J. Pharm. Pharmac., 1976, **28**, 552–554 *Received November 7, 1975*

A method for screening diuretic agents in the mouse: an investigation of sexual differences

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Acetazolamide, aminophylline, frusemide, ethacrynic acid and triamterene were tested for diuretic action at dosages of 3, 10 and 30 mg kg^{-1} (s.c.) in male and female mice. Each drug significantly raised sodium excretion and all but acetazolamide elevated urine volume and chloride excretion. Potassium excretion was significantly raised by acetazolamide and frusemide. Acetazolamide and triamterene evoked urinary alkalinization whereas frusemide and ethacrynic acid reduced urinary pH. Female mice were markedly more sensitive than males to the diuretic, natriuretic, chloruretic and urinary acidifying actions of ethacrynic acid.

In contrast to the rat (Ginsburg, 1964), the mouse is rarely used to examine compounds for diuretic activity, despite the observation that many drugs do not exhibit a common renal effect in the rat and man (Beyer, Baer, & others, 1965). It is known, however that in the mouse, diuretic responses result from water and electrolyte loading and natriuretic and anti-kaliuretic effects occur after spironolactone (Stewart, 1968, 1969). Further, ethacrynic acid significantly elevates urine, sodium, potassium and chloride excretion (Baker, Hook & Williamson, 1965).

As the oestrous cycle affects electrolyte excretion (Ginsburg, 1964) male rats are often preferred for diuretic tests (Lipschitz, Hadidian & Kerpcsar, 1943; Baer, 1964). However, Hwang & Goldberg (1959) consider female rats to give slightly more uniform excretion, finding several diuretics to have comparable effects in both sexes. We have investigated the value of the mouse for diuretic tests and compared the sensitivities of the two sexes.

MATERIALS AND METHODS

Mice of either sex (120/OLA strain) matched for age (mean weight 23 g) were deprived of food overnight, but allowed water to drink until commencing the experiment. On the following morning, the numbers of females in each stage of oestrus were determined from vaginal smears (Austin, 1969) in half the population selected at random. Acetazolamide B.P. (Lederle), aminophylline B.P. (Sigma Chemical), ethacrynic acid B.P. (Merck, Sharp & Dohme), frusemide B.P. (Hoechst) and triamterene (Dytac, Smith, Kline and French) were suspended in 0·25% methyl cellulose (Celacol) in distilled water by ball-milling for 1 h before injection. Two groups of animals (10 mice per group) were used for controls and at each dose level of a drug. The animals were weighed and given an oral saline (0·9%) load of 20 ml kg^{-1} followed by the drug (3, 10 or 30 mg kg^{-1}) or vehicle (20 ml kg^{-1}) subcutaneously. Each group was housed in a metabolic cage (Urimax, W. Eckold, St. Andreasberg, West Germany) and urine volumes were recorded over 6 h. The pH of each 6 h urine sample was immediately measured with a pH meter. The concentrations of sodium and potassium ions were measured with a flame photometer, and chloride ion estimations with a chloride meter (Models 150, 920, Corning-EEL, U.K.).

Statistical analysis took the form of analysis of variance of urine volumes, sodium, potassium and chloride excretion and urine pH. Three sources of variation were identified from control results and assessed for significance. These were: 'among experiments', 'between sexes' and 'experiment × sex interaction'. Similarly, five sources of variation were extracted from drug-treated groups: 'among doses', 'between sexes', 'drug × sex interaction', 'error within drug-treated groups' and 'control versus drug-treated groups'.

RESULTS

A high proportion (72·5 to 92·5%) of the females were in dioestrus. Urine volumes of controls varied significantly ($P < 0.001$) among experiments. Male animals excreted significantly larger volumes of urine ($P < 0.001$) in 6 h and significantly more potassium ($P < 0.01$) than did females (Table 1). The mean excretions of sodium and chloride ions, like that of potassium in males were approximately double those for females but these differences were not significant because of between group variances. Urine pH did not vary significantly with sex, but varied significantly ($P < 0.05$) among experiments.

