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Effects of chronic, urea-induced osmotic diuresis on kidney weight and function in rats

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Summary To study the effects of chronic osmotic diuresis which were not associated with hyperglycaemia on the rat kidney, osmotic diuresis was induced by i. v. infusion of urea. A 5 mol/l urea solution was continuously infused at a rate of 100 ml · kg^{-1} · day^{-1} on the basis of body weight on day 0. Duration of infusion was 2, 6, 10 or 14 days. Control rats received continuously infused Ringer's solution. Urea-treated groups developed osmotic diuresis (urine flow = about 0.04 ml · min^{-1} · 100 g body weight^{-1}) comparable to that in rats with experimental diabetes mellitus induced by i.v. streptozotocin (55 mg/kg), however urea-induced osmotic diuresis was not associated with blood glucose level increases. Compared with their controls, rats receiving urea for 2–14 days had markedly increased kidney weight. Rats receiving urea for 10 days showed greatest kidney weight increase, 0.565 ± 0.044 g/100 g body weight (mean ± SD), representing a 53 % increase compared with the control (0.369 ± 0.034 g/100 g body weight). Kidney weight was associated with increases in kidney protein content. In contrast, none of control kidney weight values differed significantly from day 0 values (= normal rats; 0.387 ± 0.028 g/100 g body weight). Creatinine clearance values in urea-treated groups were also higher than those in controls. The maximum value, 0.65 ± 0.17 ml · min^{-1} · 100 g body weight^{-1}, was recorded in the 14-day group and was significantly higher than the corresponding control value (0.34 ± 0.07 ml · min^{-1} · 100 g body weight^{-1}) ($p < 0.001$). Urea clearance values were also significantly higher in urea-treated groups than in respective controls. This study suggests that osmotic diuresis may induce renal hypertrophy/hyperplasia and glomerular hyperfiltration immediately after development of diabetes. [Diabetologia (1994) 37: 225–231]

Key words Osmotic diuresis, urea, renal hypertrophy, glomerular hyperfiltration, diabetes, rat.

Renal hypertrophy and glomerular hyperfiltration develop early in the course of diabetic nephropathy [1–3]. Mogensen et al. [4] delineated five stages of diabetic nephropathy in insulin-dependent diabetes mellitus and defined the first phase as the appearance of renal hypertrophy and glomerular hyperfiltration. Studies have confirmed that similar phenomena appear in animal models [5]. Renal hypertrophy and glomerular hyperfiltration are also considered bases of the progression of diabetic nephropathy to the next phase [6]. It is noteworthy that these phenomena appear within a short period of 1.5–4 days after the development of hyperglycaemia [5, 7, 8]. However, it still is not certain whether these conditions are caused by the effects of the glucose itself, other events secondary to hyperglycaemia, or unrelated factors. It is also unknown whether renal hypertrophy precedes [9] or follows [10] glomerular hyperfiltration, and whether the causes of these phenomena are identical.

High blood glucose levels and osmotic diuresis due to hyperglycaemia are the undisputed findings observed immediately after the development of diabetes.

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Abbreviations: DM, Diabetes mellitus; ID, inner diameter; OD, outer diameter.

What is not known, however, is whether the osmotic diuresis has negative effects on the kidney, or whether those effects are due to the hyperglycaemia itself. By inducing osmotic diuresis independent of hyperglycaemia, the effects of osmotic diuresis itself may be observed.

The present study induced continuous osmotic diuresis which was not associated with hyperglycaemia. By observing its effects on the kidney, we hoped to determine how osmotic diuresis is related to the renal hypertrophy and glomerular hyperfiltration that occurs in early diabetes.

Materials and methods

Animals

Normal male Wistar rats (Charles River Japan, Inc., Kanagawa, Japan), weighing from 235 to 305 g were used. Rats were housed individually in metabolic cages and received standard rat chow (Clea Japan Inc., Tokyo, Japan) and tap water ad libitum.

Protocol

The rats were assigned to three groups. One group received a continuous i.v. infusion of 5 mol/l urea solution (Urea group); the second group received an i.v. injection of streptozotocin to induce diabetes (DM group), and the controls received a continuous i.v. infusion of Ringer's solution. A group consisting of normal rats ($n = 7$) served as a day 0 group for each of the three treatments.

