

Diuretic Effects of a Novel Uricosuric Antihypertensive S-8666 in Rats, Mice, Monkeys, and Dogs: Comparison With Furosemide and Trichlormethiazide

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ABSTRACT

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Oral dose-response relationships of S-8666 to Na excretion were established in male and female Sprague-Dawley rats (3–100 mg/kg), male and female ddY mice (3–300 mg/kg), female cynomolgus monkeys (2–50 mg/kg), and female beagle dogs (3–100 mg/kg). The natriuretic potency of S-8666 was almost comparable to that of furosemide in rats but was lower in mice, monkeys, and dogs. The maximal effects were similar for S-8666 and furosemide, which had higher ceiling values than trichlormethiazide (TCM) in rats, mice, monkeys, and dogs. Thus, S-8666 seems to have a "loop diuretic" property like furosemide. After oral administration of S-8666 to rats (20–100 mg/kg), the onset of natriuresis was rapid, with the peak effect occurring within 1–2 hr. TCM showed a longer lasting pattern of natriuresis than S-8666 and furosemide. With intravenous administration (1–10 mg/kg) to rats, the $t_{1/2}$ for natriuresis was 16–20 min for S-8666 and 8–9 min for furosemide. Probenecid inhibited the diuretic action of S-8666 and furosemide in rats. S-8666 showed enantioselectivity; its (S)-(–)-form was natriuretic, whereas the (R)-(+)-form was inactive. These results indicate that S-8666 is a diuretic with a high ceiling property that has a lower potency than TCM and a longer lasting effect than furosemide.

Key words: loop diuretics, uricosuric, antihypertensive, natriuresis, stereospecificity

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INTRODUCTION

Hyperuricaemia is well known as one of the most frequent side effects encountered in the therapeutic use of all commonly available thiazides and related drugs. Therefore, drugs that do not raise blood urate concentration, but which retain their saluretic and antihypertensive potencies are desired. Thus, we anticipate much promise for our new diuretic S-8666, which has been found to enhance urate excretion in chimpanzees and rats [Yonetani et al., 1987a,b]. S-8666, 6,7-dichloro-5-N,N-dimethylsulfamoyl 1-2,3-dihydrobenzofuran-2-carboxylic acid, as shown in Figure 1, possesses an asymmetric carbon and exists as a racemic mixture [Harada et al., 1987]. The present study compared the diuretic effect of S-8666 with the standard thiazide diuretic trichlormethiazide and the loop diuretic, furosemide as to the time course of the diuretic response and characteristics of its effects. The natriuretic potencies of the enantiomers of S-8666 were also examined in monkeys.

MATERIALS AND METHODS

Drugs and Chemicals

S-8666 and its (S)-(-)- and (R)-(+)-enantiomers were synthesized in this laboratory. For intravenous administration, the sodium salt of S-8666 was used. The following drugs were obtained commercially: Furosemide, triamterene, acetazolamide, probenecid (Sigma, St. Louis, MO) and trichlormethiazide (TCM, Shionogi). All other chemicals were of analytical grade.

Experiments With Rats

Male Sprague-Dawley rats, 7–8 weeks of age, had free access to sucrose cubes that were substituted for the ordinary diet on the night before the experiment. These rats were given 20 ml/kg of 5% sucrose by gavage in the evening of the day before the assay. Tap water was available ad libitum. On the morning of the assay, rats were placed in metabolism cages and received orally 20 ml/kg of 2% gum arabic containing the compound to be tested. Urine was collected for 5 hr. Probenecid (50 mg/kg, dissolved in 1.0 ml of 1% gum arabic with the aid of NaOH) was given intraperitoneally where indicated.

For the time-course study, the rat was given 10 ml/kg of water by gavage at 1 hr before dosing. Next, the test compound was administered as a solution in 10 ml/kg of 2% gum arabic orally or in 1.0 ml/kg of 0.9% NaCl intravenously. Control rats received the vehicle alone. In the oral dose study, the urine was collected hourly for 8 hr. In the intravenous study, the urine, excreted over 120 min, was collected in six fractions for 20-min periods. After each collection period, the urinary losses were compensated for by giving by gavage 10 ml/kg of water per hour or 5.0 ml/kg of water per 20 min.

