seeds. We would also like to acknowledge the able technical support of Ms C. Dushin, Mr E.L. Bowman, Dr C.C. Gagne, Mr R.F. Borysewicz and Dr P. Mowery for his assistance in obtaining and interpreting the carbon-13 NMR spectra.

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0014-4754/86/060611-03$1.50 + 0.20/0

Renal effects of the inhibitor of thromboxane A_2-synthetase OKY-046[1]

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Summary. Acute renal failure (ARF) was associated with increased urinary thromboxane (TXA_2) excretion and lessened excretion of sodium ($U_{Na}V$) and fractional excretion of sodium (FE_{Na}%). The inhibitor of thromboxane A_2-synthetase OKY-046 enhanced sodium excretion and fractional excretion of sodium in normal and saline loaded animals whereas it partially prevented the reduction in sodium excretion and creatinine clearance and significantly increased fractional excretion of sodium in glycerol treated rats suggesting a partial protection against the development of acute renal failure.
Key words. OKY-046; thromboxane A_2; prostaglandin E_2; prostaglandin I_2; sodium excretion; volume expansion; acute renal failure.

The role of prostaglandins in sodium excretion and in the development of ARF has been widely investigated, since the prostaglandin system has been found to be involved in the regulation of renal hemodynamics[3]. In particular, it was observed that both infusion and increased biosynthesis of vasodilator prostaglandins, PGE_1, PGE_2 and PGI_2 cause enhanced sodium excretion by increasing renal hemodynamics and by inhibiting the tubular reabsorption of sodium[4-11].

In the case of glycerol-induced ARF, it has been shown that, in its early phase, ARF is accompanied by increased renal vascular resistance and diminished renal plasma flow, glomerular filtration rate and sodium excretion[12-15]. The mechanism responsible for these hemodynamic changes is not yet clearly established. the role of catecholamines has been found to be disputable[14,16], and that of the renin-angiotensin system remains controversial[17]. On the other hand, the prostaglandin system seems to be implicated in the early phase of ARF, as is shown by the following findings: a) chronic use of prostaglandin-synthesis inhibitors leads to the development of ARF[18,19], b) PGE_1, PGE_2 and PGI_2 infusions protect rats against ARF[13,20-23] and c) TXA_2 biosynthesis was found to increase during glycerol-induced ARF[24,25].

However, the role of TXA_2 in sodium excretion or in ARF has not been investigated. TXA_2 is a vasoconstrictor[26] and platelet aggregating agent[27], and thus it has physiological action opposite to PGE_1, PGE_2 and PGI_2. It was thus plausible that TXA_2 also plays an opposite role in the case of sodium excretion and ARF development. In order to examine this hypothesis, we used a newly synthetized selective inhibitor of TXA_2-synthetase, OKY-046 (Ono and Kissei Pharm. Co Osaka, Japan)[28].

Our results suggest that TXA_2 has an antinatriuretic action and is implicated in the early phase of glycerol-induced ARF.

Material and methods. All studies were carried out with female Wistar rats weighing 220–235 g. Tap water and standard rat chow were available ad libitum till the day of the experiment. The room temperature at which the animals were maintained was between 22–25 °C and humidity was 35–40%. The animals were randomly allocated to 9 groups; each group contained 9 rats.

Group 1 (Normal Rats) (NR). One hour before the beginning of the experiment the animals were injected i.p. with 1 ml/kg isotonic saline.

Group 2 (NR+OKY-046 2.5 mg/kg). One hour before the beginning of the experiment the animals were injected i.p. with 2.5 mg (10 µmol)/kg OKY-046 dissolved in 1 ml isotonic saline.

Group 3 (NR+OKY-046 25 mg/kg). One hour before the beginning of the experiment the animals were injected i.p. with 25 mg (100 µmol)/kg OKY-046 dissolved in 1 ml isotonic saline.

Group 4 (Volume expanded rats) (VE). One hour before volume expansion with 75 ml/kg isotonic saline s.c., the animals received i.p. 1 ml/kg isotonic saline.

Group 5 (VE+OKY-046 2.5 mg/kg). One hour before volume expansion as in group 4, the animals received i.p. 2.5 mg (10 µmol)/kg OKY-046 dissolved in 1 ml isotonic saline.

Group 6 (VE+OKY-046 25 mg/kg). One hour before volume expansion as in group 4, the animals received i.p. 25 mg (100 µmol)/kg OKY-046 dissolved in 1 ml isotonic saline.

Group 7 (Glycerol treated animals). One hour before the s.c. injection of 10 ml/kg, 50% v/v glycerol in isotonic saline, the animals received i.p. 1 ml/kg isotonic saline.

