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SPECIAL FEATURES OF THE ACTION OF A NEW DIURETIC

BY

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Among the most important of the new therapeutic agents introduced in the last few years are the substituted heterocyclic sulphonamides, most of them thiazide compounds. Reports have recently appeared of pharmacological investigations (Muschawek & Hajdu, 1964) and clinical trials (Kleinfelder, 1963) of a new diuretic which, while being a substituted sulphonamide, has not the same chemical constitution as the thiazides. This compound is frusemide (Lasix; Farbwerke Hoechst AG). We have investigated its diuretic effect by various routes of administration, its influence on carbohydrate metabolism and lastly its effects after liver by-pass. Hydrochlorothiazide was used as the standard of comparison.

METHODS

Male albino rats weighing from 200 to 250 g were used. They were fed on rat cubes (Lembeck, 1953) and were given water *ad libitum*.

The dose/effect relationship was worked out using groups of ten rats. For the testing of each individual dose, at least two different groups were used, each dose being administered twice to each group. During the diuresis testing period the animals were maintained without food or water on wire grids in metabolism cages made of stainless steel sheet. The urine produced in the course of 6 hr was collected in measuring cylinders and its potassium and sodium contents were determined by flame photometry and chloride by titration.

Frusemide was suspended in a 1% solution of carboxymethylcellulose in water; 50 ml./kg of this suspension was given by oesophageal tube. For comparison the same amount of pure carboxymethylcellulose solution was also given. Each group of rats was used for a test only on alternate weeks; in the intervening week the values were recorded to provide a baseline which was taken as 100. When, after a given dose of frusemide, a sodium output of, for example, 500 is shown in the illustrations, this means that five-times more was excreted than in the control experiments.

The dose/effect relationship for the intraperitoneal route was worked out by the same method as for the oral route. The substance was injected in aqueous solution in a uniform volume of 1.0 ml./100 g body weight, the concentration being adjusted to provide the required dose. Immediately after intraperitoneal injection of the substance being tested, the rats were given 50 ml./kg of distilled water by mouth. In the control experiments the rats received the same quantity of water alone by mouth.

During the experiments devised to test the effect of frusemide on carbohydrate metabolism, the animals were kept on wire grids in individual metabolism cages made of glass, and the urine was collected. Blood was obtained either by opening the great vessels of the neck or, if several samples were necessary, by puncturing the retrobulbar venous plexus. The glucose contents of serum and urine were determined by the enzymatic method of Keston (1956) and Teller (1956). The glucose (20% aqueous solution) and the frusemide (suspended in 1% carboxymethylcellulose solution) were administered to the rats by an oeso-

phageal tube; insulin (soluble insulin " novo," 0.08 to 0.16 I.U./kg body weight) was given by intraperitoneal injection. A uniform dose of frusemide, 40.0 mg/kg, was given in all instances. This had previously been shown to be the optimum oral dose for diuretic effect. The values given in Results are the means and standard deviations for groups of five animals.

Experimental isolation of the liver from the circulation was achieved. The liver was excluded from the circulation by tying off its blood supply. In order to avoid congestion in the portal area, the entire blood supply of the gut had to be cut off at the same time. The animals were injected intraperitoneally with ethylurethane, 1.2 g/kg body weight, as a 20% aqueous solution, and 30 min later the operation was started, when opening the peritoneum caused no reaction in the animal.

With the anaesthesia described, the rate of respiration as well as the cardiac action remained constant for the whole period between completion of the operation and the end of the experiment. After midline laparotomy the first step was to tie the coeliac and superior mesenteric arteries which arise close together from the aorta. The small inferior mesenteric artery which runs upwards along the rectum was ligated together with its companion vein and the rectum itself. After the blood flow into the portal area had been cut off by these ligatures, the hepatoduodenal ligament was tied together with the vessels running in it. A ligature was also tied round the cardia so as to cut off the last venous connexions between the portal and systemic circulations.

In addition to its simplicity, this method had the advantage of being free from the haemodynamic disturbances which are unavoidable after the establishment of an acute Eck fistula, especially in small animals. The operation took approximately 1 to 2 min, and for the next 2 hr the arterial blood pressure remained practically unchanged from the preoperative level; subsequently it fell gradually.