In the urea and control groups, a modified version of the method described by Steiger et al. [11] was used for continuous i.v. infusion. Briefly, each rat was placed supine on an operating board and restrained under diethyl ether anaesthesia. Using sterile apparatus and aseptic technique, a longitudinal incision was made over the right supraclavicular region. The right jugular vein was exposed and ligated with 5-0 silk at 0.5–1.0 cm distal to the right clavicle. The vein was bound with another suture above the clavicle and was not ligated until a catheter was inserted through the phlebotomy between the two sutures. The catheter was composed of a 43-cm long polyethylene tube (0.72 mm ID, 1.12 mm OD, No.15; Igarashi Ika Kogyo Co., Ltd., Tokyo, Japan) connected with a 4-cm long polyethylene tube (0.28 mm ID, 0.61 mm OD, Intramedic polyethylene tubing; Becton Dickinson and Co., Franklin Lakes, NJ, USA) and with a 2.5-cm long silicone rubber tube (0.30 mm ID, 0.64 mm OD, Silastic medical grade tubing; Dow Corning Corporation, Midland, Mich., USA). The catheter was positioned in the vein so that the tip of the silicone rubber tube reached either the superior vena cava or the right atrium. The base of the catheter was passed subcutaneously using a metal stylet and emerged out of the midscapular region. The basal side of the catheter was passed through a specially composed metal harness and a stainless steel spring stock welded to the harness, and connected at the lower part with a sterile swivel infusion apparatus (Model 375 series; Instech Laboratories, Philadelphia, Pa., USA). The swivel was suspended just above each metabolic cage and connected at the upper part with silicone rubber tubing (1.3 mm ID, 3.33 mm OD, Model 2030-969; LKB-Produkter AB, Bromma, Sweden) to a continuous infusion pump (Model 2232 LKB Microperpex S peristaltic pump; Pharmacia LKB Biotechnology, Uppsala, Sweden) connected to a sterile bottle for the transfusion solution. The harness was sutured with 2-0 silk to the back of each rat at four points. After these procedures, each rat was able to move without any restraint within the metabolic cage.

The solutions infused were a 5 mol/l urea solution (composed of 300.3 g/l of urea, 1.46 g/l of NaCl, 1.12 g/l of KCl, 1.68 g/l of sodium lactate, and 20.25 g/l of glucose) and the Ringer's solution (composed of 1.46 g/l of NaCl, 1.12 g/l of KCl, 1.68 g/l of sodium lactate, and 20.25 g/l of glucose). These solutions were deaerated and injected into the transfusion bottle through a filter (pore size: 0.45 μm; Millipore Corporation, Bedford, Mass., USA).

The infusion pump was calibrated using a 0.1 ml volumetric pipette so that the infusion rate was 100 ml · kg body weight^{-1} · 24 h^{-1} on the basis of body weight of each rat at day 0. Each rat received continuous i.v. infusion from the calibrated pump.

The duration of infusion was 2, 6, 10 or 14 days. The groups were termed the Urea/2-day group ($n = 7$); Urea/6-day group ($n = 7$); Urea/10-day group ($n = 7$); Urea/14-day group ($n = 6$); Ringer/2-day group ($n = 7$); Ringer/6-day group ($n = 9$); Ringer/10-day group ($n = 9$); and Ringer/14-day group ($n = 8$).

Each rat assigned to the DM group was rendered diabetic by a single i.v. dose of 55 mg/kg of streptozotocin (Sigma Chemical Co., St. Louis, Mo., USA) dissolved in 0.1 mol/l citrate buffer (pH 3.8) and injected into the tail vein. The rats were classified into the DM/2-day group ($n = 6$); DM/6-day group ($n = 8$); DM/10-day group ($n = 6$); and DM/14-day group ($n = 9$), with time periods referring to the number of days, at the completion of the experiment, since the initial i.v. injection of streptozotocin. Rats with a negative glucose urine test 24 h post-streptozotocin injection and those with a blood glucose level of no more than 17 mmol/l at the completion of the experiment were excluded from the analysis.

A 24-h urine sample was collected beginning 24-h prior to the scheduled time of completion of the experiment. Each rat from the continuous infusion groups underwent laparotomy under pentobarbital anaesthesia (40 mg/kg, i.p.), and a blood sample was taken at the bifurcation of the abdominal aorta. Rats were killed by exsanguination, and both kidneys and the liver were immediately isolated. The kidneys were cleaned and the liver freed from the attached vasculature before being weighed.