Group 8 (Glycerol+OKY-046 2.5 mg/kg). One hour before the s.c. injection of glycerol as in group 7, the animals received i.p. 2.5 mg (10 µmol)/kg OKY-046 dissolved in 1 ml isotonic saline.

Group 9 (Glycerol+OKY-046 25 mg/kg). One hour before the s.c. injection of glycerol as in group 7, the animals received i.p. 25 mg (100 µmol)/kg OKY-046 dissolved in 1 ml isotonic saline.

Intraperitoneal injections were carried out one hour before subcutaneous injections, in order to allow OKY-046 to exercise its inhibitory action. Two different doses of the inhibitor were used. In all cases, 6-h urine collections were made, using individual metabolic cages. The animals then were anesthetized and 3 ml of blood were collected from a femoral artery.

The following parameters were measured: 1) Urinary and plasma creatinine concentrations by a method using Fuller's earth in order to eliminate chromogens. 2) Urinary and plasma sodium concentrations by flame photometry. 3) Urinary thromboxane B_2 (TXB_2), 6ketoprostaglandin $F_{1\alpha}$ (6ketoPGF$_{1\alpha}$) (the stable metabolites of TXA_2 and PGI_2 respectively) and PGE_2 by radioimmunoassay.

Clearance of creatinine (C_{Cr}), sodium excretion rate ($U_{Na}V$), and fractional excretion of sodium (FE_{Na}%) were calculated as usual. C_{Cr} was utilized to represent glomerular filtration rate (GFR).

Analytical methods. Prostaglandins and TXB_2 were measured directly in the dilute urine using specific antibodies. Lyophilized Anti-TXB_2 and Anti-PGE_2 antibodies were provided by Institut Pasteur, Paris, and Anti-6ketoPGF$_{1\alpha}$ antibodies by Dr. Hornych, Hôpital Broussais, Paris. Tritium-labeled PGE_2 (160 Ci/mmol), 6ketoPGF$_{1\alpha}$ (120 Ci/mmol) and TXB_2 (163 Ci/mmol) were provided by New England Nuclear, Boston Massachusetts. The samples were incubated overnight with antibodies and tritiated prostaglandins diluted in 50 mM phosphate buffer (pH 6.8 for 6ketoPGF$_{1\alpha}$ and TXB_2, and pH 7.4 for PGE_2) containing 0.1% gelatin, at 4°C. Separation of bound from free prostaglandins was accomplished by adding 1% dextran coated charcoal suspension in 10 mM phosphate buffer (Dextran T 70 Pharmacia, Charcoal activated Norit) and incubating at 0°C for 10 min. The assay tubes were then centrifuged at 4000 rpm, at 0°C for 15 min. The supernatant was decanted into scintillant fluid (Scintillator 299 TM Packard) and counted in a Packard Tri-Carb Scintillation spectrometer, model 3380. Blanks for each urine sample (without antibodies) were also processed as described above. All measurements were made in duplicate.

The amount of radiolabeled ligand added in each sample was 18.4 pg for H^3-TXB_2, 23.2 pg for H^3-PGE_2, and 32.5 pg for H^3-6ketoPGF$_{1\alpha}$ (about 3100–3600 cpm). The total binding was found to be 33% for TXB_2, 40% for PGE_2 and 30% for 6ketoPGF$_{1\alpha}$. The amount of unlabeled (cold) prostaglandin required to displace 50% of the bound tracer was 34 pg for TXB_2, 17 pg for PGE_2 and 90 pg for 6ketoPGF$_{1\alpha}$. Measurements were restricted to the interval corresponding to 20%–90% displacement of bound tracer. The counted radioactivity for each blank urine sample was less than 4% of total radioactivity (120–180 cpm).

We, as well as other investigators[29–31], chose to measure prostaglandins (PGs) without any prior extraction, in order to avoid drawbacks such as the introduction of unspecific interfering factors (solvent impurities, leakage from columns, impurities in gas used for evaporation) and the pitfalls in estimation of recovery[29].

Results and Discussion. In this study we used the early (pre-renal) phase of acute failure (ARF) induced with glycerol to investigate the role of the endogenous TXA_2 in sodium excretion and the development of acute renal failure. As is well known, the early phase of acute renal failure is accompanied by increased renal vascular resistance and diminished renal plasma flow and glomerular filtration rate, sodium excretion and fractional excretion of sodium (FE_{Na}%)[12–15]; volume expansion with intravenous fluids may restore GFR and RBF[13,15]. On the contrary, after 12 or more hours of glycerol injection FE_{Na}(%) has usually risen and at this point volume expansion does not restore GFR[15]. Finally, the role of the endogenous TXA_2 in sodium excretion has also been investigated in normal and saline loaded rats and the results were compared to those obtained in glycerol treated animals.

