Hyporeactivity of Renal Artery to Angiotensin II in Septic Rats

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Abstract

Although reduced vascular reactivity to vasoconstrictors has been well documented, it is not clear whether renal blood flow (RBF) and renal vascular response exhibit the same pattern following sepsis. We examined RBF and renal vascular response during early sepsis. Male Sprague-Dawley rats underwent cecal ligation and puncture (CLP)-induced sepsis. At 5 h after CLP or sham operation, RBF and plasma nitric oxide (NO) concentration were measured. Moreover, angiotensin II (50 ng/kg body weight) was employed to evaluate the renal vascular response (n = 12 rats/group). The results showed that CLP caused higher heart rate (HR), RBF and lower renal vascular resistance (RVR). In addition, plasma nitrite-nitrate (NOx) increased significantly after CLP. After angiotensin II infusion, maximal response in mean blood pressure (MBP), RBF and RVR were less in CLP rats. Thus, we found that CLP induced hyporeactivity of renal artery together with overproduction of NO during an early stage of sepsis.

Key Words: cecal ligation and puncture, sepsis, renal blood flow, vascular response, nitric oxide.

Introduction

Endotoxemia or sepsis causes multiple organ failure, including injury of the kidney (10, 13, 22). In return, renal dysfunction contributes to the progression of septic shock in critically ill patients (13, 22). Although the precise mechanisms underlying the infection-induced impairment of renal function remain to be elucidated, nitric oxide (NO), which is overproduced in sepsis, has been considered to play a pivotal role in pathogenesis (7, 25). In sepsis, endotoxin and inflammatory cytokines also induce expression of inducible nitric oxide synthase (iNOS) protein, leading to excessive production of NO (16).

Furthermore, septic shock is characterized by marked circulatory changes, including high cardiac output, low vascular resistance and severe hypotension. Circulating vasodilators, downregulation of α-adrenoreceptors, and hyporeactivity to vasoactive agonists are believed to be the basis for the hypotension and low vascular resistance (23). The mechanism of endotoxin-induced vascular hyporeactivity appears to involve the induction of NO synthesis in endothelial and smooth muscle cells (17). Evidence that increased NO synthesis is responsible for decreased response to vasoconstrictors has been previously demonstrated in femoral and mesenteric vessels isolated from rats treated with endotoxin (20, 21). The renal microvasculature is known to be sensitive to NO (25).

In the rat model of polymicrobial sepsis (as

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induced by cecal ligation and puncture, CLP), the cardiovascular response includes an early, hyperdynamic phase characterized by increased cardiac output, increased tissue perfusion (26), increased oxygen delivery and oxygen consumption (30), and decreased peripheral resistance (26). It is not known whether renal vascular response is decreased in hyperdynamic stage of sepsis, and if so, whether those effects are related to overproduction of NO. The aim of the present study was to perform a direct measurement of renal blood flow, renal vascular response after angiotensin II infusion, and NO during hyperdynamic stage of sepsis.

Materials and Methods

Materials

Plasma (HAES-steril® 6%) was purchased from Fresenius (Hamburg, Germany). Other drugs and chemicals were all purchased from Sigma (St. Louis, MO, USA).

Animal Preparation and Group Assignment

The current study was approved by the animal care review board of Chang Gung University School of Medicine. Male Sprague-Dawley (SD) rats were obtained from the National Science Council and maintained in the Animal Center of Chang Gung University. The care and handling of the animals were done in accordance with the National Institutes of Health guidelines. Rats (350-400 g) were fasted overnight before the experiment but were allowed water *ad libitum*. The animals were randomly assigned into two groups (sham and CLP; n = 12 in each group). One septic rat was excluded from the analysis because of death during the experiment. Rats in the sham group underwent sham laparotomy, and animals in the CLP group underwent laparotomy followed by CLP. All operations were performed using a sterile technique during general anesthesia with pentobarbital (30 mg/kg, intraperitoneally). The animals were permitted to freely move about and access to water *ad libitum* post-operatively.

