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Probenecid potentiates the hyperglycaemic effect but reduces the diuretic effect of frusemide in mice

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1 The effect of probenecid on frusemide-induced diuresis and hyperglycaemia was studied in mice.

2 Probenecid, a known inhibitor of tubular secretion of organic anions in the kidney, strongly reduced the diuretic response to frusemide (25 or 200 mg kg^{-1} body weight). This effect of probenecid appeared to be dose-dependent up to 240 mg kg^{-1} body weight, at least at the lower concentration of frusemide.

3 Pretreatment with probenecid (240 mg kg^{-1} body weight) potentiated the hyperglycaemic effect of frusemide (25 or 200 mg kg^{-1} body weight).

4 The results show that probenecid has opposite effects on frusemide-induced diuresis and hyperglycaemia in mice. It is suggested that the acute hyperglycaemic effect of frusemide is not directly linked to diuresis.

Introduction

Frusemide is extensively used in clinical practice as a potent diuretic drug (Atkins, 1966; Cutler & Blair, 1979; Valmin *et al.*, 1980). However, it is well known that it also induces hyperglycaemia (for review, see Furman, 1981) and in some cases causes diabetes mellitus (Mustala & Toivonen, 1965; Jones & Pickens, 1967; Walsh & O'Sullivan, 1974).

Several studies on the diabetogenic action of frusemide in laboratory animals have been published. The early investigations by Senft (1966) showed that neither single-dose nor long-term administration of frusemide (200 mg kg^{-1} body weight) to rats affected serum glucose or serum insulin levels. Later studies have indicated that single-dose administration to mice (Foy & Furman, 1971) or rats (Wales *et al.*, 1968; Aynsley-Green & Alberti, 1973; Wexler, 1981) can elicit a transient hyperglycaemia. The precise reasons for the diabetogenic action in laboratory animals are unclear, although several mechanisms have been proposed. Thus it has been suggested that hyperglycaemia occurs secondary to renal effects; diuresis-induced hypotension could result in the release of catecholamines followed by glycogenolysis (Aynsley-Green & Alberti, 1973; Foy & Furman, 1973). An alternative suggestion is that diuresis-induced disturbances of serum electrolyte concentration may cause the change in carbohydrate metabolism (Aynsley-Green & Alberti, 1973).

Frusemide is actively secreted from the plasma into the renal tubuli (Bowman, 1975), where it exerts its major action by inhibiting the active re-absorption of chloride in the thick ascending loop of Henle (Burg *et al.*, 1973). The secretion of frusemide from plasma into the tubuli can be blocked by co-administration of probenecid (Bowman, 1975), which is an effective blocker of tubular secretion of organic anions. Pretreatment with probenecid abolishes the diuretic response to frusemide in dogs (Hook & Williamson, 1965) and in cats (Friedman & Roch-Ramel, 1977). In humans, pretreatment with probenecid decreases renal and non-renal clearance of frusemide (Homeida *et al.*, 1977; Honari *et al.*, 1977; Chennavasin *et al.*, 1979) with an increase in the plasma concentration and half-life of the drug (Homeida *et al.*, 1977; Honari *et al.*, 1977).

The hypothesis that the diabetogenic effect of frusemide might be secondary to the renal action of the drug can be tested by investigating whether blockage of the diuretic effect of frusemide affects the diabetogenic action of the drug. In the present study

we have therefore investigated whether pretreatment with probenecid affects the diuretic and hyperglycaemic effect of frusemide in mice.

Methods

Animals

Adult non-inbred, female mice (lean litter-mates from the breeding of Umeå *ob/ob* mice) were used in all experiments. The animals were kept alone in cages. They were allowed free access to water and food both before and during the experiments, except during the actual experimental handlings (injections, sampling of blood or weighing). All experiments were performed between 07 h 00 min and 12 h 00 min.

Drug treatment

Frusemide was dissolved in distilled water (final pH 9.0) and injected intraperitoneally at a final dose of 25 or 200 mg kg^{-1} body weight (about 0.1 ml per animal depending on body weight). Probenecid was dissolved in distilled water (final pH 9.0) and injected intraperitoneally at final doses of 60 to 240 mg kg^{-1} body weight (about 0.3 ml per animal depending on body weight). Saline adjusted to equal pH was used as control solution for the injected drugs.