Experiments With Mice

DDY mice, males and females, 5–6 weeks of age, were used. The animals were deprived of food overnight but had free access to water. At the start of the experiments, they were placed in metabolism cages in a group of five mice per cage and received orally 30 ml/kg of 2% gum arabic containing the test compound. Urine was collected for 4 hr.

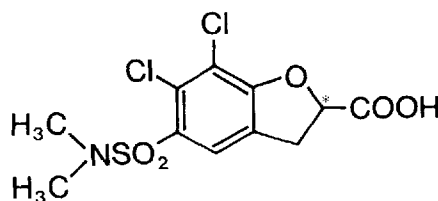


Fig. 1. Chemical structure of S-8666. The asterisk denotes the chiral center.

Experiments With Monkeys

Female cynomolgus monkeys weighing 3–4 kg, housed in individual cages, were given a standard diet and water until the experiment. On the morning of the assay, each monkey was restrained in a specially designed monkey chair after the insertion of an Argyle Foley No. 8 catheter (Nihon Sherwood Medical Ind. Inc., Tokyo) into the bladder. The monkeys were given 10 ml/kg of 10% glucose 1.0 hr by gavage before the start of the experiment and at every 2 hr after that for 6 hr. After a control period of 2 hr, they received the test compound orally as a solution in 1 ml/kg of 2% gum arabic with 9 ml/kg of 10% glucose. Urine was collected in three fractions; 0–2 hr for the control period and 0–2 and 2–4 hr after dosing. Each monkey underwent these separate assays 2 weeks apart. The potencies of the compounds were compared by cross-over design for six monkeys.

Experiments With Dogs

Nine female beagle dogs weighing 8–12 kg, housed in individual cages were given a standard diet and water until the experiment. On the morning of the assay, the dogs were given 10 ml/kg of water by gavage at 1.5 hr before the start of the experiment and then at every 2 hr for 6 hr. Urine spontaneously voided was collected in the individual cages and the bladder was emptied using an Argyl Foley No. 8 catheter at the end of every period. After a control period of 2 hr, the dogs received the test compound orally as a solution of 1 ml/kg of 2% gum arabic with 9 ml/kg of water. Urine was collected in four fractions; 0–2 hr for the control period and 0–2, 2–4, and 4–20 after dosing. At the end of the 0–4 hr experimental period, the animals were given the ordinary diet. Each dog underwent these separate assays 2 weeks apart.

Urine Analysis

Urine volume was measured and assayed for sodium and potassium by flame photometry (Instrumentation Laboratory, Model 943), for chloride by coulometry (Hiranuma Chloride Counter, Model CL 2, Tokyo), and for creatinine by a modification of the method of Jaffe [Heinegard and Tiderströme, 1973].

Statistical Methods

The urinary excretion values were expressed per kg body weight and the results were presented as the means \pm standard error of the mean. The difference between the control and experimental groups was tested by Student's *t*-test and that between the control and experimental periods was tested by the methods of Scheffé, Tukey, and Dunnett for multiple comparisons following analysis of variance. $P < 0.05$ was taken as significant.

RESULTS

Studies With Rats

Dose-response curves for S-8666, furosemide, and TCM. A linear log dose-response relationship was observed with S-8666 (3–100 mg/kg), furosemide (10–100 mg/kg), and TCM (0.03–1 mg/kg) for 5-hr excretion of Na in both male and female rats (Fig. 2). In rats, S-8666 and furosemide produced a similar rise in natriuresis and were about 1/100 less active than TCM. In the natriuretic response to these three diuretics, no significant difference was found between male and female rats.

