J. Pharm. Pharmac., 1976, **28**, 609–612 *Received January* 14, 1976

Effects of various diuretic agents in the mouse

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Diuretic effects of seven orally-acting diuretic agents have been examined in the mouse. The following compounds, examples of various types of orally active compound available, produced their characteristic diuretic effects: bendrofluazide, frusemide, ethacrynic acid, acetazolamide, triamterene, aminophylline and Su 15049A. The diuretic effects of the various agents were demonstrated under both water and saline-loading conditions. After allowing for differences in baseline sodium excretion, all diuretics except acetazolamide caused a further enhancement of sodium excretion after saline-loading compared with water-loading tests. The mouse possesses several advantages over the more commonly used rat since the range of diuretic responsiveness is greater. These results suggest that the mouse is a suitable species for diuretic testing.

The rat has been extensively used as the standard rodent species for the evaluation of diuretic agents but no systematic studies appear to have been carried out into the effects of currently-used diuretic agents in the mouse. We decided to investigate the oral effects of these agents in the mouse in order to establish if a technique for the evaluation of diuretic activity could be developed in this species. Such a method should be capable of demonstrating the effects of all known agents and the knowledge that ethacrynic acid had little activity in the rat (Beyer, Baer & others, 1965) but was active in the mouse (Baker, Hook & Williamson, 1965) indicated a possible advantage for the latter species.

To reduce experimental error, the evaluation of diuretic activity in the rat has usually been conducted under widely-varying conditions of water or saline loading (Ginsburg, 1964). We therefore compared the effects of a number of diuretics with differing modes of action, in the mouse under conditions of both water and saline loading.

MATERIALS AND METHODS

Materials and animals

Acetazolamide (Diamox powder), ethacrynic acid B.P., frusemide B.P. and triamterene B.P. were gifts from Cyanamid of Great Britain Ltd., Merck Sharp and Dohme Ltd., Hoechst Pharmaceuticals and Smith, Kline and French Laboratories Ltd., respectively. Su 15049A, the citrate of Su 15049 (2-[2,6-diphenyl-4(1-pyrrolidinyl)cyclohexyl]-pyridine), was a gift from Ciba-Geigy Ltd. The cellosize used was hydroxyethylcellulose, grade QP 15 000 obtained from Union Carbide U.K. Ltd.

Male mice of the strains and weights below were used:—Hough/Porton strain, 17–25 g; Tuck/T.O. strain, 15–27 g; Schofield/Schofield strain, 20–25 g; Scientific Products Farm/CS1 strain, 18–22 g.

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The cage, approximately 130 mm diameter by 130 mm high was made of stainless steel 6 mm square mesh with 25 mm wide (1 mm thick) stainless steel strips around the inner and outer sides of the base. The detachable lid was constructed of similar mesh with a similar strip as a lip. The cage was placed in a 300 mm diameter polythene funnel with a shortened stem (stem length 30 to 40 mm) and the funnel was supported in a frame. A baffle constructed from a watchglass by adding 3 glass 'legs' was placed near the apex of the funnel to prevent splashing and direct urine onto the funnel sides. Urine was collected in a conical graduated tube as it dripped from the tip of a hollow pear shaped glass urine/faeces separator suspended below the funnel outlet.

Methods

Mice were fasted overnight in groups of 35 but allowed free access to water. On the morning of the experiment the animals, which were used once only, were randomized into groups of 5 and placed in small cages for 1–2 h before dosing. The diuretic agents were given orally in a volume of 30 ml kg^{-1} as a suspension or solution in 0·25% cellosize in glass-distilled water (water-loading tests) or in 0·25% cellosize in 0·9% saline (saline-loading tests). Controls received a similar volume of vehicle alone. After dosing, the mice were placed in simple metabolism cages and urine collected in a graduated centrifuge tube for 3 h. The animals were then removed, the urine volume recorded, and urinary pH determined using suitable close-range indicator papers. The cages were rinsed with glass-distilled water and the washings added to the urine and made up to 25 ml with glass-distilled water. Aliquot samples were analysed for sodium and potassium content (Autoanalyser method II/07). Sodium and

potassium output have been expressed as mequiv kg^{-1} and urinary volume as % of the administered volume. For comparison all dose levels and several control groups were tested on the same day and the experiment repeated at least five times.

