. Interaction between adenosine and vasoconstrictors in renal vascular beds. (abstract) Nephrology 3 (Suppl. 1), S46.

Badr, K.F., Kelley, V.E., Rennke, H.G., Brenner, B.M., 1986. Roles for thromboxane A2 and leukotrienes in endotoxin-induced acute renal failure. Kidney Int. 30, 474–480.

Baranowski, R.L., Westenfelder, C., 1994. Estimation of renal interstitial adenosine and purine metabolites by microdialysis. Am. J. Physiol. 267, F174–F182.

Churchill, P.C., Bidani, A.K., 1987. Adenosine mediates hemodynamic changes in renal failure. Med. Hypotheses 8, 275–285.

Churchill, P.C., Bidani, A.K., Schwartz, M.M., 1987. Renal effects of endotoxin in the male rat. Am. J. Physiol. 253, F244–F250.

Groeneveld, A.B.J., Tran, D.D., Meulen, J., Nauta, J.J.P., Thijs, L.G., 1991. Acute renal failure in the medical intensive care unit: predisposing, complicating factors and outcome. Nephron 59, 602–610.

Gullichsen, E., 1991. Renal perfusion and metabolism in experimental endotoxin shock. Acta Chir. Scand., Suppl. 560, 7–31.

He, H., Miura, K., Aki, Y., Kimura, S., Tamaki, T., Iwao, H., Abe, Y., 1995. Adenosine metabolism in the renal interstitial space during ischemia and hypertonic saline infusion. (abstract) Am. J. Hypertens. 8, 156A.

Henrich, W.L., Hamasaki, Y., Said, S.I., Campbell, W.B., Cronin, R.E., 1982. Dissociation of systemic and renal effects in endotoxemia: prostaglandin inhibition uncovers an important role of renal nerves. J. Clin. Invest. 69, 691–699.

Hinshaw, L.B., Bradley, G.M., 1957. Alterations in kidney weight produced by *Escherichia coli* endotoxin. Am. J. Physiol. 189, 329–330.

Hinshaw, L.B., Spink, W.W., Vick, J.A., Mallet, E., Finstad, J., 1961. Effect of endotoxin on kidney function and renal hemodynamics in the dog. Am. J. Physiol. 201, 144–148.

Isakson, P.C., Shofer, F., Mcknight, R.C., Feldhaus, R.A., Raz, A., Needleman, P., 1977. Prostaglandins and the renin–angiotensin system in canine endotoxemia. J. Pharmacol. Exp. Ther. 200, 614–622.

Keeler, R., 1981. Effects of methysergide or phenoxybenzamine on the renal and cardiovascular responses to endotoxin in rats. Circ. Shock 8, 361–368.

Knight, R.J., Bowmer, C.J., Yates, M.S., 1993. Effect of the selective A_1 adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine on acute renal dysfunction induced by *Escherichia coli* endotoxin in rats. J. Pharm. Pharmacol. 45, 979–984.

Kusunoki, T., Terai, T., Akahane, A., Shiokawa, Y., Minoura, H., Kohno, Y., Yoshida, K., Senoh, H., Tanaka, H., 1995. FK352, a novel, water-soluble, A1-selective, adenosine antagonist. (abstract) Jpn. J. Pharmacol. 67 (Suppl. I), 318P.

Levy, E.M., Viscoli, C.M., Horwitz, R.I., 1996. The effect of acute renal failure on mortality: a cohort analysis. JAMA 275, 1489–1494.

Miura, K., Okumura, M., Yukimura, T., Yamamoto, K., 1991. Fluorometric determination of plasma adenosine concentrations using high-performance liquid chromatography. Anal. Biochem. 196, 84–88.

Murray, R.D., Churchill, P.C., 1985. Concentration dependency of the renal vascular and renin secretory responses to adenosine receptor agonists. J. Pharmacol. Exp. Ther. 232, 189–193.

Nishiyama, A., Miyatake, A., Aki, Y., Fukui, T., Kiyomoto, H., Kimura, S., Abe, Y., 1997. Continuous monitoring of the renal interstitial adenosine concentration in conscious rats. (abstract) J. Am. Soc. Nephrol. 8, 351A.

Nishiyama, A., Miyatake, A., Kusudo, K., Syokoji, T., Yue, W., Fukui, T., Aki, Y., Kimura, S., Abe, Y., 1999. Effects of halothane on renal hemodynamics and interstitial nitric oxide in rabbits. Eur. J. Pharmacol. 367, 299–306.

Nykiel, F., Glaviano, V.V., 1961. Adrenal catecholamines in *E. coli* endotoxin shock. J. Appl. Physiol. 16, 348–350.

Osswald, H., Schmitz, H.J., Kemper, R., 1977. Tissue content of adenosine, inosine and hypoxanthine in the rat kidney after ischemia and postischem recirculation. Pfluegers Arch. 371, 45–49.

Osswald, H., Spielman, W.S., Knox, F.G., 1978. Mechanism of adenosine-mediated decrease in glomerular filtration rate in dogs. Circ. Res. 43, 465–469.

Pawlowska, D., Granger, J.P., Knox, F.G., 1987. Effects of adenosine infusion into renal interstitium on renal hemodynamics. Am. J. Physiol. 252, F678–F682.

Petrucco, O., Rao, P., Cavanagh, D., 1972. Endotoxin shock in the dog. Am. J. Obstet. Gynecol. 114, 1060–1065.

Siragy, H.M., Linden, J., 1996. Sodium intake markedly alters renal interstitial fluid adenosine. Hypertension 27, 404–407.

Spielman, W.S., Thompson, C.I., 1982. A proposed role for adenosine in the regulation of renal hemodynamics and renin release. Am. J. Physiol. 242, F423–F435.