a) *Inhibition of TXA_2 biosynthesis in normal rats (table, group 1, 2, 3).* The administration of the selective inhibitor of TXA_2 biosynthesis, OKY-046, to normal rats significantly diminished urinary excretion of TXB_2. This decrease of TXB_2 excretion was associated with increased excretion of sodium ($U_{Na}V$) and fractional excretion of sodium (FE_{Na}%) without any significant change in creatinine clearance (C_{Cr}) and urinary excretion of PGE_2 and 6ketoPGF$_{1\alpha}$. Thus the observed increase in sodium excretion could be related to the diminished release of TXA_2.

b) *Role of volume expansion in PGE_2, PGI_2 TXA_2 release and in renal function (table, group 1, 4).* Volume expansion of the animals with isotonic saline significantly augmented urinary PGE_2 and 6ketoPGF$_{1\alpha}$ excretion[13,14,32–37]. These increases were accompanied by enhanced urine flow (V), sodium excretion rate, creatinine clearance and fractional excretion of sodium, while urinary TXB_2 (TXA_2) excretion was not affected. Thus the increase in sodium excretion could be related to the enhanced release of natriuretic-vasodilator[3–11] PGE_2 and PGI_2, because a) infusion of these PGs augments sodium excretion by increasing renal plasma flow (RPF) and mainly non-cortical plasma flow (NCPF)[3–7,10] and b) TXA_2 release was not affected.

The increase in fractional excretion of sodium suggests that a fraction of sodium excretion was dissociated from creatinine clearance. Since it has been established that PGs augment sodium excretion by directly inhibiting sodium reabsorption[11], the increase in fractional excretion of sodium observed could be related to the enhanced release of PGE_2 and 6ketoPGF$_1$ (PGI_2).

c) Inhibition of TXA_2 biosynthesis in saline loaded animals (table, group 4, 5, 6). The administration of the inhibitor in saline loaded rats, as in normal animals, significantly decreased urinary excretion of TXB_2. This decrease in TXB_2 excretion was again associated with increased sodium excretion and fractional excretion of sodium without significant changes in creatinine clearance and PGE_2 and 6ketoPGF$_{1\alpha}$ release. Thus one could suggest that the enhanced excretion of sodium was related to the decreased release of TXA_2.

d) Role of glycerol in PGE_2, PGI_2, TXA_2 release and in renal function (table, group 1, 7). The administration of glycerol in the animals dramatically diminished creatinine clearance[12–15], sodium excretion (suggesting the development of acute renal failure (ARF) in the animals) and fractional excretion of sodium (suggesting that a fraction of the decreased excretion of sodium was independent of creatinine clearance). These decreases were accompanied by significant increases in urinary TXB_2, PGE_2 and 6ketoPGF$_{1\alpha}$ excretions. Since TXA_2 is a potent vasoconstrictor agent, it could be implicated in the development of ARF in the animals. Another important observation is the decrease in fractional excretion of sodium following glycerol administration and the simultaneous increase in urinary excretion of TXB_2. If TXA_2 is related with this diminution in fractional excretion of sodium, then TXA_2 must be a potent antinatriuretic factor which decreases sodium excretion not only by diminishing the

The effect of OKY-046 in normal, saline loaded and glycerol treated animals. Values are means ± SEM

	Group 1 Normal rats	Group 2 OKY-046 10 μmol/kg	Group 3 OKY-046 100 μmol/kg	Group 4 Volume expansion	Group 5 Volume expansion OKY-046 10 μmol/kg	Group 6 Volume expansion OKY-046 100 μmol/kg	Group 7 Glycerol	Group 8 Glycerol OKY-046 10 μmol/kg	Group 9 Glycerol OKY-046 100 μmol/kg
PGE_2	168 ± 18	214 ± 23	194 ± 30	$404 \pm 83^{1+}$	425 ± 40	465 ± 100	$406 \pm 85^{1+}$	563 ± 98	542 ± 79
6kPGF$_{1\alpha}$	114 ± 15	146 ± 25	141 ± 8	$170 \pm 18^{1+}$	228 ± 39	239 ± 36	$240 \pm 22^{2+}$	212 ± 23	241 ± 30
TXB_2	102 ± 14	$52 \pm 6^{2+}$	$48 \pm 7^{2+}$	131 ± 14	47 ± 5^{3+}	$48 \pm 8^{3+}$	$175 \pm 19^{2+}$	$48 \pm 8^{3+}$	$46 \pm 4^{3+}$
V	18.6 ± 2.5	21.7 ± 3.9	19.2 ± 3.6	$72.5 \pm 12.8^{3+}$	58.9 ± 10.3	76.1 ± 8.9	$43.9 \pm 4.7^{3+}$	46.1 ± 2.5	49.7 ± 4.7
$U_{Na}V$	1.95 ± 0.10	$2.31 \pm 0.11^{1+}$	$2.38 \pm 0.11^{2+}$	$9.50 \pm 0.72^{3+}$	$12.25 \pm 0.78^{1+}$	$12.25 \pm 0.64^{2+}$	$0.34 \pm 0.07^{3+}$	$1.51 \pm 0.17^{3+}$	$1.53 \pm 0.25^{3+}$
C_{Cr}	3.19 ± 0.23	3.28 ± 0.19	3.35 ± 0.24	$4.74 \pm 0.36^{3+}$	5.06 ± 0.38	4.80 ± 0.34	$0.75 \pm 0.16^{3+}$	$1.51 \pm 0.21^{2+}$	$1.46 \pm 0.21^{2+}$
FE_{Na}(%)	0.42 ± 0.01	$0.47 \pm 0.02^{1+}$	$0.48 \pm 0.02^{2+}$	$1.39 \pm 0.10^{3+}$	$1.67 \pm 0.05^{1+}$	$1.75 \pm 0.05^{2+}$	$0.32 \pm 0.04^{1+}$	$0.66 \pm 0.04^{3+}$	$0.64 \pm 0.03^{3+}$