RESULTS AND DISCUSSION

In this paper we relate potency to the amount of drug required to produce a particular degree of sodium excretion, that is, a highly-potent drug is active at low dosage. Efficacy is related to the maximal sodium excretion which the diuretic is capable of inducing, that is, a highly-efficacious diuretic is capable of causing high sodium excretion.

Initially the effects of ethacrynic acid in different mouse strains were studied since different animal species vary widely in their response to this diuretic (Beyer & others, 1965) and strain differences in the diuretic response of mice to an oral water load have been reported by Stewart (1968). Table 1 shows the effect in four mouse strains of a submaximal dose of ethacrynic acid after water-loading. There was some variation in response, Hough/Porton mice showed the greatest sensitivity, but all responses were in the same general range. Therefore in subsequent experiments the Hough/Porton strain were used.

Table 1. *Diuretic effect of ethacrynic acid 30 mg kg^{-1} orally in different strains of male mice under water-loading conditions.*

Strain	Na^+ mequiv kg^{-1}	K^+ mequiv kg^{-1}	Urine vol. % fluid dosed	pH
Hough/Porton (6)	3·63 ± 0·11	1·33 ± 0·06	119 ± 6	6·4–6·8
Scientific Products Farm/CS1 (6)	2·83 ± 0·33	1·40 ± 0·20	92 ± 4**	6·4–6·7
Tuck/T.O. (10)	2·74 ± 0·12***	0·87 ± 0·06***	70 ± 5***	6·1–6·8
Schofield/Schofield (6)	2·19 ± 0·37**	0·87 ± 0·11**	67 ± 10***	6·4–7·1

Ethacrynic acid was given in a volume of 30 ml kg^{-1} in 0·25% cellosize in glass-distilled water. Urine was collected for 3 h. Figures in parentheses indicate the number of groups of 5 male mice. Values are mean ± s.e.; *** $P = <0·001$; ** $P = <0·01$; other values are not significantly different from Hough/Porton results.

The effects of the different types of diuretic agents after water-loading are shown in Table 2. Bendrofluazide, a thiazide diuretic thought to act at a site

Table 2. *Effects of various diuretic agents in water-loaded male Hough/Porton mice.*

Group	Dose mg kg^{-1} orally		Na^+ mequiv kg^{-1}	K^+ mequiv kg^{-1}	Urine vol. % fluid dosed	pH
Control	—	(15)	0·20 ± 0·03	0·45 ± 0·04	60 ± 4	5·8–6·8
Bendrofluazide	1	(5)	1·17 ± 0·22*	0·72 ± 0·12	70 ± 11	5·8–6·8
	10	(5)	1·58 ± 0·31*	1·06 ± 0·14**	87 ± 8**	5·8–6·4
	100	(5)	2·48 ± 0·33**	1·24 ± 0·13**	98 ± 3***	6·1–6·7
Frusemide	1	(5)	0·45 ± 0·07**	0·61 ± 0·13	60 ± 10	6·4–6·7
	10	(5)	3·97 ± 0·31***	1·30 ± 0·11***	160 ± 9***	6·1–6·7
	100	(5)	7·88 ± 0·23***	2·30 ± 0·08***	246 ± 4***	5·5–6·7
Ethacrynic acid	1	(5)	0·32 ± 0·10	0·44 ± 0·06	56 ± 9	6·1–6·7
	10	(5)	2·55 ± 0·30**	0·76 ± 0·06***	104 ± 6***	5·8–6·8
	100	(5)	5·74 ± 0·11***	1·71 ± 0·08***	162 ± 6***	5·8–6·4
Acetazolamide	1	(5)	0·38 ± 0·10*	0·70 ± 0·07**	56 ± 5	6·7–8·7
	10	(5)	1·59 ± 0·15***	1·28 ± 0·08***	89 ± 4**	8·0–8·7
	100	(5)	3·10 ± 0·51**	2·27 ± 0·22**	104 ± 4***	8·4–8·7
Triamterene	1	(5)	0·42 ± 0·08**	0·31 ± 0·08	59 ± 5	6·4–7·4
	10	(5)	1·19 ± 0·19**	0·33 ± 0·05	75 ± 9	6·7–7·4
	100	(5)	2·98 ± 0·33**	0·43 ± 0·07	90 ± 7**	7·1–7·7
Aminophylline	1	(5)	0·18 ± 0·04	0·49 ± 0·09	58 ± 6	6·4–7·1
	10	(5)	0·74 ± 0·20	0·72 ± 0·17	80 ± 10*	6·4–6·7
	100	(5)	2·60 ± 0·28***	1·20 ± 0·14**	101 ± 6***	6·1–6·8
Su 15049A	1	(5)	0·29 ± 0·08	0·50 ± 0·03	58 ± 4	6·1–6·8
	10	(5)	1·31 ± 0·30*	0·55 ± 0·08	91 ± 8**	6·1–6·8
	100	(5)	2·73 ± 0·33**	0·52 ± 0·04	112 ± 13*	6·1–7·4

