INVOLVEMENT OF ADRENERGIC MECHANISM IN THE DIURETIC ACTION OF o-CHLOROBENZYL METHYL SULFOXIDE (DS-30) IN THE RAT

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Abstract—Involvement of the adrenergic mechanism in the diuretic action of o-chlorobenzyl methyl sulfoxide (DS-30) was investigated in the rat. Norepinephrine and metaraminol induced diuresis, which was blocked by pretreatment with tolazoline. Isoproterenol induced antidiuresis, which was blocked by pretreatment with propranolol. The diuretic effect of DS-30 as well as that of epinephrine was reversed by pretreatment with tolazoline, but not with propranolol. The diuretic action of hydrochlorothiazide and furosemide was not influenced by pretreatment with tolazoline. The diuretic effect of DS-30 was not affected by reserpinization of rats.

Catecholamines influence greatly metabolic processes in laboratory animals and humans. The amines promote the formation of cyclic 3', 5'-AMP, which brings about conversion of inactive phosphorylase to an active one (1) and in turn alters cellular functions (2). Schwartz et al. (3) have concluded from the radioautographic study of the kidney in the rat treated with tritiated ADH that the hormone binds preferentially with the distal convolution and the collecting duct, and that previous treatment of the rats with cysteine (3) and a number of sulfhydryl inhibitors (4) decreases this binding capacity resulting in decreased permeability of the affected tubular epithels to water due to molecular distortion. ADH has been shown to increase cyclic AMP production in several tissues presumably by activation of adenyl cyclase. This concept lead Orloff and Handler (5) to suggest that cyclic AMP is an essential intermediate in the antidiuretic action of ADH in the mammalian kidney.

In a previous report (6), it was demonstrated that o-chlorobenzyl methyl sulfoxide (DS-30) and its sulfone (DS-72) exerted a diuretic activity in the rats and antagonized the antidiuretic effect of ADH, however, the diuretic effect of DS-compounds was abolished by the previous hypophysectomy and adrenalectomy. In order to elucidate the mode of action of the DS-compounds on the kidney, an attempt was made to correlate the diuretic effect with the adrenergic mechanism in the rat.

METHODS AND RESULTS

Male Sprague Dawley rats weighing from 150 to 230 g were used in all experiments. Groups of 5 to 6 rats allowed free access to drinking water only for 18 hr before the ex-
Experiments were treated with various agents and urinary outputs of water, sodium and potassium were measured up to 3 or 5 hr. The rats were deprived of drinking water during the experimental periods.

1. Effect of adrenergic agents and adrenergic blocking agents on the urine formation

Norepinephrine and metaraminol served as the alpha-adrenergic agent. Isoproterenol was used for beta-adrenergic stimulation. Epinephrine was used for stimulation of both alpha and beta receptors. Tolazoline was selected as the alpha-adrenergic blocking agent and propranolol was employed as the beta-adrenergic blocking agent. The blocking agents were injected 10 min prior to the injection of the sympathomimetic amines. Except for metaraminol, which was used as the bitartrate, all drugs were used in the form of the hydrochloride salt. All agents were dissolved in 0.9% saline in various concentrations adjusted to give a total volume of approx. 0.5 ml per rat and were injected subcutaneously. Control rats were injected with 0.5 ml of 0.9% saline alone. The dosages of the various agents employed were indicated in the individual experiments. The rats were orally loaded with 25 ml/kg of 0.9% saline immediately after the administration of sympathomimetic amines.

As shown in Fig. 1, s.c. injection of tolazoline alone did not affect all of the urinary parameters. The injection of epinephrine, norepinephrine and metaraminol caused

| Drug (S.C.) | Tolazoline, 5mg/kg |
|-------------|--------------------|
| A           | —                  |
| B           | +                  |
| C           | —                  |
| D           | +                  |
| E           | —                  |
| F           | +                  |
| G           | —                  |
| H           | +                  |

FIG. 1. Effects of pretreatment with tolazoline on the diuretic action of sympathomimetic amines.

Sympathomimetic amines were injected s.c. 10 min after injection of tolazoline and immediately after 25 ml/kg of 0.9% saline was administered orally. Statistical significance between control (A) and experimental groups: *0.05>P>0.01, **0.01>P. Each column shows the mean with standard deviation.
DIURETIC MECHANISM OF DS-30

| Drug (S.C.) | Propranolol 25mg/kg | -- |
|-------------|---------------------|--|
| A           | --                  | S.C. |
| B           | +                   |       |
| C           | --                  | Isoproterenol 0.5mg/kg |
| D           | +                   | Epinephrine 1mg/kg |
| E           | --                  | Norepinephrine 0.25mg/kg |
| F           | +                   |       |
| G           | --                  | Metaraminol 1mg/kg |
| H           | +                   |       |
| I           | --                  |       |
| J           | +                   |       |

Fig. 2 shows the effect of pretreatment with propranolol on the diuretic action of sympathomimetic amines.