The protein content in the left kidney was determined in each rat in the day 0 group (normal rats), the Urea/14-day group, the Ringer/14-day group, and the DM/14-day group. The Lowry method [12] with bovine albumin as a standard was used to measure protein content.

Kidney function was assessed on the basis of the 24-h urine samples. Endogenous creatinine clearance and urea clearance were measured. Qualitative testing for urine sugar was performed using Testape (Eli Lilly and Co., Indianapolis, Ind., USA).

The blood glucose level was measured with the Dextrometer II (Ames, Miles Laboratories, Inc., Elkhart, Ind., USA) and Dextrostix (Ames, Miles-Sankyo Co., Ltd., Tokyo, Japan). All values were obtained with whole arterial blood samples collected during non-fasting periods (from 11.00 hours to 14.00 hours).

Serum blood urea nitrogen and creatinine as well as urine urea nitrogen and creatinine were determined using an autoanalyzer (Model 736; Hitachi, Ltd., Tokyo, Japan).

Plasma osmotic pressure was determined by the cryoscopic method with an automatic osmometer (Model OSA-21; Nikkiso Co., Ltd., Tokyo, Japan).

Statistical analysis

Statistical analyses were performed using Student's t-test and paired Student's t-test. The difference was regarded as statistically significant if the p value was not more than 0.05. All data were expressed as the mean ± SD.

Results

Body weight

Body weights in the three experimental groups are shown in Table 1. In control groups, with the exception of the 6-day group, the mean body weight values increased from baseline; weight increase was greater in rats treated for longer durations with Ringer solution. On the other hand, the Urea/2-day group and DM/2-day group showed slight decreases in mean body weight from their respective baseline values (the decrease in each of these groups was within 4% of baseline). Since there were great differences among the groups in terms of body weight at the completion of the experiment, some of the data obtained were corrected for body weight (converted in terms of 100 g body weight).

Blood glucose levels

Blood glucose levels recorded in all interval groups are shown in Figure 1 A. All DM groups (from the 2-day to the 14-day groups) had significantly higher glucose levels than those of the day 0 group. No significant differences were observed between the Ringer and the urea groups. Neither the Ringer nor urea groups showed any significant changes compared with the day 0 group.

Blood urea nitrogen

The blood urea nitrogen values recorded in all interval groups are shown in Figure 1 B. All urea groups had significantly higher values than those of the respective controls, and showed continuing azotaemia. None of the Ringer groups showed any significant changes compared with the day 0 group. The DM/6-day, DM/10-day and DM/14-day groups showed significantly higher values than that in the day 0 group, but the differences were small.

Serum creatinine

No serum creatinine values in any of the three treatment/four interval groups showed any changes compared with the day 0 group (Table 2).

Table 1. Body weight in the three treatment groups (2, 6, 10, 14 days)

Group	Day	n	Body weight (g)	
			Before treatment (= day 0)	After treatment
Normal	0	7	279.6 ± 15.0	–
Ringer	2	7	256.0 ± 10.0	267.0 ± 12.6[a]
	6	9	255.1 ± 18.6	265.7 ± 32.5
	10	9	254.0 ± 14.9	316.4 ± 20.1[a]
	14	8	260.0 ± 10.4	348.5 ± 20.3[a]
Urea	2	7	273.2 ± 23.5	262.7 ± 25.3[a]
	6	7	266.9 ± 16.0	260.4 ± 16.7
	10	7	251.0 ± 11.1	247.1 ± 27.9
	14	6	274.2 ± 6.1	297.3 ± 18.9[a]
DM	2	6	260.3 ± 21.2	251.0 ± 18.3[a]
	6	8	264.9 ± 15.8	256.6 ± 24.1
	10	6	253.3 ± 11.1	298.2 ± 23.9[a]
	14	9	274.2 ± 15.2	301.1 ± 19.9[a]