Our previously described rat model of CLP-induced sepsis was used in this study (31). The cecum was ligated and punctured twice with a sterile 22-guage needle on the antimesenteric border. Gentle pressure was applied to the cecum until a small amount of feces exuded. Normal saline (30 ml/kg body weight) was given subcutaneously in CLP or sham operation. Feeding was restricted in all groups after surgery to avoid differences in nutrition supplement among the groups because of difference in food intake (15). No analgesics were administered.

Experimental Protocol

At four hours after surgery, the animals were injected with pentobarbital (50 mg/kg, intraperitoneally) and placed on a servo-controlled heated table that maintained body temperature at 37°C. A tracheostomy was performed, and a tracheal catheter (PE-90) was inserted to facilitate free breathing. Right carotid artery and left femoral vein were cannulated with PE-50 tubing for monitoring of mean blood pressure (MBP) or fluid infusion. A constant infusion at a rate 180 μl/kg body weight per minute was given throughout the experiment. This infusion contained 4% plasma in normal saline. This infusion rate was selected to ensure fluid resuscitation needed and recommended in septic conditions (1). Additional doses of pentobarbital were given intravenously as required. Carotid arterial pressure was measured by a pressure transducer driven by a PowerLab amplifier (ADInstruments Inc., Springs, CO, USA). The left kidney was approached by a midline incision extended to the left flank. Furthermore, the left renal artery was gently dissected and isolated from the renal vein for determination of renal blood flow (RBF) using a noncannulating transducer connected to an ultrasonic flowmeter (Transonic system TS106, R1 probe, Ithaca, NY, USA) (1). Bladder was cannulated to ensure free flow of urine. All the above procedures took about 45 min. After completion of surgery, the animals were allowed to stabilize for at least 15 min before measurements commenced. Baseline MBP, heart rate (HR), RBF and renal vascular resistance (RVR) were recorded in sham and CLP groups. Blood sample (100 μl) was withdrawn from right carotid artery *via* a 1-ml syringe for plasma nitrite-nitrate (NOx) detection. Immediately following withdraw of blood sample, 100 μl normal saline was infused *via* catheter of right carotid artery for replacement of blood loss. Ten min later, when MBP, HR, RBF and RVR were returned to baseline, bolus injection of angiotensin II (50 ng/kg body weight) was administered. The dose of angiotensin II was chosen to elevate MBP approximately 30 mmHg in controls (unpublished data). The maximum changes over baseline level in MBP, RBF and RVR (data expressed as %) in both groups were also recorded. At the end of the experiment, the rats were sacrificed by an overdosed pentobarbital.

Measurement of Plasma NOx Concentrations

Calculation of plasma NOx concentrations was described previously (28). The blood samples were immediately centrifuged at 3,000 rpm for 10 min at 4°C. Plasma was then stored at -80°C. Within 2 weeks, plasma samples were thawed and deproteinized by ethanol (95%) at 4°C for 30 min. These samples were subsequently centrifuged for 10 min at 14,000 rpm and the total NOx concentration in plasma was

determined. The amounts of nitrate in the plasma (5 µl) were measured by adding a reducing agent (0.8% VCl_3 in 1 N HCl) to the purge vessel to convert nitrate to NO, which was stripped from the plasma using a helium purge gas. The NO was then drawn into the Nitric Oxide Analyzer (Sievers 280i NOA, Boulder, CO, USA). Nitrate concentrations were calculated by comparison with standard solutions of sodium nitrate (Sigma, St. Louis, MO, USA).

Statistical Analyses

Statistical analyses were performed using a SigmaStat software package. Results are presented as means ± SE. Student's unpaired *t* test was employed for the comparison between CLP and corresponding sham groups. The differences were considered significant at $P < 0.05$.

Results

Alterations in Systemic Hemodynamic Parameters, Renal Blood Flow and Renal Vascular Resistance

As shown in Fig. 1A, there was no significant difference in MBP between sham and septic rats. However, CLP induced a significant (9.5%) increase in HR (391.5 ± 3.7 beats/min in sham-operated animals *vs*. 428.8 ± 4.0 beats/min in septic animals) (Fig. 1B).

