

## Antihypertensive Effects of KW-3902, an Adenosine A<sub>1</sub>-Receptor Antagonist, in Dahl Salt-Sensitive Rats

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**ABSTRACT**—We determined the effects of KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine), a novel adenosine A<sub>1</sub>-receptor antagonist, on the development of hypertension in Dahl salt-sensitive (Dahl-S) rats. KW-3902 (0.00017% w/w–0.017% w/w), fed with the diet, prevented the development of hypertension at 2–6 weeks in response to the high (8% w/w) NaCl diet. KW-3902 increased urine volume and sodium excretion and attenuated cardiac hypertrophy. In another series of the experiments employing the clearance method, KW-3902 (0.1 mg/kg, i.v.) increased urine volume, sodium excretion and lithium clearance in anesthetized Dahl-S rats. These results suggest that the antihypertensive effect of KW-3902 in Dahl-S rats is mediated via its natriuretic effect, the site of action being, at least partly, the proximal tubule. The adenosine A<sub>1</sub>-receptor antagonist may be effective for the treatment of salt-sensitive hypertension.

**Keywords:** Kidney, Hypertension, Dahl rat, Diuresis, Adenosine

The development of hypertension results from an interaction of environmental factors and genetic factors (1, 2). Dahl salt-sensitive (Dahl-S) rats are genetically predisposed to hypertension; however, the added environmental factor of excess dietary salt intake is necessary for the development of hypertension (3–5). The Dahl-S rat, thus, seems to be a useful model for human salt-sensitive hypertension. Although the etiology for the salt-sensitivity of Dahl-S rats is not fully elucidated, the Dahl-S rat, as compared to the Dahl salt-resistant (Dahl-R) rat, exhibits a reduced capacity to excrete salt, suggesting that this natriuretic dysfunction might be the reason for the salt-sensitivity (6–8). On the other hand, sodium (Na) reabsorption at the proximal tubule is reported to be greater in Dahl-S rats than in Dahl-R rats (9). Thus, the Na retention in Dahl-S rats seems to, at least partly, be due to dysfunction of the proximal tubular Na reabsorption.

KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine) is a selective and potent adenosine A<sub>1</sub>-receptor antagonist (10), which induces significant increases in urine volume and Na excretion with little change of potassium (K) excretion in saline-loaded rats (11). It is assumed that the diuretic effect of KW-3902 is caused by the inhibition of water and Na reabsorption at tubular sites, but not by the change of renal hemodynamics (12). From the

lithium-clearance study, the main site of action for KW-3902 has been suggested to be the proximal tubule (12). These observations suggest a possibility that KW-3902 could improve the proximal tubular dysfunction, resulting in the attenuated development of salt-sensitive hypertension, in Dahl-S rats. Prior to the present study, however, the effects of KW-3902 on the development of hypertension has not been reported.

In the present study, we investigated possible antihypertensive effects of KW-3902 in Dahl-S rats, in comparison with those of furosemide and trichlormethiazide. Additionally, we investigated the effects of KW-3902 on the Na reabsorption and the lithium clearance in response to saline-loading in anesthetized Dahl-S rats.

### MATERIALS AND METHODS

#### *Experimental animals*

Male 5-week-old Dahl-S rats (Seiwa Experimental Animals, Ltd., Fukuoka) were used to determine the effects of drugs on the development of hypertension. Male 8-week-old Dahl-S and Dahl-R rats were used for the clearance study. The animals were kept at 22°C with a 12-hr light-dark cycle. They had free access to tap water and commercial chow prior to the experiments.

### Drugs used

KW-3902 was synthesized in our laboratories. Furosemide (Furo) and trichlormethiazide (TCM) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo) and Sigma Chemical Co. (St. Louis, MO, USA), respectively. Inulin (INU) and lithium (Li) carbonate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka). All other chemicals and solvents were used in their analytical pure form.

### Effects on the development of hypertension

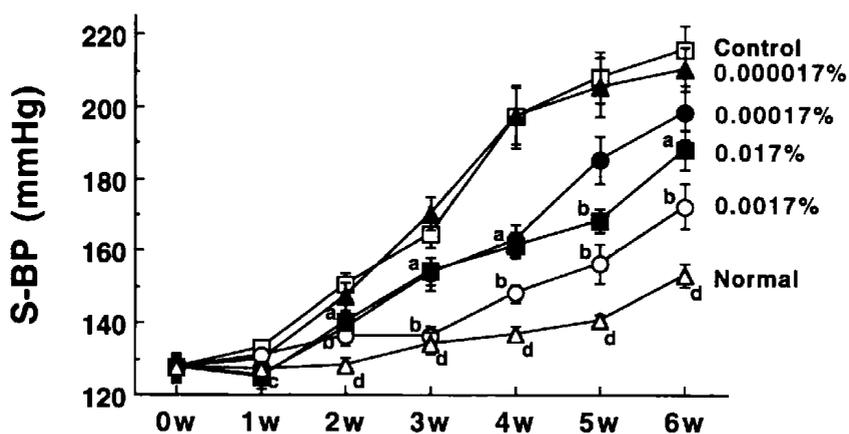
Fifty-six Dahl-S rats (5 weeks) were fed a high salt diet containing 8% (w/w) NaCl and were divided into 7 groups, each consisting of 8 rats, as follows: 1) control group, 2) 0.000017% (w/w) KW-3902-treated group, 3) 0.00017% (w/w) KW-3902-treated group, 4) 0.0017% (w/w) KW-3902-treated group, 5) 0.017% (w/w) KW-3902-treated group, 6) 0.085% (w/w) Furo-treated group and 7) 0.017% (w/w) TCM-treated group. Eight Dahl-S rats (5 weeks) were fed a normal diet (normal group). Drug treatment was started at the same time as the beginning of feeding with the high salt diet. KW-3902, Furo or TCM was mixed with the diet and was given to the rats in each group. Systolic blood pressure was determined once every week by the tail-cuff method (Multichannel sphygmomanometer; Riken Kaihatsu, Tokyo). After 6 weeks, a 24-hr urine sample was collected for the analysis of urinary excretion of Na. Thereafter, under anesthesia with pentobarbital (30 mg/kg body wt., i.p.), the blood sample was drawn from the abdominal aorta. Concentrations of Na and K in the serum and urine were determined by flame photometry (775-A; Hitachi, Ltd., Tokyo). Concentrations of glutamic pyruvic transaminase (GPT), glu-