To confirm the complete exclusion of the liver from the circulation, Indian ink was injected intravenously into a few animals. In animals which had not been operated on there was a distinct blackening of the liver visible to the naked eye. When the liver had been excluded from the circulation no macroscopic discoloration was observed. Histologically there was very slight carbon deposition near the central veins. This carbon was presumably derived from retrograde blood flow driven into the hepatic veins by the massaging effect of diaphragmatic movements.

When the liver had been excluded from the circulation, the urethra was clamped, the bladder opened and a polyvinyl catheter to which a pipette was connected was tied into the bladder. This enabled all the urine excreted to be collected and its quantity to be measured. Finally, the abdomen was closed with two or three sutures. The control animals were anaesthetized in the same way as the others; laparotomy was then carried out and a catheter tied into the bladder. The substances to be tested were given intravenously.

As the amount of urine excreted by anaesthetized rats in the course of 3 hr does not contain sufficient electrolytes, sodium, potassium and chloride for quantitative estimation, the animals were given an intravenous infusion of 0.9% saline into a jugular vein at a rate of 0.2 ml./min. This infusion was maintained for 3 hr and the urine produced in this period was examined. We did not consider it advisable to continue the experiments for longer periods as most of the animals under these conditions began to show a fall in blood pressure, and it was no longer a valid assumption that urine production was normal.

RESULTS

Relationship between oral dose of frusemide and diuretic action

As can be seen from Fig. 1, frusemide in doses of up to 5 mg/kg had no significant diuretic effect. In a dose of 10 mg/kg slight diuresis was observed; the maximum effect was obtained with a dose of 40 mg/kg. At this dose sodium excretion rose to approximately ten times the control level, while potassium excretion increased by only approximately 3.5-times. In a dose of 80 mg/kg the effect of frusemide on sodium excretion was considerably less than in doses of 40 mg/kg. However, at this dose level potassium excretion was still approximately three times higher than in the control animal. In the range of effective diuretic dosage (20 to 40 mg/kg) the ratio of sodium to potassium excretion was unequivoc-

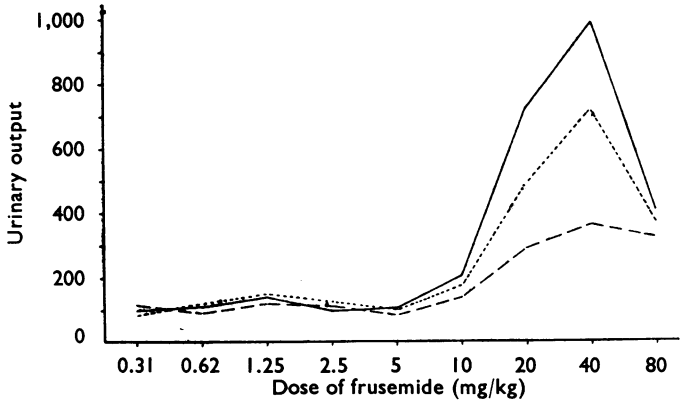

Fig. 1. Sodium, potassium and chloride excretion after oral administration of various doses of frusemide (the excretion of these three ions in untreated control animals is taken as 100). ——, Sodium; – – – –, potassium; and . . . . . , chloride.

ably shifted in favour of sodium excretion or, in other words, the excretion of potassium remained significantly lower than that of sodium. When the dose was increased above this optimum range, the effect on this ratio was distinctly unfavourable, that is sodium excretion became considerably less while potassium excretion remained almost as high as before.

Relationship between intraperitoneal dose of frusemide and diuretic action

Given by this route, frusemide had a slight diuretic effect even in doses as low as 0.15 mg/kg (Fig. 2). The maximal effect was observed after injection of 2.5 mg/kg, when sodium excretion was approximately ten times and potassium excretion approximately five times higher than in controls. The administration of higher doses produced a distinct reduction in sodium and potassium excretion, both electrolytes being affected to approximately the same extent.