$^{1+,2+,3+}$ = $p < 0.05, 0.01, 0.001$. Group 2, 3, 4 and 7 compared with the group 1. Group 5 and 6 compared with the group 4. Group 8 and 9 compared with the group 7. PGE_2, 6kPGF$_{1\alpha}$, TXB_2 (pmol · 6 · h^{-1} · kg^{-1}), V (Urine flow, μl · min^{-1} · kg^{-1}), $U_{Na}V$ (Sodium excretion, μmol · min^{-1} · kg^{-1}), C_{Cr} (Clearance of creatinine, ml · min^{-1} · kg^{-1}), FE_{Na}(%) (Fractional excretional of sodium %).

clearance of creatinine (GPR) but also by directly enhancing sodium reabsorption.

e) Inhibition of TXA_2 biosynthesis in glycerol treated rats (table, group 7, 8, 9). The relationships observed between increased urinary excretion of TXB_2 and the development of ARF and sodium excretion were further reinforced when the selective inhibitor of TXA_2 synthesis was used. Thus the administration of OKY-046 in glycerol treated animals significantly diminished urinary TXB_2 excretion. This inhibition of TXA_2 synthesis was associated with a lesser decrease in creatinine clearance and sodium excretion than in untreated animals suggesting a partial protection of the rats against ARF. Since OKY-046 did not significantly change urinary excretion of the vasodilator – natriuretic[3-11] PGE_2 and $6ketoPGF_{1\alpha}$, during the 6 h of the experiment, the protection afforded by OKY-046 must not be related to these PGs but to the decreased release of TXA_2.

The increased fractional excretion of sodium after TXA_2 biosynthesis inhibition (table) could be related to the diminished release of TXA_2 because the urinary excretion of the vasodilator-natriuretic PGs did not change significantly.

In conclusion: These results suggest that 6 h after glycerol injection 1) increased TXA_2 release is accompanied by decreased creatinine clearance (C_{Cr}), sodium excretion ($U_{Na}V$) and fractional excretion of sodium (FE_{NA}%), suggesting the development of the early phase of acute renal failure (ARF), 2) The use of a selective inhibitor of thromboxane A_2-synthetase enhanced sodium excretion and fractional excretion of sodium in normal and saline loaded animals and partially prevented the decrease in creatinine clearance and sodium excretion and significantly increased fractional excretion of sodium in glycerol treated rats suggesting a partial protection against the development of acute renal failure. These relationship between TXA_2 and sodium excretion will be reinforced by further investigation to exclude actions of OKY-046 in sodium excretion unrelated to its inhibitory effect on the enzyme system.

1 Acknowledgment. This work was supported by the Public Benefit Foundation 'Alexander S. Onassis' (G-73). We thank ONO Pharmaceutical Co. Ltd, Kissei Pharmaceutical Co. Ltd, Osaka, Japan, and Dr A Hornych for their generous donations of OKY-046 and anti-6keto-PGF_{1a} antibodies respectively.

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0014-4754/86/060613-03$1.50 + 0.20/0

Pituitary gonadotropin releasing hormone (GnRH) receptor levels in intact and ovariectomized-adrenalectomized female golden hamsters on a short photoperiod

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Summary. Both intact and ovariectomized + adrenalectomized hamsters on a short photoperiod, had a daily surge in plasma LH at approximately 16.00–18.00 h. The number of pituitary GnRH receptors was generally lower in ovariectomized + adrenalectomized hamsters than in intact animals, but both intact and ovariectomized + adrenalectomized hamsters had a decrease in the number of