RBF also increased significantly (58%) following sepsis (6.2 ± 0.2 ml/min/g tissue in sham group *vs*. 9.8 ± 0.3 ml/min/g tissue in CLP group) and thus RVR decreased significantly (57%) following sepsis (17.7 ± 0.5 mmHg/ml/min/g tissue in sham group *vs*. 11.3 ± 0.3 mmHg/ml/min/g tissue in CLP group) (Fig. 1, C and D).

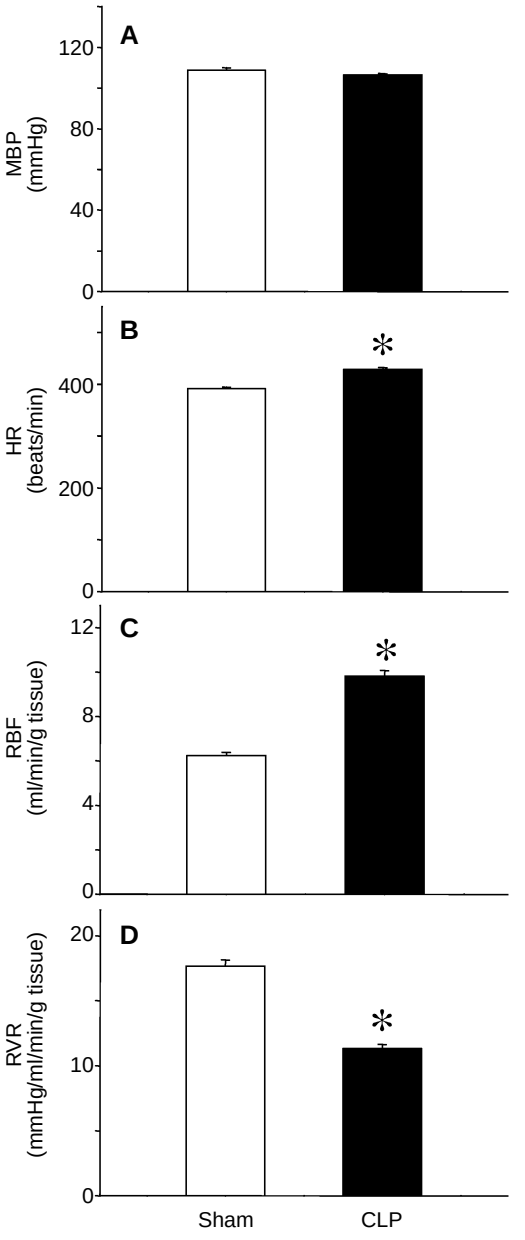

Fig. 1. Alterations in mean blood pressure (MBP, A), heart rate (HR, B), renal blood flow (RBF, C) and renal vascular resistance (RVR, D) at 5 h after CLP or sham operation. There were 12 animals in each group. Data are expressed as means ± SE, and were compared by Student's *t* test. *$P < 0.01$ versus corresponding sham group.

Alterations in Systemic and Renal Response after Angiotensin II Infusion

Angiotensin II infusion (50 ng/kg body weight) caused an increase in MBP and a decrease in RBF, thus an increase in RVR, in both sham (Fig. 2A) and CLP rats (Fig. 2B). Also shown in the typical recordings were the apparently smaller responses to angiotensin II in CLP rats (Fig. 2). Fig. 3 illustrates the mean responses (as % change) induced by angiotensin II in MBP (Δ MBP), RBF (Δ RBF), and RVR (Δ RVR) in both sham and CLP rats. The increases in MBP of CLP rats (11.1 ± 1.2%) following angiotensin II infusion were significantly less than that of the sham rats (25.0 ± 2.2%) (Fig. 3A). The angiotensin II-induced decrease in RBF was also significantly less than that of the sham rats (23.0 ± 2.4% in sham-operated rats and 15.4 ± 2.3% in CLP rats) (Fig. 3B). Thus, although RVR increased in both sham and CLP rats following angiotensin II, vascular resistance increased in sham rats (65.8 ± 4.05%) was significantly more than that of CLP rats (34.8 ± 4.5%) (Fig. 3C).

Plasma NOx Concentration

As shown in Table 1, we found that CLP treatment increased plasma NOx concentration by 77% in comparison with sham treatment.