Sympathomimetic amines were injected s.c. 10 min after injection of propranolol and immediately after 25 ml/kg of 0.9% saline has been administered orally. Statistical significance between control and experimental groups: *0.05>P>0.01, **0.01>P.

marked increases in urine volume and sodium excretion without affecting potassium excretion. The diuretic effects disappeared with a previous administration of tolazoline. Epinephrine, after pretreatment with tolazoline, showed antidiuresis with concomitant decrease in urinary sodium and potassium.

Fig. 2 shows the effect of pretreatment with propranolol on the diuretic action of sympathomimetic amines. The s.c. injection of propranolol showed no influence on any of the urinary parameters. The injection of isoproterenol caused antidiuresis, which was completely inhibited by previous administration of propranolol. The diuretic effects of epinephrine, norepinephrine and metaraminol were not affected by a previous injection of propranolol, except for the potassium-excreting effect of epinephrine which appeared following pretreatment with propranolol.

2. Effects of adrenergic blocking agents on the diuretic action of DS-30

Groups of 6 rats were treated with s.c. injection of DS-30 or propranolol, and with s.c. injection of DS-30 5 min after the injection of propranolol. All the rats were orally loaded with 25 ml/kg of 0.9% saline immediately after the injection of DS-30. Urinary outputs of water, sodium and potassium were examined for 3 hr.

As shown in Fig. 3, DS-30 caused a pronounced increase in urine volume, sodium
FIG. 3. Effects of pretreatment with propranolol on the diuretic action of DS-30. DS-30 was injected s.c. 5 min after injection of propranolol and immediately after 25 ml/kg of 0.9% saline had been orally loaded. Statistical significance between control and experimental groups: *0.05>P>0.01, **0.01>P.

FIG. 4. Effects of pretreatment with tolazoline on the diuretic action of DS-30. DS-30 was injected s.c. 5 min after injection of tolazoline and immediately after 25 ml/kg of 0.9% saline had been orally loaded. Urine was collected every hr. Statistical significance between control and experimental groups: *0.05>P>0.01, **0.01>P.
and potassium excretion. The previous injection of propranolol, having no influence on urinary parameters, did not influence the diuretic action of DS-30.

Groups of 5 rats were treated with s.c. injection of DS-30 or tolazoline, and also with s.c. injection of DS-30 5 min after the injection of tolazoline. All the rats were simultaneously loaded orally with 25 ml/kg of 0.9% saline. Urine was collected for 3 hr.

As shown in Fig. 4, DS-30 produced a pronounced diuretic effect. The pretreatment of the rats with tolazoline depressed the diuretic effect of DS-30 and conversely caused antidiuresis accompanied with a decrease in the excretions of sodium and potassium.

Groups of 5 rats were treated with s.c. injection of 10 mg/kg of DS-30 and with s.c. injection of DS-30 5 min after the injection of 0.1, 1.0, 5.0 and 10 mg/kg of tolazoline.

As shown in Fig. 5, the doses of 5 and 10 mg/kg but not 0.1 and 1.0 mg/kg of tolazoline depressed the diuretic action of DS-30.

| Tolazoline (mg/kg, s.c.) | DS-30 (mg/kg, s.c.) |
|-------------------------|---------------------|
|                         |                     |
| ---                     | ---                 |
| ---                     | ---                 |
| 0.1                     | +                   |
| 1                       | +                   |
| 5                       | +                   |
| 10                      | +                   |

Fig. 5. Effects of varying doses of tolazoline on the diuretic action of DS-30.
DS-30 was injected s.c. 5 min after injection of tolazoline and immediately after 25 ml/kg of 0.9% saline had been loaded orally. Statistical significance between control and experimental groups: *0.05>P>0.01.

3. Effect of tolazoline on the diuretic action of hydrochlorothiazide and furosemide

The urinary excretions of water, sodium and potassium in response to s.c. injection of 2 mg/kg of hydrochlorothiazide (HC) and of 5 mg/kg of furosemide (FS) were compared with the treated and untreated rats which had been given s.c. injection of
As shown in Fig. 6, pronounced diuretic effects of HC and FS were not modified by pretreatment with the adrenergic blocking agent, tolazoline.

4. Effect of the reserpinization on the diuretic effect of DS-30

Groups of 5 rats were treated with an i.p. injection of 2.5 mg/kg of reserpine 24 hr before the s.c. injection of DS-30, norepinephrine or tyramine. Urinary parameters were determined every hour x 5 after administration of the diuretic agents.