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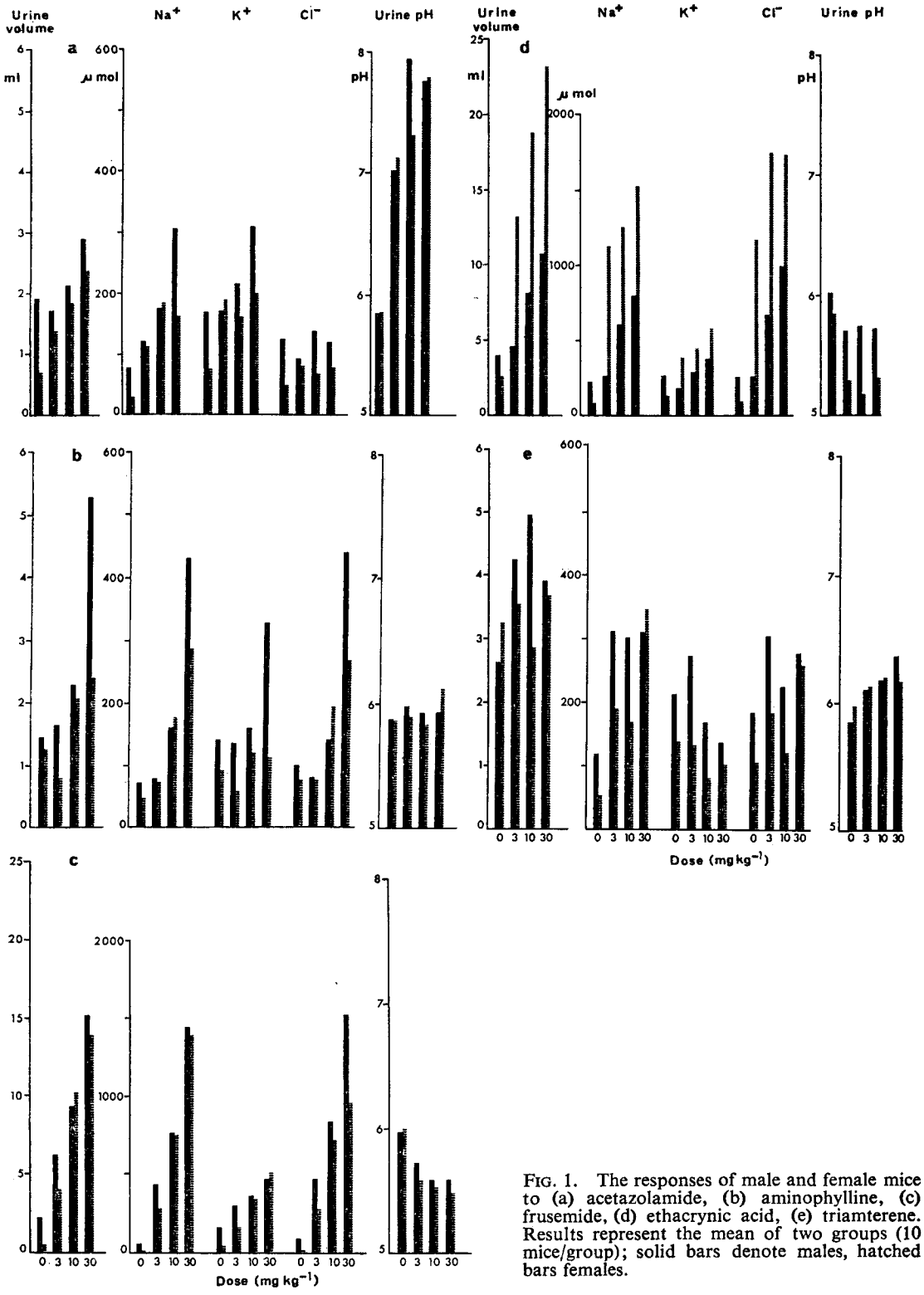

FIG. 1. The responses of male and female mice to (a) acetazolamide, (b) aminophylline, (c) frusemide, (d) ethacrynic acid, (e) triamterene. Results represent the mean of two groups (10 mice/group); solid bars denote males, hatched bars females.