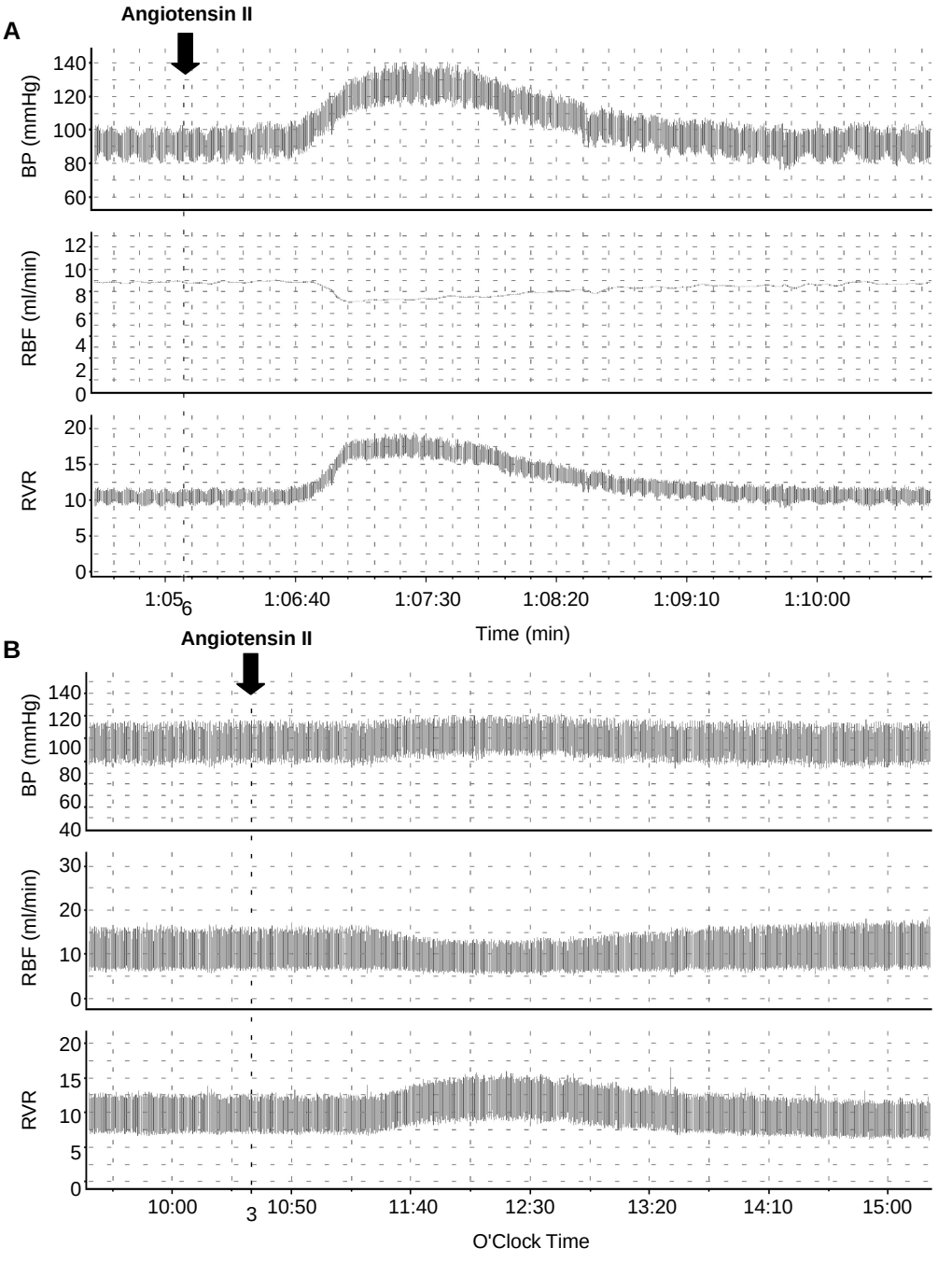

Fig. 2. Original traces of increase in mean blood pressure (MBP) and renal vascular resistance (RVR), and decrease in renal blood flow (RBF) after angiotensin II (50 ng/kg body weight) infusion in sham (A) or CLP (B) operation. Dotted lines mean the time point of angiotensin II infusion. RVR in mmHg/ml/min/g tissue.

