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On the pharmacological actions of a diuretic, fenquizone, with particular reference to its site of action

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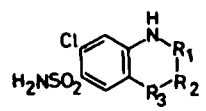
The pharmacological actions of a diuretic drug, fenquizone have been investigated and its effects compared with well characterized diuretics in rats, mice and rabbits. Changes in sodium and potassium excretion and urine volume were similar in magnitude and duration to those of the thiazide diuretics over dose range 0·05–100 mg kg^{-1}. Free water clearance in rabbits was decreased indicating an action at the cortical diluting site in the nephron and since free water reabsorption was relatively unaffected it appears unlikely to have actions at other sites. Calcium and phosphate excretion studies also suggested that the predominant effects are those occurring at the cortical diluting segment of the nephron. Additional parameters not affected by the drug were blood flow to the cortex and medulla of the kidney (and other major organs), plasma glucose concentration and plasma urate concentration.

Fenquizone [2-phenyl-6-sulphonamido-7-chloro-1,2,3,4-tetrahydro quinazoline-4-one, Idrolone, M.G. 13054, Maggioni Farmaceutici, S.p.A., Milan, Italy], a diuretic (Biressi et al 1969) with a close structural similarity to thiazide and thiazide-like diuretics, has been studied to determine whether it shared similar pharmacological properties with other drugs in its chemical group and to determine, as far as possible, the site or sites of action within the kidney at which it acts to produce its effects. These latter studies were based on clearance methods, the theoretical justifications and shortcomings of which have been discussed by Eknoyan et al (1978). An initial comparison was



	R_1	R_2	R_3
Fenquizone	$C-C_6H_5$	NH	CO
Quinethazone	C–Et	NH	CO
Hydrochlorothiazide	CH	NH	SO_2

made in small rodents using as comparators diuretics with well characterized sites of action. Then, free water clearance and free water reabsorption were examined to determine sites of action within the nephron—confirmatory data for which was obtained by studying calcium and phosphorus elimination.

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METHODS

Rat diuresis

Male Sprague-Dawley derived rats (180–245 g) were starved overnight and orally dosed the following morning with either: (i) 2·5 ml per 100 g of 1% carboxymethylcellulose/0·9% NaCl (saline); (ii) or drug at the stated concentration in the above suspension medium.

Urine was collected by placing individual rats on collecting funnels and measuring urine volume every hour over the next 3 or 5 h. Both control and drug-treated animals were housed in the same room and were subject to the same experimental conditions. The ionic composition, pH and osmolarity were determined after 3 h; urinary sodium and potassium concentrations were determined by flame photometry, osmolarity by a freezing point depression method, and pH from a standardized pH meter.

Calcium determination

The method was based on a fluorimetric technique by Waliach et al (1959), as modified in the Perkin-Elmer 1000 Fluorimeter handbook. The method is sensitive and the interference of other divalent cations, especially magnesium, is reported to be low.

Free water clearance

This was determined in a similar manner to that suggested for the rat by Stitzer & Martinez-Maldonado (1978). Female adult New Zealand white rabbits 2–3 kg, had food withdrawn overnight and

5% glucose solution substituted for drinking water. The following day a stomach tube was inserted and a water load of 50 ml was administered. After 15 min the animal was anaesthetized with 50 mg kg^{-1} pentobarbitone sodium (Sagatal M & B) administered slowly into the marginal ear vein. The jugular vein, carotid artery, trachea and either the bladder or both ureters were cannulated. The jugular cannulae served as the main route of administration for the infusion of drug + saline and the carotid cannula was connected via a three way tap to a pressure transducer so that blood pressure could be monitored and blood samples withdrawn. Urine volume was recorded by means of an electric drop counter. After surgery, the animal was allowed to become stabilized on a slow infusion of saline and then perfusion began with 0·5% NaCl and at a rate of either 2·2 ml min^{-1} or 3·3 ml min^{-1} depending on animal size. Urine osmolarity was checked at intervals and stable free water clearance assumed when successive osmolar values of less than 250 mM were obtained. At this stage a blood sample was withdrawn from the arterial cannula, centrifuged and a sample of plasma taken to determine plasma osmolarity. The drug under test was then administered via the venous cannula and the osmolarity readings repeated at urine peak flow, usually after about 10 min. Animals in which a steady free water clearance could not be obtained were rejected from the study.

Free water reabsorption

These determinations were made using a similar procedure but with the following amendments. Firstly, no glucose or water was given. Secondly, after surgery 1 unit kg^{-1} of synthetic vasopressin (Syntopressin, Sandoz) was administered by subcutaneous injection. Finally, saline infusion was transferred to 2% NaCl solution once urine flow was stabilized. Drug administration was made when urine osmolarity became established and this was always in excess of plasma osmolarity.