Table 1. *Excretion by control male and female mice after 6 h.* Results given as means with s.e. of 5 experiments each containing two groups of 10 mice. Significance levels attained in analysis of variance and N.S. denotes not significant at $P = 0.05$.

	Male	Female	Significance between sexes
Urine volume (ml)	2·47±0·46	1·47±0·40	$P<0.001$
Sodium excretion (μ mol)	110·30±27·85	45·10±11·99	N.S.
Potassium excretion (μ mol)	189·70±21·71	86·85±16·57	$P<0.01$
Chloride excretion (μ mol)	154·85±32·35	70·31±14·72	N.S.
Urine pH	5·910±0·036	5·903±0·034	N.S.

Acetazolamide significantly increased sodium and potassium excretion ($P < 0.05$) and pH ($P < 0.001$) and tended to increase urine volume (Fig. 1a). Sodium and potassium elevations tended to be greater in the males given 30 mg kg^{-1} than in females but these differences were not significant.

Aminophylline evoked mild dose-related increases in the excretion of water and ions with little change in urine pH (Fig. 1b). The diuretic and kaliuretic effects of this agent were significantly ($P < 0.05$ and $P < 0.01$ respectively) greater in males than females. Further, a slight drug × sex interaction ($P < 0.05$) was observed for the kaliuresis.

Frusemide was followed by a dose-related diuresis (Fig. 1c), natriuresis, kaliuresis and chloruresis ($P < 0.001$) accompanied by a significant reduction in urine pH ($P < 0.01$). Although no significant difference between the responsiveness of the sexes to this drug was observed at 10 or 30 mg kg^{-1}, males tended to be more sensitive to its chloruretic action.

Only ethacrynic acid (Fig. 1d) evoked significantly greater elevations in urine and electrolyte excretion in females than in males ($P < 0.05$, except for kaliuresis). Similarly, urinary acidification was more marked ($P < 0.001$) in females.

Triamterene (Fig. 1e) produced significant diuresis, natriuresis, chloruresis and urinary alkalinization though dose-response relations were ill-defined. This agent did not systematically affect output of potassium. The natriuretic effect was greater in the males at low doses whereas the response of the females appeared to be greatest with high dosage.

DISCUSSION

The results indicate that the mouse is sensitive to drugs from several classes of diuretics. A significant increase in urine volume was found with all diuretics tested except acetazolamide and all showed a natriuretic effect indicating the importance of measuring sodium excretion in a primary diuretic screen.

Ethacrynic acid is a diuretic in the rat only when administered directly into the circulation in very high doses (Zins, Walk & others, 1968) but is a powerful diuretic in the dog (Beyer & others, 1965) and man (Goldberg, McCurdy & others, 1964). The present study confirms the findings of Baker & others (1965) that ethacrynic acid is a potent diuretic in the male mouse. Further, it shows the female is markedly more responsive than the male to the diuretic, natriuretic, chloruretic and urinary acidifying actions of this drug. On this basis it would seem preferable to use mice and in particular female mice rather than rats for the screening of certain kinds of diuretic agent.

Whether the greater sensitivity of the females is dependant on their being in dioestrus has not been investigated. It is possible that our animals were in a state of anoestrus induced by a high density of housing (85 to a cage) before the experiments (Van der Lee & Boot, 1955; Whitten, 1957).

Acknowledgement

We gratefully acknowledge the guidance of Mr. P. A. Young on the statistical analysis.

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