Discussion

Renal dysfunction in sepsis is a life-, cost-, and time-consuming disease and future optimal therapy would be advanced by a better understanding of the mechanisms of hemodynamic changes that would ameliorate the outcome of septic patients. In the present study, we showed that renal microcirculation behaved the same as other vascular beds, displaying vascular hyporeactivity after angiotensin II infusion in septic condition.

Renal hemodynamic changes were studied in a standard experimental intraabdominal peritonitis model of sepsis (27, 31). There are two stages, *i.e.* hyperdynamic and hypodynamic, in the progression of this CLP-induced sepsis. To the best of our

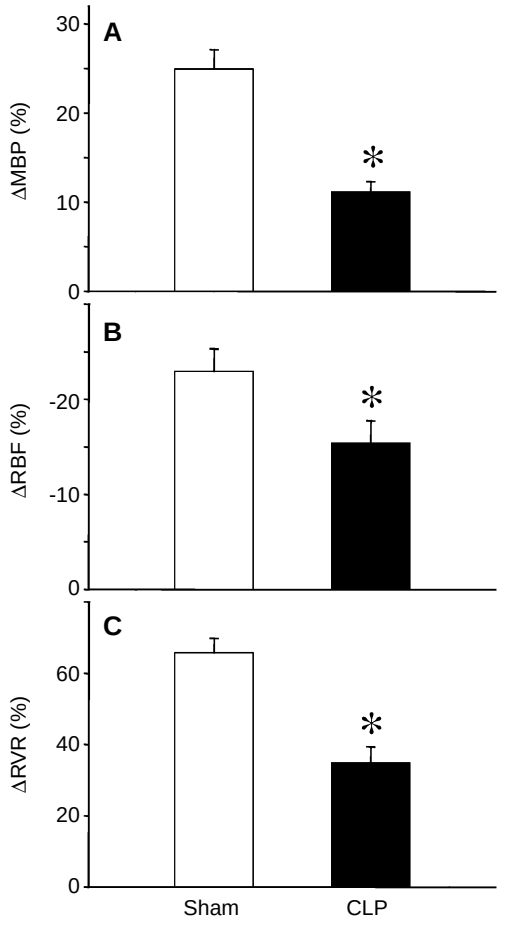

Fig. 3. Maximum increase in mean blood pressure (MBP, A) and renal vascular resistance (RVR, C) and maximal decrease in renal blood flow (RBF, B) (% over baseline) after angiotensin II addition (50 ng/kg body weight) in CLP or sham operation. There were 12 rats in each group. Data are expressed as means ± SE, and were compared by Student's *t* test. **P* < 0.05 versus corresponding sham group.

knowledge, there was no study performed previously to evaluate the renal vascular reactivity in the early, hyperdynamic stage of sepsis. For this reason, we focused on a relatively early phase of sepsis to simulate a short time-window of undiagnosed clinical sepsis. The results showed that hyporeactivity of renal vasculature was present 5 h post-CLP, although MAP was maintained and RBF was increased in this stage. Furthermore, our recent studies have shown that there was a significant increase in pro-inflammatory mediators, such as TNF-α and IL-6, in CLP rats compare with sham rats (unpublished data).

Excessive vasodilatation is one of the characteristics accompanying septic shock. The degree of this vasodilatation can be correlated with the mortality rate of patients suffering from severe sepsis (8). Explanations for this vasodilatation include increased

Table 1. Alterations in plasma nitric oxide (NO) at 5 h after CLP or sham operation.