Time course of diuretic and saluretic effects of S-8666, furosemide, and TCM. The patterns of urinary Na excretion as a function of time after a single oral administration of different doses of S-8666, furosemide, and TCM were compared (Fig. 3). The natriuretic effect S-8666 started within an hour and reached maximum at 1 to 2 hr. Furosemide caused a rapid onset of natriuresis followed by a decline as a function of time. TCM showed a longer lasting pattern of natriuresis than those of S-8666 and furosemide. Intravenous administration indicated more clearly the difference among these three diuretic compounds (Fig. 3). The

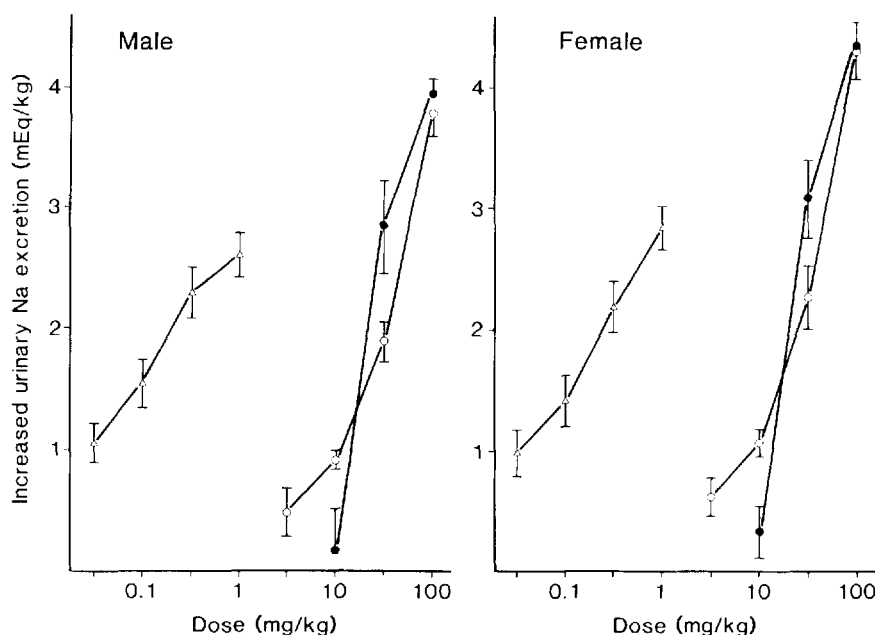


Fig. 2. Dose-response curves for S-8666 (○), furosemide (●) and TCM (△) on urinary excretion of sodium for a 5-hr period following oral administration to male and female rats. Results are mean \pm S.E. of values in eight rats.

natriuretic effects of S-8666 and furosemide started immediately, and furosemide declined more rapidly than S-8666. TCM, however, showed maximum natriuresis at 20–40 min after the intravenous administration. The effect lasted much longer with TCM than with S-8666 and furosemide. For the intravenous doses of 1–10 mg/kg in rats, the $t_{1/2}$ for natriuresis was 16–20 min for S-8666 and 8–9 min for furosemide.

Effects of probenecid on diuresis and saluresis induced by S-8666, furosemide, and TCM. The effects of probenecid on natriuresis induced by S-8666, furosemide, and TCM are shown in the Figure 4 plots of the increases in sodium excretion with time. Probenecid partially inhibited the diuretic action of both S-8666 and furosemide, but did not significantly change the natriuretic response to TCM.

Comparison of the Saluretic Effects of S-8666, Furosemide, TCM, Acetazolamide, and Triamterene

The urinary Na/K and Na/Cl ratios at different doses of S-8666, furosemide, and TCM, and those of triamterene and acetazolamide were compared in rats (Fig. 5). The urinary Na/K ratio of triamterene was higher than those of S-8666, furosemide, and TCM. Acetazolamide showed the lowest Na/K values. The urinary Na/Cl ratio was highest with acetazolamide and decreased in the order of triamterene > TCM, furosemide, S-8666. The respective value for both ratios were similar for S-8666, furosemide, and TCM.