Values are mean ± SD.
[a] $p < 0.05$ vs before each treatment

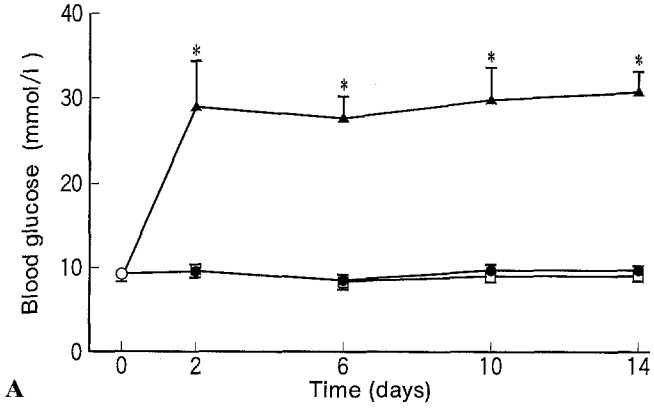
Table 2. Serum creatinine and plasma osmolality in the three treatment groups (2, 6, 10, 14 days)

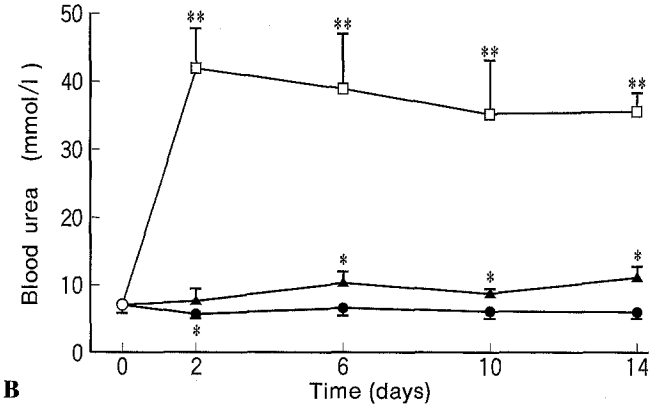
Group	Day	n	Serum creatinine (μmol/l)	Plasma osmolality (mosmol/kg)
Normal	0	7	45.1 ± 3.5	302.7 ± 7.2
Ringer	2	7	45.1 ± 3.5	296.9 ± 5.0
	6	9	46.0 ± 6.2	307.0 ± 9.7
	10	9	43.4 ± 5.3	302.6 ± 5.9
	14	8	47.8 ± 4.4	306.9 ± 8.2
Urea	2	7	41.6 ± 7.1	351.3 ± 4.8[a]
	6	7	50.4 ± 9.7	343.2 ± 10.3[a]
	10	7	41.6 ± 9.7	357.4 ± 12.1[a]
	14	6	39.8 ± 15.9	332.8 ± 5.8[a]
DM	2	6	50.4 ± 7.1	312.0 ± 6.5[b]
	6	8	48.7 ± 7.1	325.9 ± 6.0[b]
	10	6	46.0 ± 3.5	319.2 ± 7.1[b]
	14	9	49.6 ± 4.4	320.0 ± 2.9[b]

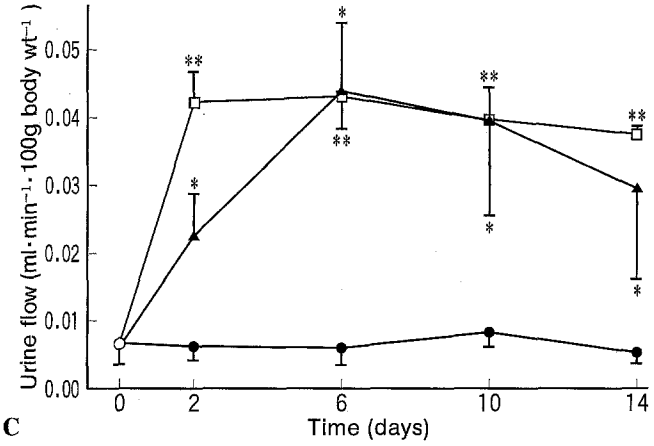
Values are mean ± SD.
[a] $p < 0.05$ vs Ringer group of respective days;
[b] $p < 0.05$ vs normal group (day 0 group)