The compounds were given in a volume of 30 ml kg^{-1} 0·25 % cellosize in glass-distilled water. Urine was collected for 3 h. Figures in parentheses indicate the number of groups of 5 male Hough/Porton mice. Values are means ± s.e.: ***$P = <0·001$; **$P = <0·01$; *$P = <0·05$, all other values are not significantly different from controls.

of urinary dilution in the cortical portion of the ascending limb of Henle's loop, was very potent and had the moderately efficacious action and kaliuretic effect typical of this class of diuretic. High potency in the mouse is similar to that seen in man for this particular compound. Frusemide and ethacrynic acid, diuretics believed to act on the medullary portion of the ascending limb of Henle's loop produced the typical highly-efficacious effects with associated kaliuresis and high urinary volume. Acetazolamide, which probably acts by inhibiting carbonic anhydrase at proximal and distal tubular sites, caused the characteristic excretion of alkaline urine, together with moderate amounts of sodium and a marked kaliuresis. Triamterene, thought to act by inhibiting sodium/potassium–hydrogen ion exchange at a site in the distal tubule, caused lower potassium excretion in association with moderate natriuresis and slightly-increased urinary pH. The mode of action of aminophylline is not clear but may involve effects on glomerular filtration, renal blood flow or tubular inhibition of sodium reabsorption. This agent was moderately potent, efficacious and kaliuretic in the mouse. Su 15049A, though not used clinically, is an interesting diuretic with a novel mode of action which seems to depend on the presence of circulating glucocorticoids (Gaunt, Renzi & others, 1967). This compound acts primarily on the proximal tubule and the potassium-sparing action of the drug is thought to result from enhanced distal-tubular reabsorption (Cohen & Cafruny, 1973). In our experiments, Su 15049A was moderately efficacious and potassium excretion remained within control values, despite increasing sodium excretion. This effect contrasts with that of triamterene which tended to cause potassium retention. Our results in the mouse regarding the lack of effect on potassium excretion, despite increasing sodium excretion are in agreement with those of other workers in rats (Gaunt & others, 1967) and dogs (Cohen & Cafruny, 1971).

Effects of diuretics after saline-loading are shown in Table 3. The characteristic actions of the various diuretic agents were also apparent in this type of test. It is interesting that, after saline-loading, triamterene caused a much higher excretion of

Table 3. *Effects of various diuretic agents in saline-loaded male Hough/Porton mice.*

Group	Dose mg kg^{-1} orally		Na^+ mequiv kg^{-1}	K^+ mequiv kg^{-1}	Urine vol. % fluid dosed	pH
Control	—	(15)	2·36 ± 0·34	0·79 ± 0·07	51 ± 7	6·1–7·1
Bendrofluazide	1	(5)	4·50 ± 0·67**	1·03 ± 0·15	82 ± 16	5·8–7·1
	10	(5)	5·99 ± 0·64***	1·13 ± 0·14*	103 ± 14**	5·8–6·8
	100	(5)	5·87 ± 0·51***	1·08 ± 0·09*	106 ± 9***	6·1–6·8
Frusemide	1	(5)	2·73 ± 0·82	0·86 ± 0·12	64 ± 15	5·8–7·1
	10	(5)	9·18 ± 0·51***	1·59 ± 0·12***	199 ± 10***	5·8–6·8
	100	(5)	13·59 ± 0·43***	2·33 ± 0·12***	307 ± 10***	6·1–6·8
Ethacrynic acid	1	(5)	2·48 ± 0·44	0·73 ± 0·15	53 ± 12	5·8–6·8
	10	(5)	4·27 ± 0·31**	0·90 ± 0·06	97 ± 6**	6·4–6·8
	100	(5)	10·75 ± 1·33**	2·28 ± 0·20***	251 ± 30**	6·1–6·7
Acetazolamide	1	(5)	2·31 ± 0·50	1·05 ± 0·26	53 ± 14	6·8–7·7
	10	(5)	3·88 ± 0·56*	1·40 ± 0·18**	68 ± 8	7·7–8·4
	100	(5)	4·53 ± 0·32**	1·91 ± 0·23**	89 ± 9*	7·7–8·4
Triamterene	1	(5)	2·37 ± 0·67	0·66 ± 0·21	53 ± 17	6·4–7·4
	10	(5)	3·68 ± 0·59	0·35 ± 0·11**	66 ± 17	6·8–7·4
	100	(5)	6·77 ± 0·67***	0·42 ± 0·07*	116 ± 7***	7·4–7·7
Aminophylline	1	(5)	2·38 ± 0·23	0·82 ± 0·16	48 ± 8	6·4–6·8
	10	(5)	4·55 ± 0·57**	1·20 ± 0·11**	88 ± 10*	6·7–7·1
	100	(5)	6·69 ± 0·49***	1·42 ± 0·17***	115 ± 14***	6·4–7·1
Su 15049A	1	(5)	3·61 ± 0·68	1·04 ± 0·29	76 ± 15	6·4–7·1
	10	(5)	4·72 ± 0·67**	0·96 ± 0·13	105 ± 20**	6·1–7·1
	100	(5)	5·89 ± 0·41***	0·73 ± 0·14	111 ± 13***	7·1–8·0