Blood samples

As anaesthetics are known to affect serum glucose and serum insulin levels (Yoshimura *et al.*, 1971), blood samples were drawn from a tail incision in unanaesthetized animals. About 20 µl of blood was drawn on each occasion.

Measurements of serum glucose

Blood samples were allowed to clot for 30 min at room temperature and then centrifuged for 3 min at 10,000 *g*. Serum was withdrawn and diluted 161–322 times in 1 mmol l^{-1} EDTA before determining the glucose concentration. The one-step assay used is built on two enzymatic reactions running in parallel. In the first reaction, glucose is phosphorylated by glucokinase with a proportional consumption of ATP and in the other reaction the remaining ATP is measured with the luciferin/luciferase system for ATP determination. In the second reaction, the emitted light is proportional to the ATP concentration. In the assay situation, 5 µl samples of diluted serum or sugar standard were mixed with 125 µl of a reagent containing ATP, glucokinase, luciferin and luciferase and incubated for 15 min at room temperature. The emitted light, being proportional to the glucose concentration, was measured in a liquid scintillation spectrometer (Packard Tri-Carb 3310). The method is described in detail elsewhere (Idahl *et al.*, 1986).

Detection of glucose in urine

The presence of glucose in urine was checked with Clinistix from Ames Division, Miles Laboratories Limited, Stoke Poges, England.

Measurements of body weight

Acute changes in whole body weight was used as an indicator of diuresis. Such measurements agree well with direct measurements of diuresis in metabolic cages (Foy & Furman, 1971). Thus, the urinary

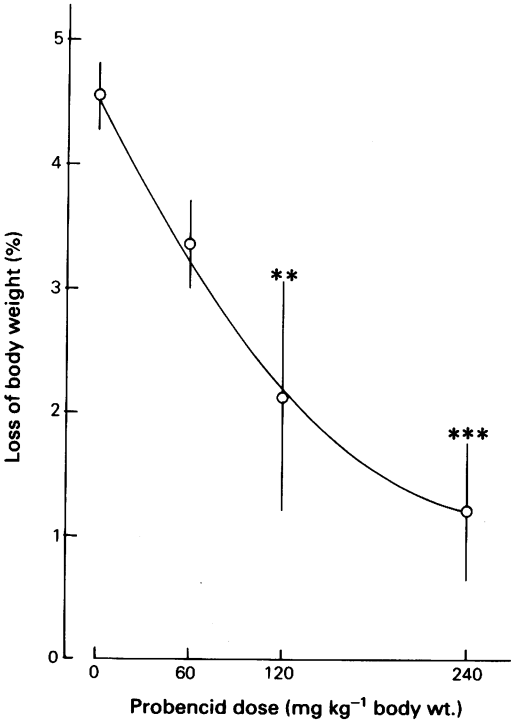

Figure 1 The effect of different doses of probenecid on the frusemide-induced reduction of body weight. Animals were injected with saline or probenecid (60, 120 or 240 mg kg^{-1} body weight) 30 min before an injection of frusemide (25 mg kg^{-1} body weight). The animals were weighed before and 60 min after the frusemide injection and the loss of body weight was calculated. Each point represents the mean, and vertical lines show s.e.means, for 3–14 animals. $^{**}P < 0.01$, $^{***}P < 0.001$ for effect of probenecid.

volume during the 60 min period following the injection of frusemide (200 mg kg^{-1} body weight) was estimated to be 4.3 ± 0.5 ml 100 g^{-1} mouse (mean ± s.d. for 6 experiments) in metabolic cages (Foy & Furman, 1971). The present corresponding value from the reduction of body weight was 5.0 ± 1.16 ml 100 g^{-1} mouse (mean ± s.d. for 15 experiments). The animals were weighed on a digital balance (Type 3716) from Sartorius-Werke, Göttingen, West Germany.