tamic oxalacetic transaminase (GOT), triglyceride (TG), total cholesterol (T-CHO), total protein (TP), albumin (ALB), creatinine (CRE), uric acid (UA), urea nitrogen (UN) and glucose (GLU) in the serum were determined by an autoanalyzer (AU510; Olympus, Tokyo). Additionally, the wet tissue weights of the heart and the kidney were measured.

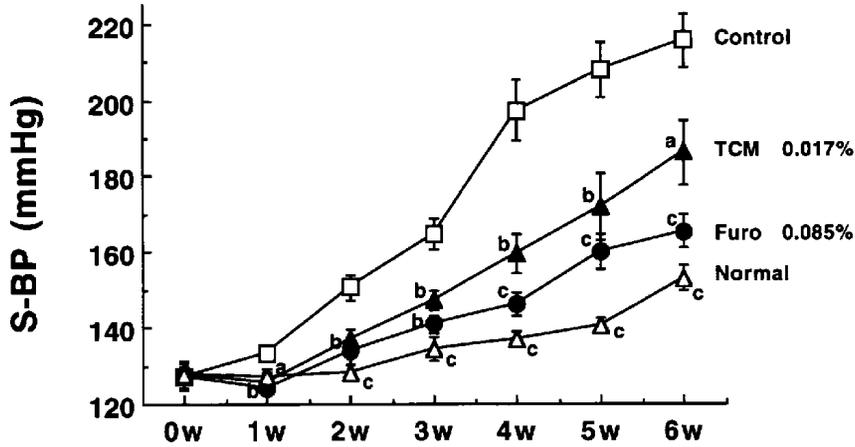
### Effects on the proximal tubule

In another series of experiments, a clearance study was performed with 10 Dahl-S rats and 5 Dahl-R rats. Under anesthesia with urethane (1.3 g/kg body wt., i.p.), a PE-50 polyethylene catheter was placed into the tail vein of each rat for intravenous infusion of INU, Li and NaCl solution. Another PE-50 catheter was placed into the bladder through a midline supra pubic incision for urine collection. Following the surgery, 0.9% (w/v) NaCl solution containing 3% (w/v) INU and 0.075% (w/v) Li carbonate was infused at a rate of 2 ml/hr/rat throughout the study (12).

After a 30-min equilibrium period following the surgery, saline containing 1% (v/v) dimethylsulfoxide and 0.01 N NaOH (the vehicle) was injected into the left femoral vein of all rats. From each Dahl-S rat, urine was collected for 60 min to determine the basal parameters, so that the rats could be divided into 2 groups as follows: Dahl-S rats were assigned to the control group and the KW-3902 group so that the average basal parameters were equal in each group. Vehicle was injected into the left femoral vein in the Dahl-R group and the Dahl-S control group, while KW-3902 (0.1 mg/kg body wt.) was injected in the Dahl-S KW-3902 group. Urine was again collected for 60 min, and then a blood sample was drawn from the

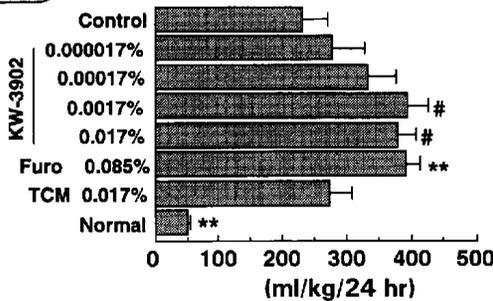


**Fig. 1.** Effects of KW-3902 on the development of hypertension in salt-loaded Dahl-S rats. Hypertension was induced by feeding the rat an 8% (w/w) NaCl-containing (high salt) diet. KW-3902 was fed the high salt diet at concentrations of 0.000017% (w/w)–0.017% (w/w). S-BP: systolic blood pressure, 0w–6w: weeks after the beginning of salt-loading, Control: control group (fed with the high salt diet), Normal: normal group (fed with the normal diet). Values are the means  $\pm$  S.E. of 7 or 8 animals. <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01 (Dunnett's test), <sup>c</sup>P < 0.05, <sup>d</sup>P < 0.001 (Student's *t*-test), when compared with the control value.