As shown in Fig. 7, a slight but significant diuresis by tyramine in intact rats was abolished by pretreatment of the rats with reserpine. In contrast, the diuretic action of DS-30 and norepinephrine was still observed in the reserpinized rats.

5. Comparison of the diuretic action of DS-30 and metaraminol in the adrenalectomized rats

It is known that the adrenalectomized rat maintained for one week or more after adrenalectomy shows reduced ability to excrete acutely hydrated water (7), therefore the
diuretic nature of DS-30 was compared with that of metaraminol in adrenalectomized rats with reduced ability of water excretion.

Firstly, the rats were bilaterally adrenalectomized as described in a previous paper (6). Thereafter, sham-operated and adrenalectomized rats were allowed free access to the tap water, 0.9% saline and the standard diet for 15 days. Groups of 6 rats, given only the drinking solutions for 18 hr before the experiment, were then orally loaded with 25 ml/kg of distilled water. DS-30 was orally administered with a load of distilled water, and metaraminol was s.c. injected just before the water load. Urine was collected...
for 3 hr. Secondly, the rats maintained as described above for 23 days after the adrenalectomy were treated for 4 days with a daily s.c. injection of 0.1 ml/rat of sesame oil alone or containing hydrocortisone acetate. Two hr after the last application of hydrocortisone, 25 ml/kg of distilled water was orally administered to the rats. The procedures of drug administration and urine collection were similar to those as mentioned above.

As shown in Fig. 8-A, the adrenalectomized rats loaded with distilled water alone showed a significant reduction of urine output in comparison to the sham-operated rats. The oral administration of DS-30 did not show any diuretic response, although in a preliminary experiment using intact rats loaded with distilled water DS-30 (10 mg/kg, p.o.) enhanced urine output, 1.66 times that of control was observed. In contrast, metaraminol caused an increase in urine volume, but the effect was less than that in the intact rats (Fig. 1). In Fig. 8-B, the impaired ability of water excretion in the adrenalectomized rats was normalized by application of hydrocortisone. In such a condition, DS-30 showed no diuretic response, but metaraminol caused a pronounced water diuresis similar to that of intact rats (Fig. 1).
6. **Comparison of the diuretic action of DS-30 with norepinephrine in the hypophysectomized rats**

The rats, which had been hypophysectomized as described in a previous paper (6), were maintained on the tap water and a standard diet ad libitum for 18 days. Groups of 6 rats, allowed free access only to the tap water during 18 hr before the experiment, were then orally loaded with 25 ml/kg of 0.9% saline alone or containing DS-30. Norepinephrine, which causes diuresis in the same manner as metaraminol (Figs. 1 and 2), was injected s.c. just before the oral load of 0.9% saline. Urine was collected for 5 hr after the drug administration.

As shown in Fig. 9, DS-30 did not show any diuretic activity in comparison with its pronounced diuretic action in intact rats (6), but norepinephrine caused a marked diuresis similar to that in intact rats (Fig. 1).

**DISCUSSION**

Theoretically, the rate of glomerular filtration may be altered by a number of factors. Drugs with marked hemodynamic action such as adrenergic and cholinergic drugs alter the filtration rate and urine flow by affecting arterial pressure, and afferent and efferent renal arteriolar resistance. There is a convincing evidence, obtained from a variety of species, that spontaneous diuresis is conditioned by endogenous release of ADH, which is directly influenced by the degree of body hydration. Electrical stimulation of supraoptic or paraventricular nuclei, injection of the cholinomimetics into the area, and
depolarization of the hypothalamoneurohypophyseal tract by a high concentration of potassium result in the endogenous release of ADH (8). The complexity of these sequences makes difficult a clear-cut elucidation of the autonomic involvement in the diuretic mechanism.

In a previous report (6), the diuretic characteristics of DS-30 in rats were as follows: 1) water diuresis with less natriuresis, 2) enhancement of diuretic activity by a combination with hydrochlorothiazide, acetazolamide and furosemide, 3) abolition of the diuretic activity in the adrenalectomized rats and antagonism against the sodium retaining activity of the mineralcorticoids in the adrenalectomized rats, 4) antagonism against the antidiuretic activity of exogenously administered ADH in intact rats, and 5) abolition of the diuretic activity in the hypophysectomized rats even if ADH had been administered previously.