Studies With Mice

Dose-response curves for S-8666, furosemide, and TCM. Oral dose-response relationships of S-8666, furosemide, and TCM to sodium excretion as well as to urine volume were established with both male and female mice (Fig. 6). The dose-response curves show that furosemide is about three times more active than S-8666 after oral administration. The high

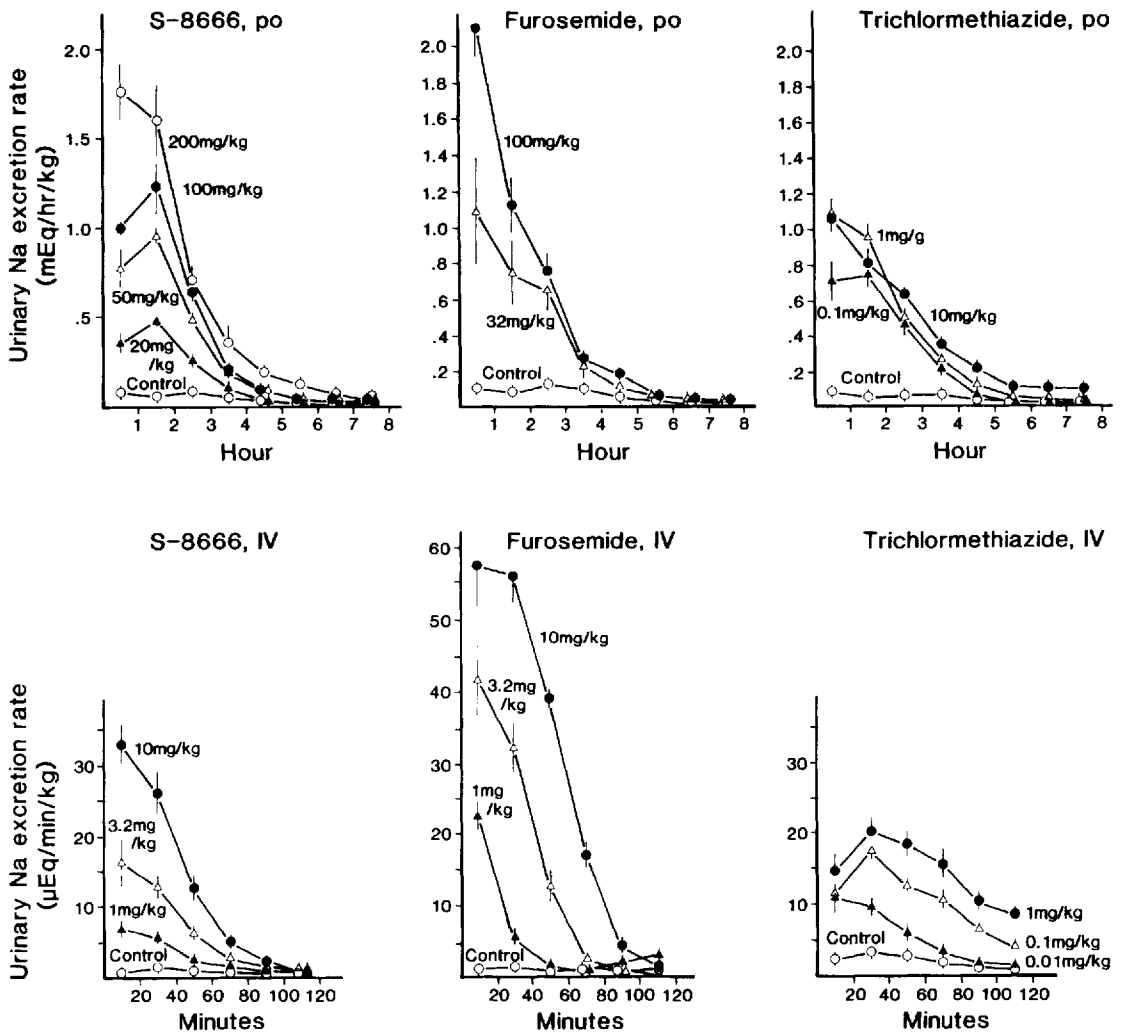


Fig. 3. Time course of the effects on urinary sodium excretion of S-8666, furosemide, and TCM after oral or intravenous administration to male rats. Results are mean \pm S.E. of values in eight rats.

“ceiling effect” found with both furosemide and S-8666 appears to be the same, the ceiling value being 100–150% higher than that of TCM. These effects were not significantly different between male and female mice.

Studies With Monkeys

Dose-response curves for S-8666, furosemide, and TCM. Furosemide was more potent than S-8666 on a weight basis but because of the nonparallelism between both curves (Fig. 7), the potency ratio on sodium excretion as well as on potassium increased 3–10 with increasing doses. TCM showed a low “ceiling” property.