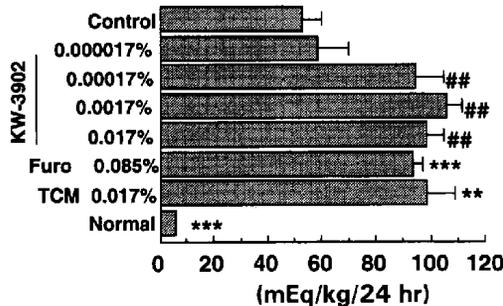


**Fig. 2.** Effects of furosemide (Furo) and trichlormethiazide (TCM) on the development of hypertension in salt-loaded Dahl-S rats. Hypertension was induced by feeding the rat an 8% (w/w) NaCl-containing (high salt) diet. Furo or TCM was fed with the high salt diet at concentrations of 0.085% (w/w) or 0.017% (w/w), respectively. S-BP: systolic blood pressure, 0w–6w: weeks after the beginning of salt-loading, Control: control group (fed with the high salt diet), Normal: normal group (fed with the normal diet). Values are the means  $\pm$  S.E. of 7 or 8 animals. <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001 (Student's *t*-test), when compared with the control value.

**Urine volume**



**Na ex.**



**Fig. 3.** Effects of KW-3902 (0.000017% (w/w)–0.017% (w/w)), furosemide (Furo, 0.085% (w/w)) and trichlormethiazide (TCM, 0.017% (w/w)) on urine volume and urinary sodium excretion (Na ex.) in salt-loaded Dahl-S rats. A 24-hr urine sample was collected at 6 weeks following the high salt diet. Values are the means  $\pm$  S.E. of 7 or 8 animals. <sup>#</sup>P < 0.05, <sup>##</sup>P < 0.01 (Dunnett's test), <sup>\*\*</sup>P < 0.01, <sup>\*\*\*</sup>P < 0.001 (Student's *t*-test), when compared with the control value.

abdominal aorta for determination of plasma INU, Li, Na and K concentrations.

Urine volume was determined gravimetrically. Li, Na and K concentrations in plasma and urine were determined by flame photometry (775-A, Hitachi, Ltd.). INU concentrations in plasma and urine were determined by the modified Anthrone method of Davidson and Sackner (13). Determination of INU, Li, Na and K in blood and urine, and urinary flow rates permitted the calculation of INU clearance (C-*INU*), Li clearance (C-*Li*), Na clearance (C-*Na*) and urinary excretions of Na and K (12).

*Statistical analyses*

Data are presented as means  $\pm$  S.E. Statistical significance was estimated by using the Dunnett's test, following analysis of variance (ANOVA) or Student's *t*-test for independence.

**RESULTS**

*Effects on the development of hypertension*

Throughout the experimental period, each rat had eaten food in the amount of about 80 g/kg/day. From the food intake, the daily dose of drug in the 0.017% (w/w) KW-3902-treated group was calculated to be 1.4 mg/kg/day. The increase in body weight was slightly inhibited at the first week in the groups of rats fed the high salt diet. In the control group and the 0.000017% (w/w) KW-3902-treated group, the body weight scarcely changed from 4 to 6 weeks following the high salt diet. In contrast, the increases in body weight were similarly observed in the other groups. Figures 1 and 2 illustrate the

alterations in systolic blood pressure over the experimental period in Dahl-S rats. In the control group, blood pressure increased from the first week following the feeding with the high salt diet. Development of hypertension

was significantly ameliorated in the KW-3902-treated groups, except for the 0.000017% (w/w) KW-3902-treated group, as compared with that in the control group (Fig. 1). In the 0.085% (w/w) Furo- and 0.017% (w/w) TCM-treated groups, the development of hypertension was also inhibited significantly (Fig. 2).

Figures 3 to 5 show the results of various measurements at the end of the experimental period, i.e., at 6 weeks following the drug treatment. In the control group, urine volume and Na excretion were higher as compared with those in the normal group. Urine volume and Na excretion were higher or tended to be higher in the KW-3902 (0.00017% (w/w)–0.017% (w/w))-, Furo- and TCM-treated groups than those in the control group (Fig. 3). The weight gain in the control group was attenuated as compared with that in the normal group (Fig. 4). In the 0.017% (w/w) KW-3902-treated group, the body weight was slightly but significantly higher than that in the control group, suggesting a slight amelioration of the sys-