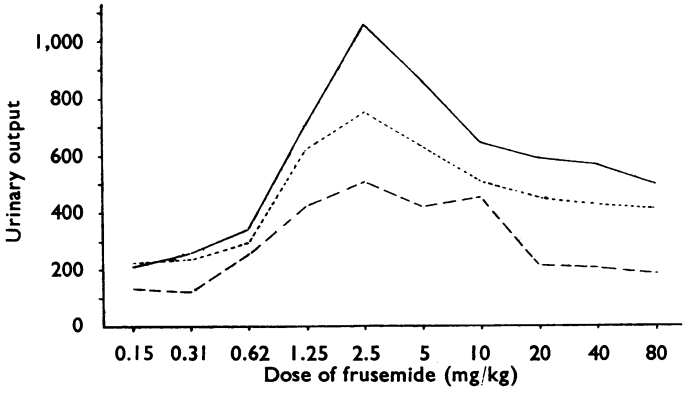

Fig. 2. Excretion of sodium, potassium and chloride after intraperitoneal administration of various doses of frusemide (the excretion of these three ions in untreated control animals is taken as 100). ——, Sodium; – – – –, potassium; and . . . . . , chloride.

The effects of frusemide on carbohydrate metabolism

Effects of frusemide on blood and urine sugar levels. The first step was to test the action of a single oral dose of 40 mg/kg of frusemide in control animals which had not received a loading dose of glucose. In this dose the substance has no effect on the blood sugar level and does not even cause glycosuria (Table 1).

TABLE 1

SERUM AND URINE GLUCOSE CONCENTRATIONS, COMPARED WITH NORMAL LEVELS, 1 HR AFTER ADMINISTRATION OF GLUCOSE, GLUCOSE AND FRUSEMIDE, AND FRUSEMIDE

Values are means and standard deviations for serum glucose, and ranges for urine glucose.
* Given in 50 ml./kg of water

Treatment	Serum glucose after 1 hr (mg/100 ml.)	Urine glucose after 1 hr (mg/100 ml.)
Glucose, 10 g/kg*	195±18	2–461
Frusemide, 40 mg/kg,+ glucose, 10 g/kg*	254±23	8–500
Frusemide, 40 mg/kg	100±3	0
None	98±2	0

After administration of 50 ml./kg of 20% glucose solution by mouth the serum glucose level rose from 98 to 195 mg/100 ml. within the first hour. The urine glucose levels were between 2 and 461 mg/100 ml. If 40 mg/kg of frusemide had been given at the same time as the glucose a mean serum glucose level of 254 mg/100 ml. was found and the urine glucose levels were between 8 and 500 mg/100 ml.

Effect of frusemide on the hypoglycaemic action of insulin in healthy animals. The hypoglycaemic action of insulin (0.08 and 0.16 U./kg, intraperitoneally) was determined in a series of preliminary experiments. At 1.5 hr after a dose of 0.08 U. of insulin the serum glucose level was 80 mg/100 ml.; after a dose of 0.16 U. it was 22 mg/100 ml. In both cases the serum glucose at the outset was 100 mg/100 ml. When frusemide, 40 mg/kg, was given at the same time as insulin, 0.08 U./kg, the serum glucose did not fall; when 0.16 U./kg of insulin was given together with 40 mg/kg of frusemide the serum glucose fell to 32 mg/100 ml., compared with the value of 22 mg/100 ml. after the same dose of insulin alone. The results of these investigations are summarized in Table 2.

TABLE 2

CONCENTRATION OF SERUM GLUCOSE BEFORE AND AFTER ADMINISTRATION OF INSULIN, OR OF INSULIN AND FRUSEMIDE

The difference between insulin (0·08 U.) and insulin (0·08 U.) + frusemide and that between insulin (0·16 U) and insulin (0·16 U.) and frusemide are highly significant ($P<0·001$). Values are means and standard deviations

Treatment	Serum glucose (mg/100 ml.)	
	Before	1·5 hr after
Insulin, 0·08 U.	102±4	80±2
Insulin, 0·08 U., +frusemide, 40 mg/kg	100±3	102±3
Insulin, 0·16 U.	99±2	22±2
Insulin, 0·16 U.,+frusemide, 40 mg/kg	100±4	32±3