Comparison of the saluretic effects of the enantiomers of S-8666. Racemic S-8666, its (S)-(–)-enantiomer and (R)-(+)-enantiomer were administered orally at a dose of 10 mg/kg (Table 1). The (–)-enantiomer was active inducing natriuresis and more potent than the racemate (S-8666). The (+)-enantiomer, however, produced no significant change in sodium excretion. Urinary excretion of creatinine was not affected.

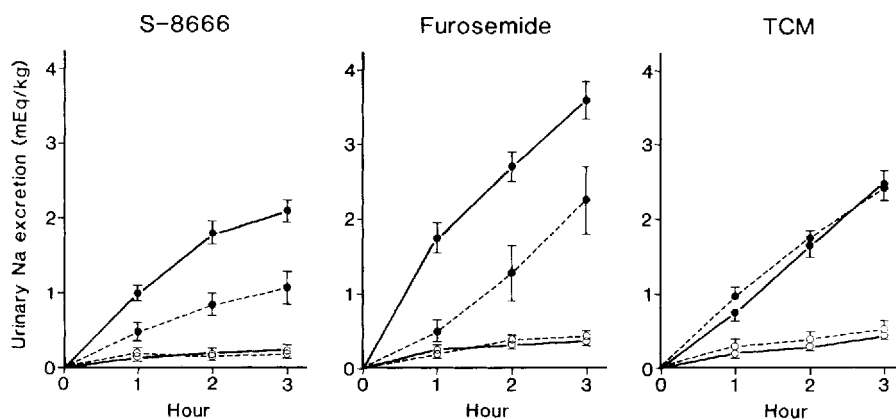


Fig. 4. Effect of probenecid (50 mg/kg, i.p.) on the natriuretic responses to S-8666 (50 mg/kg, p.o.), furosemide (25 mg/kg, p.o.), and TCM (1 mg/kg, p.o.) in male rats. Results are means \pm S.E. of values in eight rats. *, significant difference from diuretic alone. \circ — \circ control; \circ ···· \circ , probenecid; \bullet — \bullet , compound alone; \bullet ···· \bullet , compound with probenecid.

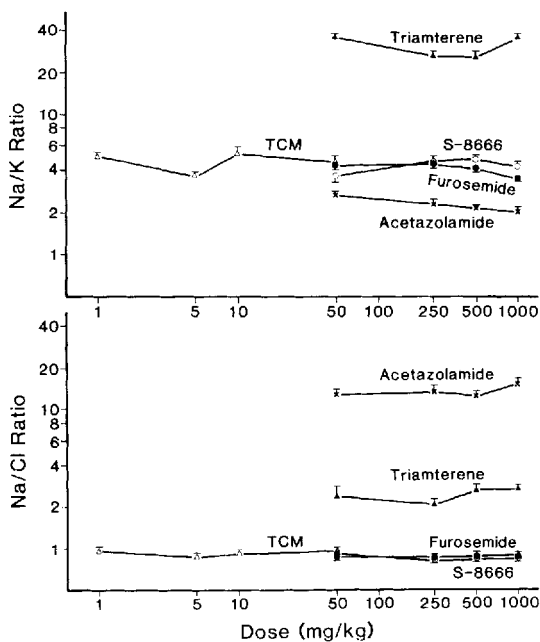


Fig. 5. Comparison of the effects of oral doses of S-8666, furosemide, TCM, triamterene, and acetazolamide on the urinary Na/K and Na/Cl ratios in male rats. Results are mean \pm S.E. of values for eight rats.