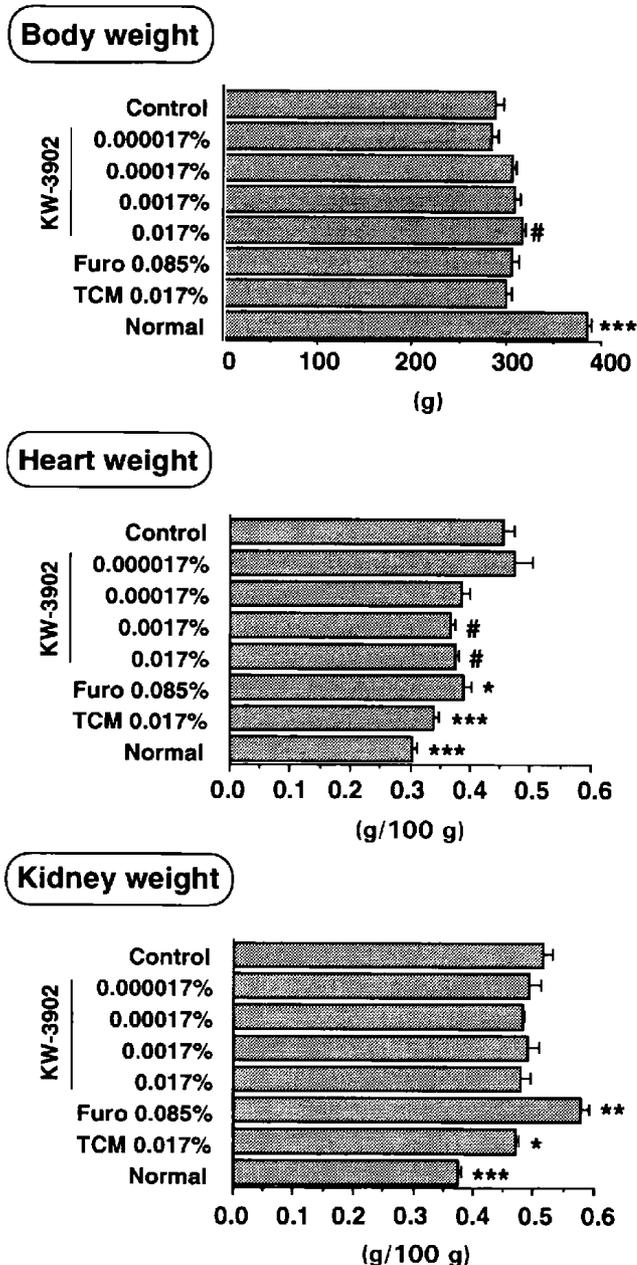


Fig. 4. Effects of KW-3902 (0.000017% (w/w)–0.017% (w/w)), furosemide (Furo, 0.085% (w/w)) and trichlormethiazide (TCM, 0.017% (w/w)) on the body weight and the wet weights of the heart and kidney in salt-loaded Dahl-S rats. The body weight (g) and the wet weight (g) per 100 g body weight were determined at 6 weeks following the high salt diet. Values are the means ± S.E. of 7 or 8 animals. #P < 0.05 (Dunnett's test), \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 (Student's *t*-test), when compared with the control value.

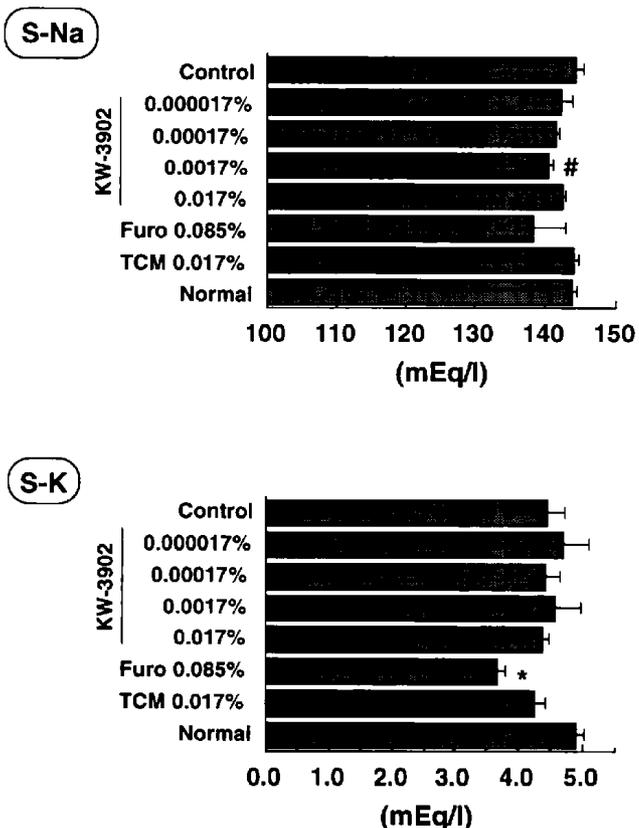


Fig. 5. Effects of KW-3902 (0.000017% (w/w)–0.017% (w/w)), furosemide (Furo, 0.085% (w/w)) and trichlormethiazide (TCM, 0.017% (w/w)) on serum concentrations of Na (S-Na) and K (S-K) in salt-loaded Dahl-S rats. Blood samples were drawn at 6 weeks following the high salt diet. Values are the means ± S.E. of 7 or 8 animals. #P < 0.05 (Dunnett's test), \*P < 0.05 (Student's *t*-test), when compared with the control value.