Comparison of the diuretic actions of frusemide and hydrochlorothiazide given intravenously to animals with liver by-pass

In untreated rats liver by-pass results in a considerable reduction in sodium and chloride excretions compared with the levels in rats without liver by-pass. This reduction in sodium and chloride excretions is probably attributable to the diminished volume of urine secreted during the period of the experiment after the establishment of liver by-pass (Table 3). Potassium excretion after liver by-pass is very low. As was expected, in animals without liver by-pass, a dose of 2 mg/kg of hydrochlorothiazide evoked a distinct diuresis compared with the response of untreated controls. Frusemide in the same doses had a considerably smaller effect. When the two drugs were compared in animals with liver by-pass it was

TABLE 3

URINE VOLUME AND TOTAL ELECTROLYTE EXCRETION DURING 3 HR AFTER THE ADMINISTRATION OF FRUSEMIDE, OR OF HYDROCHLOROTHIAZIDE

The numbers were derived from five rats with and five rats without liver by-pass. Values are means and standard deviations

Treatment	Excretion in 3 hr of			
	Sodium (μequiv)	Potassium (μequiv)	Chloride (μequiv)	Urine (ml.)
Without liver by-pass				
None	95± 7	68± 8	174±12	0·86±0·09
Frusemide, 2 mg/kg	161±11	92± 9	268±21	4·5 ±0·6
Hydrochlorothiazide, 2 mg/kg	556±36	102±10	680±42	2·5 ±0·2
With liver by-pass				
None	56± 6	8±0·5	70± 8	0·46±0·03
Frusemide, 2 mg/kg	390±28	50± 8	465±40	5·4 ±0·4
Hydrochlorothiazide, 2 mg/kg	226±15	38± 3	274±23	1·7 ±0·2

evident that sodium excretion after frusemide was considerably higher than after hydrochlorothiazide. The relative effectiveness of hydrochlorothiazide and frusemide was thus reversed by liver by-pass (Table 3).

DISCUSSION

From the results it is clear that frusemide differs in its action from hydrochlorothiazide both quantitatively and qualitatively. In earlier investigations (Formanek & Höller, 1961) it was established that hydrochlorothiazide given by mouth attained its maximum effect in a dose of 1.25 mg/kg. A few spot checks on random samples showed that our previous results were applicable to the animals used in the present experiments. Frusemide does not have the same diuretic effect on sodium excretion until the dose is raised to 20 mg/kg. It must be admitted that, when frusemide is given in this dose, potassium excretion is approximately twice as high as after hydrochlorothiazide, 1.25 mg/kg. In this connexion it should, however, be pointed out that, when hydrochlorothiazide was given in the doses used in the earlier investigations, it was not possible to attain the extraordinarily high levels of sodium output reached after frusemide, 4 mg/kg. Similar results have been published by Timmerman, Springman & Thomas (1964).

Hydrochlorothiazide gives its maximum effect on sodium output in a dose as low as 1.25 mg/kg; in this dose it increases sodium output approximately seven times compared with untreated controls. Frusemide does not exert its maximum effect until given in a dose of 40 mg/kg, but at this dose it increases sodium output by approximately ten times compared with controls. It is a striking fact that with both compounds an increase beyond the

maximum effective dose causes a distinct shift in the proportions of sodium and potassium excreted; sodium output falls sharply while potassium output remains the same or may even increase.

This investigation of the dose/effect relationships of frusemide and hydrochlorothiazide when given by mouth has thus demonstrated that frusemide, when administered to rats in the way described, must be given in doses approximately sixteen times higher than hydrochlorothiazide in order to achieve the same sodium output. It is of great interest that after intraperitoneal administration the results are totally different. Given by this route, frusemide (see Figs. 2 and 3) is distinctly more active than hydrochlorothiazide as regards