Studies in Dogs

Dose-response curves for furosemide, and TCM. At the doses studied, both furosemide and S-8666 produced a large part of natriuresis within the first 4 hr after oral administration (Fig. 8). At the end of this period, the dogs were given ordinary diet and free access to water. The diuretic effect of furosemide and S-8666 disappeared after the first 4 hr. However, the

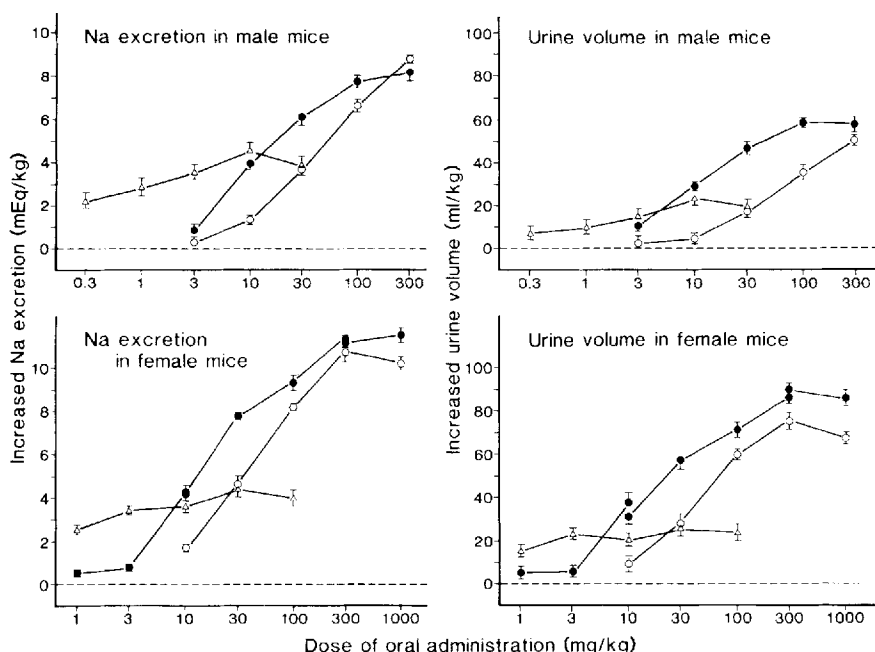


Fig. 6. Dose-response curves for S-8666 (○), furosemide (●), and TCM (△) on urinary volume and urinary excretion of sodium for 4-hr period following oral administration to male and female mice. Results are means \pm S.E. of values in five groups (a group of five mice per cage.)

natriuretic effect of TCM lasted more than 4 hr. The overall effects are shown in Figure 8. Both furosemide and S-8666 increased sodium excretion dose-dependently in a parallel manner and S-8666 was shown to be 1/25 less potent than furosemide. A comparable higher sodium value in overall response was obtained with TCM.

DISCUSSION

High potency diuretics caused extracellular fluid volume constriction, which leads to increased tubular reabsorption of urate in the kidney. As this is thought to decrease urate clearance, the diuretic potency of uricosuric diuretics should not be too high. If the potency is low, the drug can be administered in large doses that will raise the diuretic concentration in the proximal tubules. This can be expected to depress the reabsorption of urate by the nephron. For this reason the moderately potent diuretic S-8666 was chosen for development. Animal studies have shown that it does not cause urate retention in rats and chimpanzees [Yonetani et al., 1987a,b].

Studies of diuretics with animals indicate that there are many species differences in the dose-diuretic response to experimental animals; ethacrynic acid is almost inactive in the rat [Beyer et al., 1965; Zins et al., 1968] and the dog is the most sensitive to bumetanide while the rat is resistant [Ostergraad et al., 1972]. Sex-related differences have also been found [Cooling and Sim, 1977]. Our study showed that S-8666 displayed no significant differences among these animals (Fig. 9), although furosemide exhibited different potencies of natriuresis decreasing in the order of dogs > mice, monkeys > rats. TCM showed decreasing potency in the order of rats, mice > dogs. These results are mostly related to the different rates of metabolism of the diuretics in different species and gender.