**Table 1.** Effects of KW-3902, furosemide (Furo) and trichlormethiazide (TCM) on serum parameters in salt-loaded Dahl-S rats

	GPT (IU/l)	GOT (IU/l)	TG (mg/dl)	T-CHO (mg/dl)	TP (g/dl)	ALB (g/dl)	CRE (mg/dl)	UA (mg/dl)	UN (mg/dl)	GLU (mg/dl)
Control	30.03 ±4.80	85.34 ±12.28	150.40 ±53.52	152.05 ±10.62	6.05 ±0.17	2.90 ±0.12	0.66 ±0.14	1.12 ±0.17	51.54 ±21.93	126.99 ±10.59
KW-3902 0.000017%	32.41 ±2.92	78.67 ±4.79	89.39 ±11.15	157.42 ±13.03	6.28 ±0.17	2.95 ±0.13	0.58 ±0.03	1.15 ±0.25	41.37 ±2.50	119.53 ±12.93
KW-3902 0.00017%	27.48 ±1.55	61.51 ±3.56	157.43 ±26.15	134.23 ±10.68	6.94 <sup>##</sup> ±0.08	3.26 ±0.07	0.56 ±0.03	0.70 ±0.13	35.68 ±1.92	145.94 ±4.18
KW-3902 0.0017%	29.59 ±1.34	64.08 ±3.44	170.31 ±17.29	118.39 ±10.67	7.05 <sup>##</sup> ±0.07	3.43 <sup>##</sup> ±0.08	0.58 ±0.04	0.70 ±0.12	34.59 ±1.47	141.54 ±4.14
KW-3902 0.017%	28.75 ±1.50	59.20 ±1.70	187.72 ±20.13	133.80 ±11.00	6.97 <sup>##</sup> ±0.10	3.22 ±0.11	0.57 ±0.02	0.75 ±0.15	35.65 ±1.07	131.63 ±6.09
Furo 0.085%	29.80 ±3.20	65.39 ±3.03	174.09 ±13.48	142.39 ±14.42	6.95 <sup>***</sup> ±0.09	3.24 <sup>*</sup> ±0.09	0.57 ±0.04	0.86 ±0.12	33.90 ±1.36	141.83 ±5.05
TCM 0.017%	26.36 ±1.42	67.06 ±1.90	114.29 ±7.64	116.29 <sup>*</sup> ±3.31	7.13 <sup>***</sup> ±0.06	3.55 <sup>***</sup> ±0.05	0.49 ±0.01	0.74 ±0.12	44.04 ±2.34	143.96 ±6.17
Normal	22.76 ±0.94	63.40 ±3.53	209.37 ±21.97	103.92 <sup>**</sup> ±2.40	7.29 <sup>***</sup> ±0.08	3.46 <sup>***</sup> ±0.05	0.53 <sup>*</sup> ±0.02	0.64 ±0.14	24.44 ±0.60	147.10 ±9.15

GPT: glutamic pyruvic transaminase, GOT: glutamic oxalacetic transaminase, TG: triglyceride, T-CHO: total cholesterol, TP: total protein, ALB: albumin, CRE: creatinine, UA: uric acid, UN: urea nitrogen, GLU: glucose. Serum parameters were determined at 6 weeks following the high salt diet. Each value represents the mean ± S.E. of 7 or 8 animals. <sup>##</sup>P < 0.01 (Dunnett's test), \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 (Student's *t*-test), when compared with the control value.

temic abnormality following the salt loading. Kidney and heart weights in the control group were greater than those in the normal group. The cardiac hypertrophy was ameliorated by all three examined drugs. The kidney weight in the Furo-treated group was higher, whereas that in the TCM-treated group were lower than that in the control group. Figure 5 shows the drug effects on serum concentrations of Na and K. Serum Na concentrations in the 0.0017% (w/w) KW-3902- and Furo-treated groups were lower than that in the control group. In the Furo-treated group, the serum K concentration was also lower than that in the control group.

Table 1 shows the drug effects on serum parameters at 6 weeks following the drug treatment. In the control group, serum concentrations of T-CHO and CRE were higher and concentrations of TP and ALB were lower than those in the normal group. KW-3902 and TCM tended to ameliorate the elevation of T-CHO concentration. KW-3902, Furo and TCM significantly attenuated the decreases in TP and ALB concentrations. In the control group, serum concentrations of GPT, UA and UN were higher, although these changes were not statistically significant. KW-3902 and Furo tended to improve the elevated UN concentration; however, UN concentration in the TCM-treated group was slightly higher than that in the other drug treatment groups.