Chemicals

Probenecid was a gift from Astra Läkemedel AB, Södertälje, Sweden and frusemide was a gift from Svenska Hoecht AB, Stockholm, Sweden. Chrystalline, electrophoretically pure, bovine serum albumin, twice crystallized luciferase (EC 1.13.12.7), synthetic D-(−)-luciferin, and ATP were obtained from Boehringer/Mannheim, Mannheim, West Germany. Glucokinase (EC 2.7.1.2) was from Sigma Chemical Co., St. Louis, Missouri, U.S.A. Anhydrous D-glucose and EDTA were from BDH Chemicals Ltd, Poole, England. Inorganic chemicals were commercially available and of analytical grade. Twice distilled water was used throughout.

Results

Effect of probenecid on frusemide-induced diuresis

To investigate whether probenecid affects frusemide-induced diuresis, animals were injected with saline or probenecid (60, 120 or 240 mg kg^{-1} body weight) 30 min before the injection of frusemide (25 mg kg^{-1} body weight). The animals were weighed before and 60 min after the frusemide injection and the loss of body weight, which was used as an estimate of diuresis, was determined. As shown in Figure 1, doses of up to at least 240 mg probenecid kg^{-1} body weight reduced the frusemide-induced diuresis in a dose-dependent manner.

To investigate further the effect of probenecid on frusemide-induced diuresis, 240 mg probenecid kg^{-1} body weight was injected 30 min before the injection of 25 or 200 mg frusemide kg^{-1} body weight and the weight loss was determined 30, 60, 120 and 180 min after the frusemide injection. The data presented in Table 1 show that the diuresis induced by either 25 or 200 mg frusemide kg^{-1} body weight was significantly reduced by the probenecid pretreatment at all times. After correction for the slight diuresis (data not shown) in the relevant control groups injected with probenecid or saline but not frusemide, the reduction of frusemide-induced diuresis after 60 min amounted to 86–91% for either frusemide dose.

Effect of probenecid on frusemide-induced hyperglycaemia

The effect of probenecid on frusemide-induced hyperglycaemia was studied by injecting probenecid (240 mg kg^{-1} body weight) 30 min before the injection of frusemide (25 or 200 mg kg^{-1} body weight). Previous studies have shown that the lower dose does not clearly affect the blood sugar, whereas the higher dose of frusemide causes transient hyperglycaemia (Foy & Furman, 1971). Animals injected with saline 30 min after the probenecid injection were used as controls. As shown in Figures 2 and 3, the injection of either of the frusemide doses after probenecid pretreatment resulted in hyperglycaemia compared to saline injected controls. In the animals injected with the low frusemide concentration (25 mg kg^{-1} body

Table 1 Effect of frusemide (F) on loss of body weight after pretreatment with saline (S) or probenecid (Pb)

Time (min)	*Loss of body weight* (%) S + F (200)	Pb + F (200)	S + F (25)	Pb + F (25)
30	1.65 ± 0.22	0.48 ± 0.07[a]	1.56 ± 0.25	0.66 ± 0.15[b]
60	4.99 ± 0.31	1.32 ± 0.17[a]	4.55 ± 0.28	1.13 ± 0.21[a]
120	8.40 ± 0.31	3.33 ± 0.49[a]	7.01 ± 0.27	2.86 ± 0.24[a]
180	9.17 ± 0.36	4.96 ± 0.55[a]	8.09 ± 0.29	3.44 ± 0.25[a]

Probenecid (240 mg kg^{-1} body weight) or saline was injected at −30 min and frusemide (25 or 200 mg kg^{-1} body weight) was injected at time 0 following the protocol described in Figure 2. The animals were weighed and calculation of the relative loss of body weight was performed as indicated in the table. The initial (time 0) body weights for the different groups were as follows: S + F (200): 24.33 ± 0.28 (n = 15), Pb + F (200): 25.27 ± 0.30 (n = 15), S + F (25): 23.49 ± 0.28 (n = 14), Pb + F (25): 24.17 ± 0.35 (n = 13). The small differences between the mean values of the initial body weights of the groups are probably due to random variation and do not significantly change the calculated effects of the drugs. Results shown are means ± s.e.means for 13–15 animals (same animals as are described in Figures 2 and 3). Differences between groups pretreated with saline or probenecid were evaluated by Student's t tests on unpaired observations. [a]$P < 0.001$, [b]$P < 0.01$.