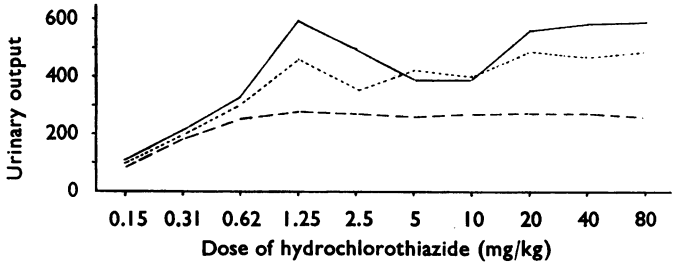

Fig. 3. Excretion of sodium, potassium and chloride ions after intraperitoneal administration of various doses of hydrochlorothiazide (the excretion of these three ions by untreated control animals is taken as 100). ——, Sodium; – – – –, potassium; and . . . . . , chloride.

sodium output. Quite apart from the considerable variations in the effects of frusemide on sodium excretion produced by changing the route of administration, the two modes of administration of the drug are remarkably different in their effects on potassium excretion. After an oral dose of 40 mg/kg of frusemide approximately ten times more sodium is excreted than in untreated controls. By intraperitoneal injection a dose of 2.5 mg/kg has approximately the same effect. Potassium excretion after oral administration of 40 mg/kg of frusemide is only about 3.5-times higher than in the controls, but after intraperitoneal administration of 2.5 mg/kg of frusemide it rises to approximately five times the control level. Intraperitoneal administration thus causes more potassium loss than does oral administration, sodium output being kept constant.

In its effects on carbohydrate metabolism frusemide shows no essential qualitative differences from hydrochlorothiazide, although it seems to be considerably less active than the latter. As was established in previous investigations (Formanek, 1963), the optimum diuretic dose of 1.25 mg/kg of hydrochlorothiazide causes a significant fall in glucose tolerance after oral glucose loading. In the earlier experiments administration of hydrochlorothiazide at the same time as the oral glucose load resulted in serum glucose levels approximately 200 mg/100 ml. higher than after administration of the same dose of glucose alone. This may be compared with a rise in serum glucose of only approximately 60 mg/100 ml. after simultaneous administration of frusemide and glucose. Moreover, the decrease in the action of insulin after frusemide is far less pronounced than after hydrochlorothiazide (Formanek, 1963).

The most noteworthy finding is the difference in diuretic efficiency between frusemide and hydrochlorothiazide in animals with liver by-pass. From the results reported above,

it is clear that the liver plays an important part in the diuretic action of hydrochlorothiazide, while this does not seem to be true for frusemide. In this connexion it must not be forgotten that the technique of liver by-pass described results in a considerable reduction of circulating blood volume and that, dosage being constant, the drug will reach the target organ, in this case the kidney, in higher concentration. Any substance which does not first require to be converted into an active form in the liver must therefore have a more powerful effect in animals with liver by-pass than in normal animals. This is obviously true of frusemide and, by contrast, not true of hydrochlorothiazide.

SUMMARY

1. Experiments in rats to investigate the actions of a new diuretic, frusemide, revealed certain unusual features in its mechanism of action and certain differences from hydrochlorothiazide.

2. Frusemide differs from hydrochlorothiazide in that the mode of administration has a marked effect on its diuretic action. To achieve approximately the same sodium output the oral dose of frusemide required is about sixteen times higher than that of hydrochlorothiazide. On the other hand, by intraperitoneal injection frusemide is distinctly more active than hydrochlorothiazide. Given in optimum doses by both routes of administration, frusemide produced considerably higher levels of sodium output than corresponding doses of hydrochlorothiazide.

3. The route of administration—oral or intraperitoneal—of frusemide also has an important influence on potassium excretion. If the doses are adjusted so that sodium output is the same, potassium loss will be considerably greater after intraperitoneal than after oral administration.

4. In its effects on carbohydrate metabolism frusemide shows no essential qualitative differences from hydrochlorothiazide. However, assuming that the reduction of glucose tolerance after oral glucose loading is a measure of decrease in insulin effect, frusemide seems to be considerably less active than hydrochlorothiazide.

5. Experiments on rats after liver by-pass showed a considerable impairment of the diuretic efficiency of hydrochlorothiazide given parenterally, though this was not true of frusemide. It is clear that the liver plays an important part in the diuretic action of hydrochlorothiazide; this does not seem to apply to frusemide.

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