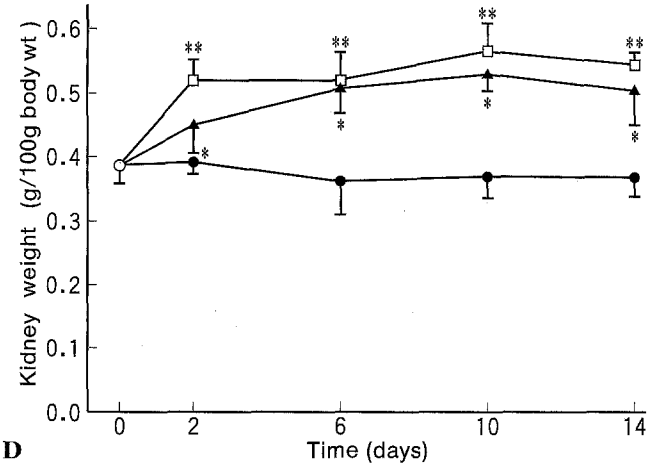
Urine flow

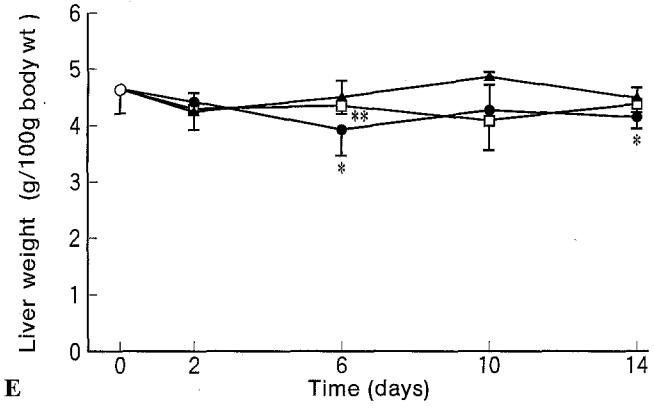
The urine flow values in all urea, Ringer and DM groups are shown in Figure 1 C. The urine flow values for the day 0 group were obtained from normal rats. There were no significant differences in urine flow rates between any of the Ringer groups and the day 0 group. In all urea groups, the urine volume began to show a marked increase immediately after the start of the experiment; significantly higher urine flow values than those of the controls (values in the Ringer groups) were maintained throughout the experimental period. Compared with the day 0 group, all DM groups

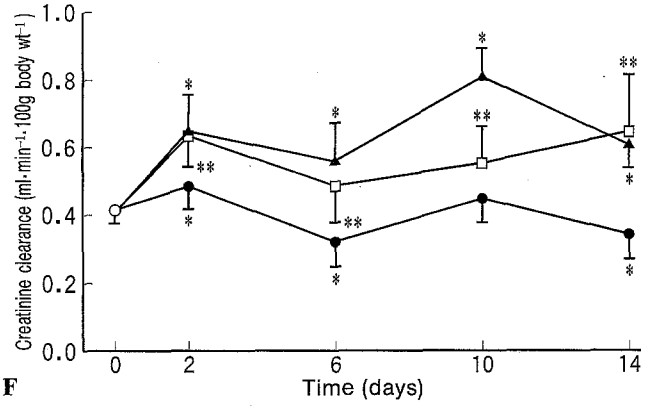












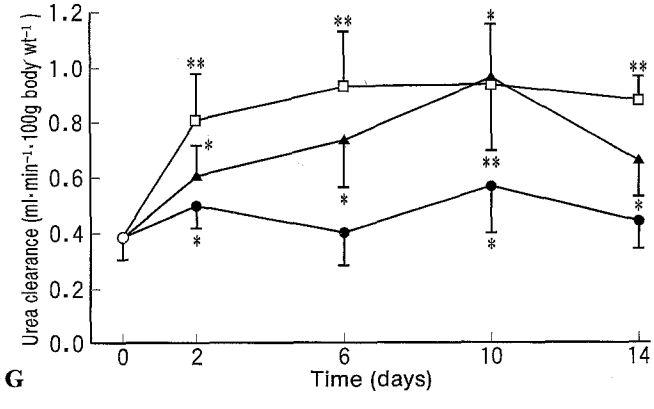


Fig. 1 A–G. Changes in **A** relative blood glucose, **B** blood urea nitrogen, **C** relative urine flow, **D** relative kidney weight (averages of wet weights of the left and right kidney relative to 100 g body weight), **E** relative liver weights, **F** relative creatinine clearance, **G** relative urea clearance, in the three treatment groups (2, 6, 10, 14 days). ○, normal group (day 0, $n = 7$); □, urea group ($n = 7, 7, 7, 6$); ●, Ringer group ($n = 7, 9, 9, 8$); ▲, DM group ($n = 6, 8, 6, 9$). * $p < 0.05$ vs normal group, ** $p < 0.05$ vs Ringer group of respective days

also showed significantly higher urine flow values, which were very similar to those observed in the urea groups.