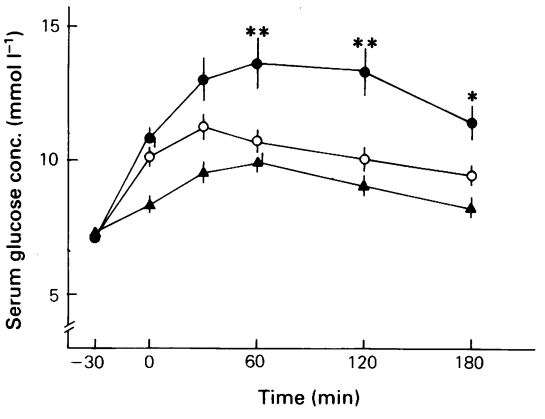

Figure 2 Effect of a low frusemide dose (25 mg kg^{-1} body weight) on serum glucose after pretreatment with probenecid (240 mg kg^{-1} body weight). Serum glucose was measured at intervals indicated in the figure. The following experimental groups were used: animals injected with saline at −30 min and frusemide at 0 min (▲), animals injected with probenecid at −30 min and saline at 0 min (○) and animals injected with probenecid at −30 min and frusemide at 0 min (●). Each point represents the mean, and vertical lines show s.e.means, for 13–15 animals. $*P<0.05$, $**P<0.01$ for difference from respective controls (○).

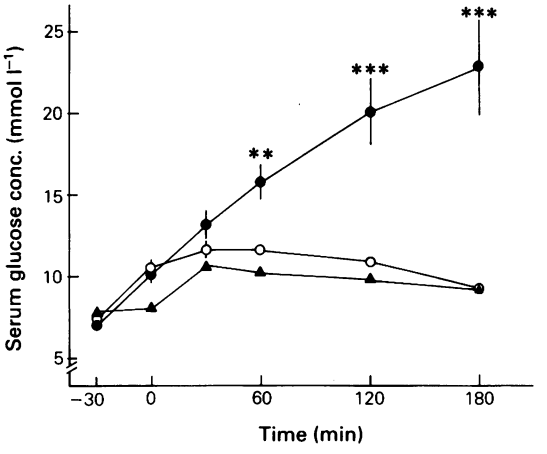

Figure 3 Effect of a high frusemide dose (200 mg kg^{-1} body weight) on serum glucose after pretreatment with probenecid (240 mg kg^{-1} body weight). Serum glucose was measured as indicated in the figure. The following experimental groups were used: animals injected with saline at −30 min and frusemide at 0 min (▲), animals injected with probenecid at −30 min and saline at 0 min (○) and animals injected with probenecid at −30 min and frusemide at 0 min (●). Each point represents the mean, and vertical lines show s.e.means, for 15 animals. $**P<0.01$, $***P<0.001$ for difference from respective controls (○).

weight) (Figure 2) after probenecid pretreatment an apparent maximum serum glucose level was achieved after 60 min followed by a significant decline. Animals injected with the high frusemide concentration (200 mg kg^{-1} body weight) (Figure 3), after probenecid pretreatment showed a potentiated hyperglycaemia over the whole experimental period. In this group 9 out of 15 animals showed glucosuria 180 min after the frusemide injection. No glucose could be detected in the urine of any of the control mice at that time. As shown in Figures 2 and 3, treatment with probenecid alone for 30 min slightly increased the serum glucose level.

The specific effects of frusemide on serum glucose were estimated by subtracting the serum glucose values of control animals run in parallel and not treated with frusemide from those of frusemide-treated animals (Table 2). In the group pretreated with probenecid and subsequently injected with frusemide this was done by subtracting the serum glucose values

Table 2 Specific effect of frusemide (F) on serum glucose after pretreatment with saline (S) or probenecid (Pb)