#### Effects on the proximal tubule

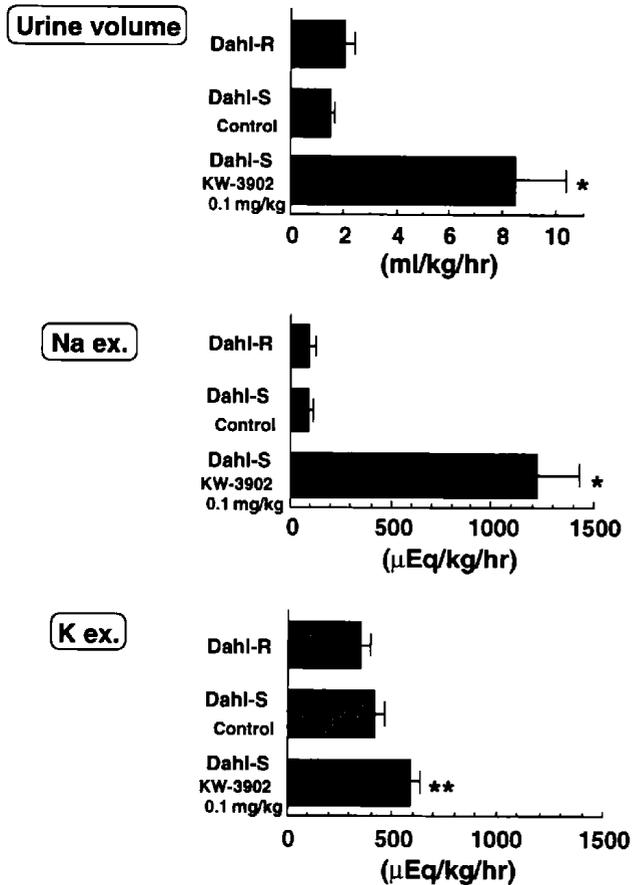
Figure 6 illustrates the effects of KW-3902 on urine volume and electrolyte excretions in anesthetized Dahl-S

rats. KW-3902 significantly increased urine volume and excretions of Na and K as compared with those in the Dahl-S control group. The increase in Na excretion was more prominent than that in K excretion. Figure 7 illustrates the effects of KW-3902 on various clearance parameters. In the Dahl-S control group, C-INU and C-Li were lower or tended to be lower than those in the Dahl-R group. KW-3902 significantly increased C-Li and C-Na.

#### DISCUSSION

The present study demonstrated that the adenosine A<sub>1</sub>-receptor antagonist KW-3902 attenuated the salt-induced hypertension in Dahl-S rats, as was the case with Furo and TCM. The antihypertensive effect of another adenosine A<sub>1</sub>-receptor antagonist, FK838, has already been reported in an abstract (14). Moreover, all the three drugs inhibited the increase in heart weight, which was observed in vehicle-treated Dahl-S rats. The attenuation by KW-3902, Furo and TCM of cardiac hypertrophy seems to be due to the antihypertensive effect of these drugs.

In the present study, KW-3902 induced diuresis and natriuresis in Dahl-S rats. This observation is in accordance with the previous one in normal rats (11, 12). Furo and TCM also induced natriuresis in Dahl-S rats. In fact, it is reported that one of the mechanisms for salt-induced hypertension in Dahl-S rats is Na retention (6–8) and that methyclothiazide, one of the thiazide diuretics, attenuated the development of hypertension (15). These observa-

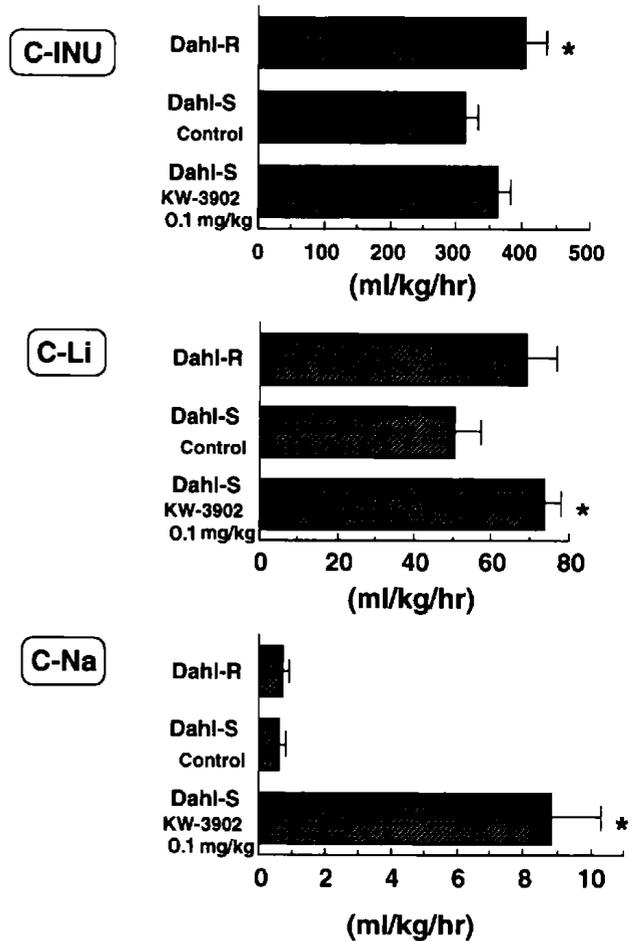


**Fig. 6.** Effects of KW-3902 (0.1 mg/kg, i.v.) on urine volume, excretion of sodium (Na ex.) and potassium (K ex.) in anesthetized Dahl-R and -S rats. Dahl-R: Dahl salt-resistant rat, Dahl-S: Dahl salt-sensitive rat. Values are the means  $\pm$  S.E. of 5 animals. \* $P < 0.05$ , \*\* $P < 0.01$  (Student's *t*-test), when compared with the Dahl-S control value.

tions suggest that the antihypertensive effect of KW-3902 is due to its natriuretic effect, resulting in the attenuated Na retention.