Probenecid, an inhibitor of renal tubular secretion of organic acids, inhibits the natriuretic actions of furosemide [Hood and Williamsburg 1965], bumetanide [Smith and Lau,

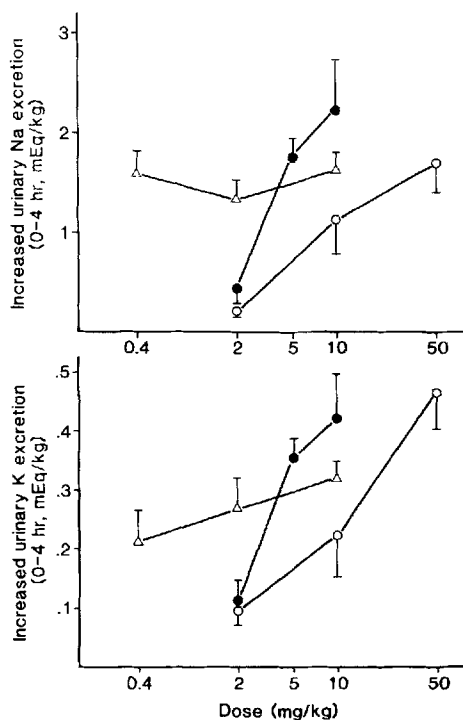


Fig. 7. Dose-response curves for S-8666 (○), furoseide (●), and TCM (△) on urinary excretion of sodium for 4-hr period following oral administration to female cynomolgus monkeys. Results are mean \pm S.E. of values for six monkeys.

TABLE 1. Diuretic Effects of S-8666, Its (S)-(-)-Enantiomer and (R)-(+)-Enantiomer After Oral Administration (10 mg/kg) to Cynomolgus Monkeys*

	Urine volume (ml/kg)	Na (μ Eq/kg)	K (μ Eq/kg)	Cl (μ Eq/Kg)	Creatinine (mg/kg)
S-8666					
Before 2 hr	9.1 \pm 2.1	19 \pm 9	88 \pm 15	19 \pm 5	3.20 \pm 0.35
After 0-2 hr	13.9 \pm 3.7 NS	720 \pm 229 ^{a,b,c}	236 \pm 41 ^{a,b,c}	763 \pm 182 ^{a,b,c}	3.75 \pm 0.29 NS
2-4 hr	9.6 \pm 1.9 NS	427 \pm 138 ^{a',b',c'}	169 \pm 33 ^{c'}	381 \pm 64 NS	3.40 \pm 0.21 NS
(-)-Enantiomer					
Before 2 hr	6.7 \pm 2.5	25 \pm 12	68 \pm 23	25 \pm 10	2.77 \pm 0.49
After 0-2 hr	18.4 \pm 3.3 ^{a,b,c}	1,127 \pm 225 ^{a,b,c}	212 \pm 31 ^{a,b,c}	1,295 \pm 237 ^{a,b,c}	3.67 \pm 0.24 NS
2-4 hr	9.7 \pm 1.6 NS	480 \pm 108 ^{a',b,c}	172 \pm 32 ^{a',b',c'}	853 \pm 364 ^{a,b,c}	3.16 \pm 0.21 NS
(+)-Enantiomer					
Before 2 hr	13.8 \pm 2.2	13 \pm 5	116 \pm 49	24 \pm 11	3.77 \pm 0.32
After 0-2 hr	10.4 \pm 1.1 NS	12 \pm 4 NS	68 \pm 5 NS	17 \pm 6 NS	3.22 \pm 0.16 NS
2-4 hr	9.8 \pm 2.2 NS	14 \pm 7 NS	71 \pm 8 NS	17 \pm 7 NS	3.30 \pm 0.20 NS

*Values represent the mean \pm S.E. (n = 6). Data were analyzed by analysis of variance and the comparisons were performed using Scheffé's method (a), Tukey's test (b), and Dunnet's procedure (c) for multiple comparisons. a,b,c, $p < 0.01$; a',b',c', $p < 0.05$; different from control period (before 2 hr). NS, not significant.