Sham (n = 12)	CLP (n = 12)
28.4 ± 2.9 μM	50.2 ± 1.4* μM

CLP = cecal ligation and puncture; n = number of animals. Data are expressed as mean ± SE, and were compared by Student's *t* test. **P* < 0.01 versus sham group.

circulating levels of vasodilators, decreased α-adrenoreceptors, and hyporeactivity to vasopressors (23). The responses to vasoactive agonists in regional circulations have not been completely characterized. In renal artery, we found a significant hyporeactivity to the vasopressor angiotensin II in renal vasculature during early sepsis (Figs. 2 and 3). Furthermore, increased NO production (Table 1) was likely involved in this hyporeactivity, since plasma NOx concentration was also increased at 5 h post-CLP. Therefore, the renal circulation appears to take part in the systemic hyporeactivity to vasopressors observed during this stage of sepsis. In addition, NO is thought to cause cell damage by interacting with superoxide anion to form peroxynitrite, a potent oxidizing agent (3). Because the reaction of NO and superoxide anion is essentially diffused (3), increased superoxide anion and NO production could amplify the interaction and of these two molecules and divert NO from its physiologic homeostatic functions. Consistent with these findings, our previous studies have shown that CLP induced significant increase in serum NO, aortic NO, and aortic superoxide production and decrease in aortic NO bioavailability (31).

Vascular hyporeactivity has been described by Julou-Schaeffer *et al*. (11, 12), who showed that the mean arterial pressure increase after norepinephrine injection is significantly reduced in animals previously challenged with endotoxin. *In vitro* studies also demonstrated hyporeactivity to vasopressors in aorta excised from rats injected with endotoxin or normal aorta incubated in organ chambers with endotoxin or cytokines (12, 14). So far vascular hyporeactivity associated with endotoxemia has been studied in the macrocirculation (aorta, femoral or mesenteric arteries) or microcirculation (liver perfused *via* the portal vein) (18). In this study, the hyporeactivity was demonstrated in the whole kidney *via* the renal artery. The vasopressors chosen in this study was angiotensin II because angiotensin II showed significant vascular reactivity in renal and non-renal vascular beds (1). Furthermore, the renal sympathetic nervous system and renin-angiotensin system interact at multiple levels to regulate vascular tone (5, 9), and angiotensin II augments the ability of renal sympathetic nerve stimulation to constrict the renal circulation (5, 6). Administration of angiotensin

II would not only affect renal vasculature but also have effects on systemic vascular beds. Thus, an integrated response was generated and observed in renal vasculature as in any *in vivo* study. For that reason, further study, such as administration of angiotensin II were given locally into renal artery, is needed to reduce the systemic effects of angiotensin II in non-renal vasculature. Our findings are consistent with the previously reported vascular hyporeactive responses to norepinephrine *in vivo* (12) or *in vitro* (12, 24). Additionally, the renal hyporeactivity was observed early. Our understanding of mechanisms that are responsible for this early abnormality in vascular reactivity is limited by a paucity of experimental data. Using a selective iNOS inhibitor, iNOS knockout mice, or global NOS inhibition, it has been clearly shown that iNOS/NO production mediates, if not all, of the weakened responsiveness to constrictor substances (2, 4, 19). Furthermore, measurements during endotoxemic shock reveal a relatively high stimulation of iNOS and NO production in the kidney (7). In accordance with this, we found a similar response of MBP, RBF and RVR in both groups, suggesting a homogeneous stimulated production of NO between renal and non-renal vascular beds. In addition to systemic NO, quantification of renal NO production by measuring NO in the urine would be helpful to support the hypothesis which is a homogenous stimulated production of NO between renal and non-renal vascular beds. Measurement of NO in the urine was, however, not performed and this aspect therefore remains unknown. In view of this, additional studies are needed to completely elucidate the mechanism.

There are two stages (*i.e*. hyperdynamic and hypodynamic) in CLP model. Consistent with our findings, previous studies have shown that there is normotension in 5 h after CLP (29). However, we also found hypotension in 24 h after CLP (unpublished data). It can be argued that systemic overproduction of NO is noted in a normotensive rat. Our previous studies have shown that there is a higher systemic amount of NO in septic rats 24 h after CLP compared with septic rats 5 h after CLP (unpublished data). It is, therefore, possible that the amount of NO is not increased enough and contributed to shock status in the present study. Nonetheless, further studies are needed to evaluate the precise mechanism.

In summary, vascular hyporeactivity to angiotensin II is demonstrated in rat renal artery following 5 h after CLP. These results suggest that rat renal circulation takes part in the systemic vascular hyporeactivity observed after CLP and that NO production is likely involved in the pathogenesis. Additional studies are needed to elucidate this and other mechanisms to improve renal function and overall patient outcome during septic shock.

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