Na reabsorption at the proximal tubule is reported to be greater in Dahl-S rats than in Dahl-R rats (9), indicating that the Na retention in Dahl-S rats is at least partly due to dysfunction of the proximal tubule. We have previously demonstrated that KW-3902 at the diuretic and natriuretic dose increases C-Li in anesthetized rats (12), suggesting that KW-3902 produces a diuretic effect by inhibiting the reabsorption of water and Na at the proximal tubule. Also in this study using anesthetized Dahl-S rats, KW-3902 significantly increased C-Li, which tended to be depressed as compared with that in Dahl-R rats. These results suggest that KW-3902 improved the depressed Na excretion at the proximal tubule, resulting in the natriuretic effect.

A variety of adenosine  $A_1$ -receptor antagonists, including KW-3902, FK453 and 1,3-dipropyl-8-cyclopentylxan-



**Fig. 7.** Effects of KW-3902 (0.1 mg/kg, i.v.) on inulin clearance (C-INU), lithium clearance (C-Li) and sodium clearance (C-Na) in anesthetized Dahl-R and -S rats. Dahl-R: Dahl salt-resistant rat, Dahl-S: Dahl salt-sensitive rat. Values are the means  $\pm$  S.E. of 5 animals. \* $P < 0.05$  (Student's *t*-test), when compared with the Dahl-S control.

thine, have been reported to induce diuresis and natriuresis (11, 12, 16, 17). Recently, Takeda et al. (18) reported that the adenosine  $A_1$ -receptor antagonist FK453, probably via the increased intracellular cAMP, inhibits the  $\text{Na}^+ - 3\text{HCO}_3^-$  cotransporter along the proximal tubule, suggesting that the inhibited  $\text{Na}^+ - 3\text{HCO}_3^-$  cotransport may, at least in part, account for the diuretic effect of FK453. On the other hand, KW-3902 has been shown to inhibit the Na-dependent phosphate uptake in proximal tubular cells (19). These observations, as well as the fact that KW-3902 increased C-Li, indicate that the inhibited Na reabsorption at the proximal tubule is involved in the diuretic effect of the adenosine  $A_1$ -receptor antagonist. Moreover, KW-3902 at the diuretic dose antagonized the antidiuretic effect of the adenosine  $A_1$ -receptor agonist,  $(-)-N^6$ -(2-phenylisopropyl) adenosine (20). Taken together, KW-3902 seems to have inhibited, via the blockade of the

adenosine A<sub>1</sub>-receptors, Na reabsorption at the proximal tubule, leading to natriuresis and, consequently, to the antihypertensive effect in Dahl-S rats, which are predisposed to have a dysfunctional proximal tubule.

It is assumed that patients with essential hypertension can be divided into "salt-sensitive" and "non-salt-sensitive" hypertensives according to the changes in blood pressure in response to high-salt intake (21). The factors determining salt-sensitivity or non-salt-sensitivity are probably of genetic origin, as is the case with Dahl-S or Dahl-R rats (3, 4). It is suggested that the environmental factor of high salt intake interacting with the accessory gene elevates arterial blood pressure in "salt-sensitive" hypertension (3, 4). In the present study, KW-3902 attenuated the progression of salt-induced hypertension in Dahl-S rats. Accordingly, KW-3902, like the conventional antihypertensive diuretics, seems to be useful for the treatment of "salt-sensitive" hypertension in humans.

Long-term treatment with loop diuretics or thiazide diuretics frequently causes abnormalities in blood chemistry such as hyponatremia, hypokalemia and hypercholesterolemia (22). In this study, Furo, but not KW-3902 or TCM, caused hypokalemia in Dahl-S rats at 6 weeks following the repeated treatment. The kidney weight was increased following the treatment with Furo, suggesting a possible nephrotoxicity of Furo in Dahl-S rats. In addition, serum UN tended to be higher in the TCM-treated Dahl-S rats. Our previous study examining the effects of repeated oral administration in normal rats (23) demonstrated that Furo and TCM, but not KW-3902, decreased serum concentrations of Na and K and increased serum concentrations of GPT, GOT, UN and TG. These results suggest that the side effects of KW-3902 are less common than those of Furo and TCM in terms of abnormalities in blood chemistry.

In summary, the present study demonstrates that the adenosine A<sub>1</sub>-receptor antagonist KW-3902 attenuates the salt-induced hypertension and cardiac hypertrophy in Dahl-S rats, presumably due to the natriuretic effect of this drug. Additionally, KW-3902 increased C-Li and C-Na in anesthetized Dahl-S rats. These results suggest that KW-3902 induces diuresis and natriuresis at least partly by inhibiting Na reabsorption at the proximal tubule, resulting in the antihypertensive effect. The adenosine A<sub>1</sub>-receptor antagonist may be useful for the treatment of salt-sensitive hypertension.