The compounds were given in a volume of 30 ml kg^{-1} 0·25 % cellosize in 0·9 % saline. Urine was collected for 3 h. Figures in parentheses indicate the number of groups of 5 male Hough/Porton mice. Values are means ± s.e.: *** $P = <0·001$; ** $P = <0·01$; * $P = <0·05$; all other values are not significantly different from controls.

sodium though potassium excretion was no higher than after water-loading. With Su 15049A potassium excretion was within control values in contrast to the potassium retention caused by triamterene.

As might be expected, saline loading (4·62 mequiv kg^{-1} Na^+) increased sodium excretion. The increase in excretion (2·16 mequiv kg^{-1} Na^+) compared to water-loaded animals was about half the amount of sodium given. After allowing for this baseline difference in sodium excretion it was apparent that all diuretics, with the exception of acetazolamide, caused a further increase of sodium excretion in saline-loaded compared to water-loaded animals. Acetazolamide caused similar, or less, natriuresis under saline loading conditions after allowing for the baseline difference. Many workers have demonstrated that increasing extracellular fluid space by intravenous saline loading, results in increased sodium excretion due to inhibition of reabsorption in the proximal tubule (De Wardener, 1973), an effect which may be due to a change in the concentration of a circulating hormone other than aldosterone, oxytocin or ADH. The increased sodium excretion after control oral-saline loading observed in our experiments may be due to activation of this mechanism. The further enhancement of sodium excretion by diuretics other than acetazolamide may also be related to this mechanism. The absence of enhancement after acetazolamide may perhaps be associated with its mode of inhibition of sodium reabsorption. Acetazolamide causes inhibition of carbonic anhydrase at proximal and distal tubular sites which is associated with the inhibition of the sodium/hydrogen ion exchange mechanism, whereas, the other diuretics probably inhibit the active transport of sodium or chloride by tubular cells.

Wiebelhaus, Brennan & others (1960) observed that, in the rat, both sodium output and urinary volume were similarly sensitive indicators of diuretic effect after saline-loading, but sodium output was the most sensitive after water-loading. These authors suggested that for preliminary testing, measurement of urinary volume alone was sufficient if saline loading was used. In our experiments sodium output generally tended to be the slightly more sensitive indicator and we consider that it is important to measure sodium output, potassium output and urinary volume.

The rat has been used extensively for the study of diuretics but we are not aware of a systematic study in the mouse. Our results show that the mouse responded to all the diuretics tested, including ethacrynic acid which is inactive in the rat. These agents had differing modes of action and, with those used clinically, responses in the mouse were similar to those in man. The mouse thus appears to be a suitable species for the evaluation of new diuretic agents and effects can be demonstrated under water- or saline-loading conditions.

Acknowledgements

We wish to thank the various Pharmaceutical Companies for gifts of standard diuretic agents and Dr R. Gaunt, Ciba-Geigy Ltd. for a supply of Su 15049A. The technical assistance of Mrs K. J. Foster is gratefully acknowledged. We are also indebted to Mr L. J. Hale for the electrolyte determinations.

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