Kidney weight

Changes in kidney weight are shown in Figure 1 D. These values are relative kidney weights (averages of wet weights of the left and the right kidney relative to 100 g body weight). All the urea groups had markedly higher values than those found in their respective controls (Ringer groups) at the same time periods. The maximum increase in mean kidney weight in the urea group was 53 % which was observed in the Urea/10-day group (0.565 ± 0.044 g/100 g body weight) compared with the Ringer/10-day group (0.369 ± 0.034 g/100 g body weight). All DM groups, from the 2-day (0.449 ± 0.043 g/100 g body weight) to the 14-day (0.504 ± 0.054 g/100 g body weight) groups, had higher values than those in the day 0 group (0.387 ± 0.028 g/100 g body weight). None of the Ringer-treated groups showed any significant changes compared with the day 0 group.

Liver weight

The wet weight of another parenchymatous organ, the liver was taken for each rat, and the values are shown in Figure 1 E. The mean weight in the Ringer/6-day group was significantly lower than that in the day 0 group, although the three remaining Ringer-treated groups had values similar to those in the day 0 group.

Creatinine and urea clearance

Changes in kidney function are shown in Figures 1 F and G. Each group exhibited significant changes in the 24-h endogenous creatinine clearance value compared with the day 0 group. All the DM groups had values significantly higher than those in the day 0 group (0.41 ± 0.04 ml · min^{-1} · 100 g body weight^{-1}). The value in the DM/10-day group (0.81 ± 0.09 ml · min^{-1} · 100 g body weight^{-1}) was the highest among them. All the urea groups had values significantly higher than those of their respective controls, with the value in the 14-day group being the highest (0.65 ± 0.17 ml · min^{-1} · 100 g body weight^{-1}). Some of the values in the Ringer-treated groups showed changes when compared to those in the day 0 group (0.41 ± 0.04 ml · min^{-1} · 100 g body weight^{-1}), but no definite trend was noted.

The urea clearance values obtained from the 24-h urine samples remained markedly higher in the urea groups than in the respective controls (Ringer-treated groups) throughout the experimental period (Fig. 1 G). The 10-day group had the highest urea clearance value (0.94 ± 0.24 ml · min^{-1} · 100 g body weight^{-1}). All the DM groups (from the 2-day to the 14-day groups) showed significantly higher values than those in the day 0 group. The 10-day group had the highest value (0.96 ± 0.19 ml · min^{-1} · 100 g body weight^{-1}). Some of the values in the Ringer-treated groups demonstrated changes when compared to those in the day 0 group (0.39 ± 0.08 ml · min^{-1} · 100 g body weight^{-1}), but no definite trend was seen.

Table 3. Renal total protein (left kidney) in the three treatment groups

Group	*n*	Renal total protein (mg/100 g body weight)
Normal	7	63.5 ± 5.4
Ringer/14 day	8	62.6 ± 4.9
Urea/14 day	6	84.4 ± 5.6[a]
DM/14 day	9	75.6 ± 5.4[b]

Values are mean ± SD.
[a] $p < 0.05$ vs Ringer/14 day group;
[b] $p < 0.05$ vs normal group (day 0 group)

Renal protein

Data on the protein content in the left kidney obtained from the day 0 group (normal rat group) and from the 14-day groups for each of the three treatments are shown in Table 3. The value in the Ringer/14-day group was not significantly different from that in the day 0 group, while the value in the Urea/14-day group was significantly higher than that in the Ringer group. The value in the DM/14-day group was significantly higher than that in the day 0 group.

Plasma osmolality

Changes in the osmotic pressure of plasma separated from arterial blood are shown in Table 2. All the urea groups (from the 2-day to the 14-day groups) had values significantly higher than those in the respective controls. All the DM groups also had values significantly higher than those in the day 0 group, but the values in the urea groups were higher than those in the DM group. No values in any of the Ringer groups showed any significant changes compared with the day 0 group.