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#### REFERENCES

- 1 Rapp JP: Genetics of experimental and human hypertension. *In* Hypertension Pathophysiology and Treatment, 2nd Edition, Edited by Genest J, Kuchel O, Hamet P and Cantin M, pp 582–598, McGraw-Hill Book Co, New York (1983)
- 2 Smith DG and Sing CF: Genetic-environmental interactions in the variation of blood pressure in Tecumseh, Michigan. *J Chron Dis* **30**, 781–791 (1977)
- 3 Dahl LK, Heine M and Tassinari L: Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt ingestion. *Nature* **194**, 480–482 (1962)
- 4 Aoki K: The three-way classification of hypertension: essential hypertension, environment hypertension and disease hypertension. *In* Calcium in Essential Hypertension, Edited by Aoki K and Frohlich ED, pp 9–36, Academic Press, Japan, Inc, Tokyo (1989)
- 5 Dahl LK, Heine M and Tassinari L: Effects of chronic excess salt ingestion. *J Exp Med* **115**, 1173–1190 (1962)
- 6 Maude DL and Kao-Lo G: Salt excretion and vascular resistance of perfused kidneys of Dahl rats. *Hypertension* **4**, 532–537 (1982)
- 7 Fink GD, Takeshita A, Mark AL and Brody MJ: Determinants of renal vascular resistance in the Dahl strain of genetically hypertensive rat. *Hypertension* **2**, 274–280 (1980)
- 8 Tobian L, Lange J, Azar S, Iwai J, Koop D, Coffee K and Johnson MA: Reduction of natriuretic capacity and renin release in isolated, blood perfused kidneys of Dahl hypertension-prone rats. *Circ Res* **43**, Supp 1, 92 (1978)
- 9 Roos JC, Kirchner KA, Abernethy JD and Langford HG: Differential effect of salt loading on sodium and lithium excretion in Dahl salt-resistant and -sensitive rats. *Hypertension* **6**, 420–424 (1984)
- 10 Shimada J, Suzuki F, Nonaka H and Ishii A: 8-Polycycloalkyl-1,3-dipropylxanthines as potent and selective antagonists for A<sub>1</sub>-adenosine receptors. *J Med Chem* **35**, 924–930 (1992)
- 11 Mizumoto H, Karasawa A and Kubo K: Diuretic and renal protective effects of KW-3902, a novel adenosine A<sub>1</sub>-receptor antagonist, via pertussis toxin insensitive mechanism. *J Pharmacol Exp Ther* **266**, 200–206 (1993)
- 12 Mizumoto H and Karasawa A: Renal tubular site of action of KW-3902, a novel adenosine A<sub>1</sub>-receptor antagonist, in anesthetized rats. *Jpn J Pharmacol* **61**, 251–253 (1993)
- 13 Davidson WD and Sackner MA: Simplification of the anthrone method. *J Lab Clin Med* **62**, 351–356 (1963)
- 14 Kohno Y, Minoura H, Nakano K, Hanaoka K, Horiai H, Otsuka M, Shimomura K, Terai T and Kusunoki T: Anti-hypertensive and diuretic effects of FK838, a novel adenosine A<sub>1</sub>-receptor antagonist. Abstract of 36th Japanese Congress of Nephrology, p 330 (1993) (in Japanese)
- 15 Sasaki S and Buñag RD: Methyclothiazide attenuates salt-induced hypertension without affecting sympathetic responsiveness in Dahl rats. *J Cardiovasc Pharmacol* **57**, 378–383 (1983)
- 16 Terai T, Kita Y, Kusunoki T, Andoh T, Shimazaki T, Deguchi Y, Akahane A, Shiokawa Y and Yoshida K: The renal effects of FR113453, a potent non-xanthine adenosine antagonist (Abstract). *Eur J Pharmacol* **183**, 1057–1058 (1990)
- 17 Collis MG, Shaw G and Keddle JR: Diuretic and saluretic effects of 1,3-dipropyl-8-cyclopentylxanthine, a selective A<sub>1</sub>-adenosine receptor antagonist. *J Pharm Pharmacol* **43**,

- 138–139 (1991)
- 18 Takeda M, Yoshitomi K and Imai M: Regulation of Na<sup>-</sup>-3HCO<sub>3</sub> cotransport in rabbit proximal convoluted tubule via adenosine A<sub>1</sub> receptor. *Am J Physiol* **34**, F511–F519 (1993)
  - 19 Cai H, Batuman V, Puschett DB and Puschett JB: Effect of KW-3902, a novel adenosine A<sub>1</sub> receptor antagonist, on sodium-dependent phosphate and glucose transport by the rat renal proximal tubular cell. *Life Sci* **55**, 839–845 (1994)
  - 20 Mizumoto H and Karasawa A: Effects of adenosine A<sub>1</sub>-agonist and -antagonist on urinary volume and Na excretion in IAP-treated and non-treated rats. *Jpn J Pharmacol* **63**, 257–259 (1993)
  - 21 Kawasaki T, Delea CS, Bartter FC and Smith H: The effect of high-sodium and low-sodium intakes on blood pressure and other related variables in human subjects with idiopathic hypertension. *Am J Med* **64**, 193–198 (1978)
  - 22 Dustan HR, Tarazi RC and Bravo EL: Diuretic and diet treatment of hypertension. *Arch Intern Med* **133**, 1007–1013 (1974)
  - 23 Kusaka H and Karasawa A: Effects of repeated administration of KW-3902, a novel adenosine A<sub>1</sub>-receptor antagonist, on its pharmacological actions. *Jpn J Pharmacol* **63**, 513–519 (1993)