Other measurements

Blood glucose concentrations were determined after dosing mice with either a single dose of fenquizone (10 mg kg^{-1}) or five daily doses. Blood and urine urate concentrations were also measured. Diuretic and natriuretic responses in the rat were determined after bilateral adrenalectomy. Kidney (and other organ) blood flow was determined 1 h after a single dose of 50 mg kg^{-1} fenquizone by means of a radioactively labelled microsphere technique (Foy & Lucas 1977).

RESULTS

Fig. 1(a) shows the urine output in rats over 5 h expressed as a percentage of the administered saline load. The output after fenquizone was statistically indistinguishable from that after hydrochlorothiazide or quinethazone but significantly slower and less than after frusemide—all administered at 50 mg kg^{-1}. Fig. 1(b) shows a dose-response relationship for sodium and potassium output measured at 3 h.

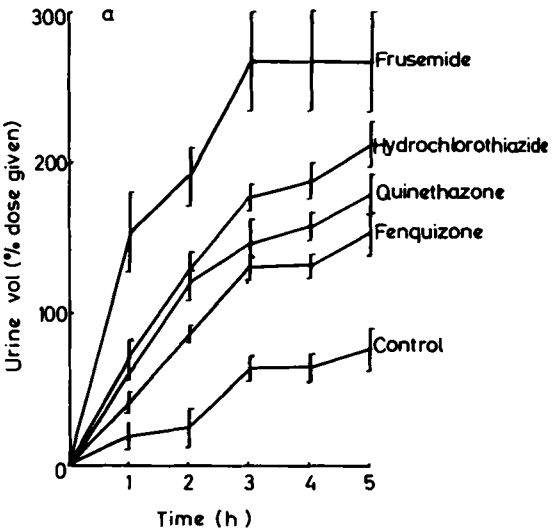

FIG. 1(a). Urine output in the rat over 5 h after receiving 50 mg kg^{-1} of the drug shown + 25 ml kg^{-1} 0·9% sodium chloride solution/1% carboxymethylcellulose. (Mean ± s.e.m., n = 6–8).

After fenquizone, calcium in the urine was below the level of detection (Table 1) and, unlike frusemide, it did not rise above the level of controls. Phosphorus output after fenquizone was similar to control values.

Table 2 shows the results for free water clearance (C_{H_2O}) and free water reabsorption ($T^C_{H_2O}$) calculated from separate rabbit experiments and divided by a suitable delivery term, in this case, volume flow (V), before and after the administration of fenquizone. In all cases C_{H_2O}/V was reduced while $T^C_{H_2O}/V$ was either not altered or rose. In the latter instance subsequent administration of either frusemide or bumetanide brought about a dramatic fall in $T^C_{H_2O}$.

After acute administration to the rat, fenquizone did not modify kidney blood flow or any other area of regional blood flow measured. Mice dosed acutely with 10 mg kg^{-1} fenquizone or with the same dose of the drug over 5 days showed no significant differences in plasma urate or glucose concentrations nor were

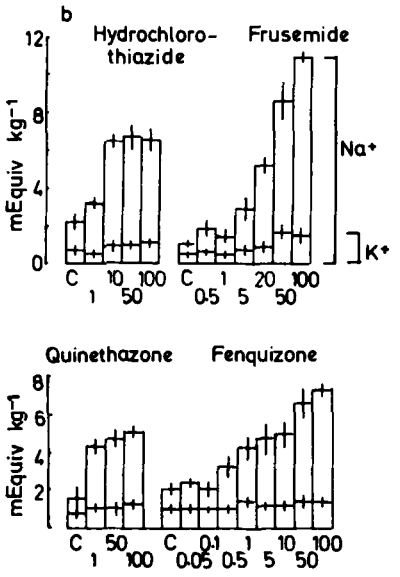

FIG. 1(b). Sodium and potassium output in the rat 3 h after receiving the dose of diuretic shown + 25 ml kg^{-1} 0·9% sodium chloride solution/1% carboxymethylcellulose. (Mean ± s.e.m., n = 6–8).

there detectable differences in the amount of urate excreted in the urine. Adrenalectomy in the rat did not abolish the diuretic or natriuretic response to fenquizone.

DISCUSSION AND CONCLUSIONS

There are believed to be four principle sites in the nephron where diuretics exert their action: Site 1—the proximal nephron which includes the proximal convoluted tubule and pars recta; site 2—the medullary diluting segment comprising the thick and possibly the thin ascending limb of Henle; site 3—the cortical diluting segment and includes the cortical portion of the ascending limb which runs into the first portion of the distal convoluted tubule; site 4—the collecting duct which contributes to both urinary concentration and dilution. By comparing the clearance of suitable substances the sites of action of diuretic drugs may be determined (Eknoyan et al 1978).