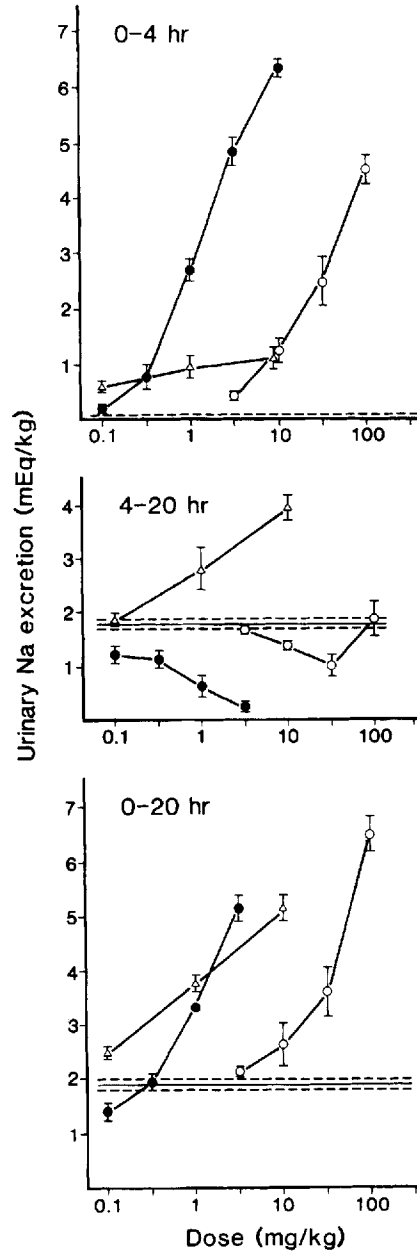


Fig. 8. Dose-response curves for S-8666 (○), furosemide (●), and TCM (△) on urinary excretion of sodium for 0-4, 4-20, and 0-20-hr periods following oral administration to female beagle dogs. Results are mean \pm S.E. of values for six dogs.

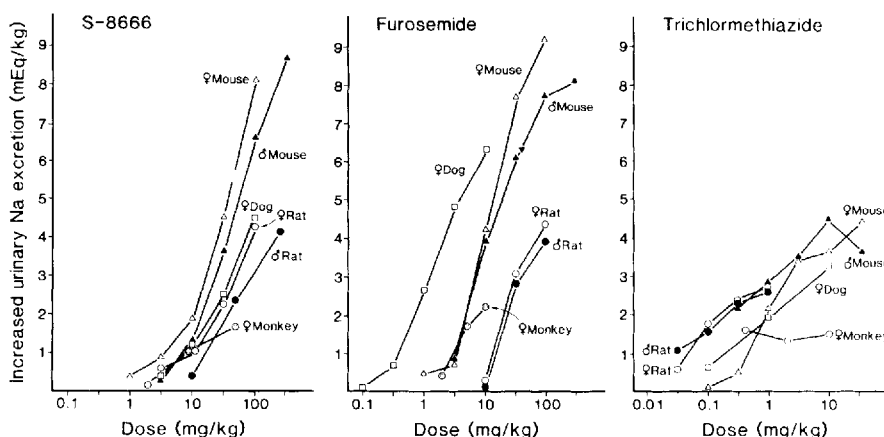


Fig. 9. Summary of the natriuretic responses as functions of the amount of diuretic compounds administered orally to the experimental animals.

1983], ethacrynic acid [Beyer et al., 1965], and chlorothiazide [Beyer and Baer, 1961]. As shown in the results, probenecid was found to block the natriuretic action of S-8666. Thus, an adequate concentration of S-8666 in the luminal fluid appears to be necessary for its natriuretic action. TCM was not affected by probenecid. This may be due to the lack of effective blockage of tubular secretion of TCM, since the dose of TCM to elicit natriuresis was significantly lower than that of furosemide. Taylor and Maren [1963] showed that TCM was extensively bound to plasma protein and its renal clearance was three to four times the calculated value for the amount filtered in dogs.

S-8666 showed enantioselectivity. In cynomolgus monkeys, the (–)-enantiomer showed natriuretic potency, whereas the (+)-enantiomer was inactive. Similar stereospecificity of the diuretic action has been demonstrated with the optically active isomer of ozolone [Greven et al., 1980] and indacrinone [Field et al., 1984]. The (–)- and (+)-enantiomers of S-8666 both seem to affect the tubular secretion of each other but not in competition with diuretic action.

In conclusion, this study has shown that S-8666 is a loop diuretic with a high ceiling property and has a lower potency than TCM and a longer lasting effect than furosemide and displays no marked species differences. Further studies to elucidate its mode of action using isolated tubules are now in progress.

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