Discussion

To study the effects of continuous osmotic diuresis which were not associated with hyperglycaemia on the rat kidney, we infused male Wistar rats with a hypertonic urea solution. Brown et al. [13] suggested that the effects of osmotic diuresis observed in diabetic nephropathy should be studied in isolation from hypergly-

caemia, using a simple model that excludes other variables. For example, mannitol injections have been used to experimentally induce osmotic diuresis in normal rats [14]. However, osmotic diuresis associated with hyperglycaemia has been shown to be markedly different from osmotic diuresis induced by mannitol or a hypertonic sodium chloride solution [15–17]. The increase in plasma osmotic pressure due to hyperglycaemia is unlikely to cause an effective osmotic stimulus at osmoreceptors. Thus, the osmotic diuresis associated with hyperglycaemia does not stimulate vasopressin secretion as does the osmotic diuresis induced by mannitol [15–17]. Urea is an endogenous, slightly toxic substance which is also a final metabolite in urea-excreting animals such as mammals. When used to induce osmotic diuresis, urea does not cause vasopressin secretion, leading several researchers to suggest that urea-induced osmotic diuresis is similar to the osmotic diuresis caused by glucose in the diabetic state [15–17]. Because of this similarity, urea was used to induce osmotic diuresis in this study.

All previous studies using urea to induce osmotic pressure [18, 19] addressed the effects of acute osmotic diuresis; to the best of our knowledge, this is the first investigation on the effects of continuous urea-induced osmotic diuresis.

It has also been pointed out that different kinds of protein, even when administered in similar amounts, cause different degrees of renal hypertrophy and glomerular hyperfiltration. The cause for these differences has not been explained [20, 21]. It has also been reported that different kinds of protein cause similar increases in the excretion of urea [22]. These studies suggest that nitrogen loading or increased excretion of urea itself is an unlikely cause of renal hypertrophy or glomerular hyperfiltration. Therefore, the effects of exogenous urea observed in this experiment may not have been due to nitrogen loading or increased urea excretion, but the result of osmotic diuresis.

The urea groups also showed a marked increase in plasma osmotic pressure. Thus, we think it reasonable to conclude that osmotic diuresis and a hyperosmotic state can result from urea administration. These two phenomena are difficult to evaluate independently because they are seemingly inseparable.

In this experiment, continuous administration of a large dose of urea (30.03 g · kg body weight^{-1} · day^{-1}) induced osmotic diuresis and produced a urine flow rate (about 0.04 ml · min^{-1} · 100 g body weight^{-1}) comparable to that observed in rats with diabetes (induced by 55 mg/kg of streptozotocin, i.v.).

Forced continuous osmotic diuresis induced by urea caused a marked increase in kidney weight and glomerular hyperfiltration as early as experiment day 2. These increased levels were maintained throughout the 14-day experimental period. This pattern was very similar to the pattern of renal hypertrophy and glomerular hyperfiltration observed in the DM groups. Hyperfiltration in clinical diabetes has been attributed partly to acute and rapidly reversible haemodynamic changes and partly to renal hypertrophy. Our study considered only the mechanisms responsible for renal hypertrophy.

The relative liver weight (relative to 100 g body weight) remained almost constant during the experimental period in all three treatment groups, indicating that dehydration was not a factor in body weight changes. Thus, the increase in relative kidney weight (relative to 100 g body weight) observed in the study cannot be attributed to dehydration.

Because of the simultaneous increase in the protein content in the kidney, the increase in the relative kidney weight may not have been due to fluid accumulation, but to renal hypertrophy/hyperplasia. These results suggest that osmotic diuresis may play a major independent role in the pathogenesis of renal hypertrophy and glomerular hyperfiltration observed early in the development of diabetes.

This experiment does not explain the mechanism by which osmotic diuresis causes renal hypertrophy and glomerular hyperfiltration. Nor does it determine which of these two phenomena appeared first, because administration of urea resulted in the simultaneous occurrence of both. Furthermore, our study does not rule out the possibility of other factors besides osmotic diuresis as a causative factor of these phenomena. However, the study does suggest that a single factor, osmotic diuresis, may induce these two phenomena. The effects of osmotic diuresis on the kidney are worthy of further investigation.

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