The results show that the excretion of sodium is increased after fenquizone in a dose-related manner. Both the magnitude of the increase in sodium excretion and the duration and pattern of the diuresis more closely resembles that following quinethazone and hydrochlorothiazide than that due to frusemide. Similarly, potassium excretion resembles that following quinethazone and hydrochlorothiazide, is modest and does not parallel sodium output with increase in

Table 1. Excretion of calcium, sodium and phosphate in rat urine after either saline alone (control) or 50 mg kg^{-1} fenquizone or frusemide 3 h previously. Mean ± s.e.m., number of animals in parentheses.

	Control	Fenquizone	Frusemide
mg Ca^{2+}	0·99 ± 0·76 (3)	Below detect. (3)	1·58 ± 0·12 (6)
m equiv Na^+ kg^{-1}·13	1·59 ± 0·66 (6)	6·64 ± 0·81 (6)	11·0 ± 0·22 (6)
mg PO_4 kg^{-1}	82·6 ± 14 (6)	79·4 ± 15 (6)	N.D.

Table 2. Free water clearance (C_{H_2O}) values from individual, hydrated anaesthetized rabbit experiments and free water reabsorption ($T^C_{H_2O}$) values from individual hydropenic anaesthetized rabbits.

	$\frac{C_{H_2O}}{V}$ Before drug	$\frac{C_{H_2O}}{V}$ After drug
Fenquizone 1 mg	0·13	—0·13
,,	0·38	0·12
,,	0·40	0·11
,,	0·57	—0·57
,,	0·15	0·03
Fenquizone 10 mg	0·32	0·01
Bumetanide 0·25 mg	0·32	0·07
	$\frac{T^C_{H_2O}}{V}$ Before drug	$\frac{T^C_{H_2O}}{V}$ After drug
Fenquizone 0·1 mg	0·31	0·52
Fenquizone 1 mg	0·27	0·38
,,	0·55	0·52
,,	0·43	1·0
,,	0·36	0·41
Frusemide 0·5 mg	0·43	—0·01

Table 3. Cardiac output and regional blood flow (ml min^{-1}/100g) in the anaesthetized rat approximately 1 h after oral dosing with 50 mg kg^{-1} fenquizone or suspending agent as control. (Mean ± s.e.m., n = 6.)

	After fenquizone	Control
Heart	175 ± 32	174 ± 31
Liver	6·4 ± 1·0	5·4 ± 0·9
Pancreas	40 ± 3·3	47 ± 6·0
Spleen	59 ± 7·1	73 ± 12
Stomach	14·6 ± 0·8	17 ± 3·4
Small intestine	75 ± 5·5	94 ± 14
Large intestine	35 ± 5·4	54 ± 8·4
Abdominal skin	5·9 ± 0·9	6·4 ± 0·8
Abdominal muscle	6·3 ± 0·9	7·4 ± 1·4
Adrenals	383 ± 72	516 ± 110
Kidney cortex	504 ± 34	488 ± 39
Kidney medulla	49 ± 2·9	46 ± 5·5
Cardiac output (ml min^{-1})	49 ± 3·0	52 ± 2·7

dose. pH values are not markedly increased or decreased from control values and would not support fenquizone having any carbonic anhydrase inhibitory action as found with thiazides (Maren 1967). A similar pattern of diuretic response was also seen in the mouse. The principle site of action of the thiazides is at site 3. The free water clearance (C_{H_2O}) and reabsorption ($T^c_{H_2O}$) studies confirmed this pattern of activity. Fenquizone consistently decreases free water clearance, whereas site 1 diuretics such as acetazolamide increase free water clearance. Fenquizone therefore probably acts further along the nephron. Fenquizone does not cause a reduction in the free water reabsorption and so probably does not have a major action at sites 2 or 4 unlike frusemide which is mainly a 'loop' diuretic. All clearance studies depend for their interpretation on little or no alterations in glomerular filtration rate. Such alterations were not evident in preliminary studies carried out by the manufacturers (personal communication) nor were there significant changes in blood pressure which might bring about such alterations. Furthermore, blood flow studies reported here indicate that fenquizone is without obvious effect on blood flow to the kidney medulla or any other major organ.

The supportive studies also indicate a site 3 action. Phosphate excretion is mainly proximal and since fenquizone does not increase phosphate clearance, it is unlikely that it has a major action here. Similarly, the excretion of calcium following the administration of fenquizone is interesting. Urinary levels are low, a similar pattern to that recorded for thiazide type diuretics (Costanzo & Weiner 1974; Higgins et al 1964). The reasons for this are not fully understood but in practice the failure of a diuretic to increase calcium excretion as sodium excretion rises indicates that it probably exerts a major effect on the cortical diluting segment (Suki et al 1973). Fenquizone acts in such a manner.

The lack of any hyperglycaemic or hyperuricaemic effect in these experimental results is interesting in view of the known tendency for thiazides to reduce glucose tolerance over the long term and to precipitate gout. However, the results indicate that fenquizone has a similar site of action to the thiazides exerting its principle effects at site 3 in the nephron.

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