It is known that in the rat, alpha-adrenergic agents such as norepinephrine and metaraminol (9, 10) cause diuresis and a beta-adrenergic agent such as isoproterenol causes antidiuresis (10, 11). In the present studies, stimulation of the beta-receptors by isoproterenol in the rat produced a marked antidiuresis, which was blocked by pretreatment of the rat with propranolol, a beta-adrenergic blockade (Fig. 2). On the other hand, the diuretic actions of epinephrine, norepinephrine and metaraminol were not affected by pretreatment of propranolol, but disappeared by pretreatment of tolazoline, an alpha-adrenergic blockade (Fig. 1). The antidiuresis by epinephrine after treatment of the rat with tolazoline appeared to be due to the beta-receptor stimulating action. The diuretic action of DS-30 was abolished by pretreatment with tolazoline (Figs. 4 and 5), but not with propranolol (Fig. 3), and further DS-30 after pretreatment with tolazoline resulted in an antidiuresis (Figs. 4 and 5). DS-30 is therefore likely to have both alpha- and beta-adrenergic stimulating activities similar to epinephrine. Since DS-30 is effective in the reserpinized rats (Fig. 7), DS-30 does not appear to cause diuresis via release of endogenous catecholamines as tyramine (12). Some differences between the diuretic activities of DS-30 and the known alpha-adrenergic agents were however observed. DS-30 was ineffective in the adrenalectomized rats and hypophysectomized ones, whereas metaraminol resulted in a diuresis in the former rats while norepinephrine was effective in the latter (Figs. 8 and 9). Further studies were not performed on these different responses between DS-30 and the adrenergic agents. The diuretic action of hydrochlorothiazide and furosemide was not influenced by pretreatment with tolazoline (Fig. 6). These findings suggest that DS-30 is different from these known diuretics regarding the mode of action.

It has been reported that norepinephrine antagonizes the antidiuretic action of ADH in the intact rat (13). DS-30 antagonized the antidiuretic effect of ADH in the intact rat (6) and the hypophysectomy of the rat abolished the diuretic activity of DS-30. Regarding the mechanism of antidiuretic action of ADH, Orloff and Handler (5) have proposed that intracellular accumulation of cyclic 3', 5'-AMP is the essential factor that leads to increased permeability of amphibian bladder to water. Beta-receptor stimulating activity of epinephrine after the combined use with an alpha-receptor blockade was shown to facilitate the accumulation of cyclic 3', 5'-AMP apparently by stimulating the activity of mem-
brane-bound adenyl cyclase (13, 14). It is much more likely that stimulation of the beta-adrenergic receptor or ADH induces the antidiuresis by accumulation of cyclic 3', 5'-AMP in the epithels of the collecting duct of the kidney, while stimulation of the alpha-adrenergic receptor or DS-30 induces diuresis by interfering with accumulation of cyclic 3', 5'-AMP in the epithels of the rat. Evidence to support this assumption will be discussed in following papers.

REFERENCES

1) LIDDLE, G.W. AND HARDMAN, J.G.: New Eng. J. Med. 285, 560 (1971)
2) JOST, J.P. AND RICKENBERG, H.V.: Ann. Rev. Biochem. 40, 741 (1971)
3) SCHWARTZ, I.L., RASMUSSEN, H., SCHOESSLER, M.A., SILVER, L. AND FONG, C.T.O.: Proc. Natl. Acad. Sci. 46, 1288 (1960)
4) RASMUSSEN, H., SCHWARTZ, I.L., SCHOESSLER, M.A. AND HOCHSTER, G.: Proc. Natl. Acad. Sci. 46, 1278 (1960)
5) ORLOFF, J. AND HANDLER, J.S.: J. clin. Invest. 41, 702 (1962)
6) NISHIKAWA, K. AND KIKUCHI, S.: J. Takeda Res. Lab. 31, 331 (1972)
7) STOLTE, H., BRECHT, J.P., WIEDERHOLT, M. AND HIERHOLZER, K.: Pflügers Arch. ges. Physiol. 299, 99 (1968)
8) SAWER, W.H. AND MILLS, E.: Neuroendocrinology, Ed. by MARTINI, L. AND GANONG, W.F., Vol. 1, p. 187, Academic Press, New York (1966)
9) FÜLGRAFF, G., HEIDENREICH, O., HEINTZE, K. AND OSSWALD, H.: Arch. Pharmakol. exp. Path. 262, 295 (1969)
10) LEHR, D., MALLOW, J. AND KRUKOWSKI, M.: J. Pharmacol. exp. Ther. 158, 150 (1967)
11) LEES, P.: Br. J. Pharmacol. Chemother. 34, 429 (1968)
12) BEIN, H.J.: Pharmacol. Rev. 8, 435 (1956)
13) LIBERMAN, B., KLEIN, L.A. AND KLEEMAN, C.R.: Proc. Soc. exp. Biol. Med. 133, 131 (1970)
14) SUTHERLAND, E.W. AND ROBINSON, G.A.: Pharmacol. Rev. 18, 145 (1966)
15) TURTLE, J.R. AND KIPNIS, D.M.: Biochem. biophys. Res. Commun. 28, 797 (1967)