Time (min)	*Serum glucose* (mmol l^{-1}) S + F (200)	Pb + F (200)	S + F (25)	Pb + F (25)
30	2.49 ± 0.40	1.56 ± 0.64	1.09 ± 0.18	1.91 ± 0.55
60	2.14 ± 0.47	4.24 ± 0.67^{b}	1.49 ± 0.21	3.05 ± 0.73
120	1.68 ± 0.55	9.30 ± 1.30^{a}	0.81 ± 0.20	3.37 ± 0.71^{b}
180	1.09 ± 0.55	13.44 ± 2.37^{a}	−0.01 ± 0.19	2.09 ± 0.53^{b}

Data are calculated from the results presented in Figures 2 and 3. The specific effect of frusemide on serum glucose after the different pretreatments was calculated as described in the Results section. Results shown are means ± s.e.means for 5 separate experiments with 2–3 animals in each. Differences between groups pretreated with saline or probenecid were calculated by Student's *t* test on paired observations. $^{a}P<0.01$, $^{b}P<0.05$.

of the group injected with saline after pretreatment with probenecid. In the group pretreated with saline and subsequently injected with frusemide this was done by subtracting the serum glucose values of the same animals at time zero. The calculated values (Table 2) show that frusemide at least at the higher dose, caused a pronounced and transient hyperglycaemia and that pretreatment with probenecid indeed enhanced the hyperglycaemia in the presence of either the low or the high frusemide concentration.

Discussion

Different types of diuretics, such as thiazides and loop-diuretics, despite different cellular mechanisms of action in the kidney (Small & Cafruny, 1967) have been shown to cause hyperglycaemia (Conn, 1965; Mustala & Toivonen, 1965; Jones & Pickens, 1967; Walsh & O'Sullivan, 1974; Helderman *et al.*, 1983). It has therefore been tempting to speculate that the diabetogenic action of diuretics in general is secondary to the diuresis.

The present results are in accord with previous work on small rodents (Wales *et al.*, 1968; Foy & Furman, 1971; 1973; Aynsley-Green & Alberti, 1973; Wexler, 1981) in showing that frusemide induces acute hyperglycaemia in mice. It has been proposed that this hyperglycaemia is a secondary effect of the diuresis produced by alterations in the electrolyte balance (Aynsley-Green & Alberti, 1973) or reflex-mediated catecholamine release (Foy & Furman, 1973; Aynsley-Green & Alberti, 1973). As direct experimental evidence in support of this general idea, Foy & Furman (1973) showed that removal of the kidneys in mice prevented the hyperglycaemia. However, some results refute this hypothesis. Thus, certain diuretics (ethacrynic acid), although inducing a strong diuresis, do not induce hyperglycaemia (Foy & Furman 1971). Indeed, if excessive diuresis induced hyperglycaemia, it would be expected that inhibition of frusemide-induced diuresis by pretreatment with probenecid would abolish, or at least reduce, the frusemide-induced hyperglycaemia.

Probenecid strongly reduced the diuretic response to both the lower (25 mg kg^{-1} body weight) and the higher (200 mg kg^{-1} body weight) dose of frusemide. The reduction appeared to be dose-dependent, at least at the lower concentration of frusemide. This is in principle in agreement with the findings in dogs (Hook & Williamson, 1965) and in cats (Friedman & Roch-Ramel, 1977), and suggests that probenecid acts in mice by mechanisms similar to those described in other species. At least part of the frusemide-induced diuresis that could not be blocked by probenecid might be due to osmotic diuresis secondary to the hyperglycaemia. This is supported by the observation that the majority of the animals pretreated with probenecid and subsequently injected with a high dose of frusemide (200 mg kg^{-1} body weight) showed glycosuria 180 min after the frusemide injection.

The strong reduction of frusemide-induced diuresis by probenecid did not abolish the hyperglycaemic effect. After calculation of the specific effect of frusemide (elevation above baseline), it became apparent that probenecid pretreatment even potentiated the frusemide-induced hyperglycaemia. The results also show that frusemide at 25 mg kg^{-1} body weight, a dose which induces only a very slight, if any hyperglycaemia by itself (Foy & Furman, 1971) (Figure 2), was markedly hyperglycaemic in animals where the diuresis had been reduced by probenecid pretreatment. It is likely that this potentiation was due to increased retention of frusemide in plasma. No marked difference between the diuretic response to low (25 mgkg^{-1} body weight) or high (200 mgkg^{-1} body weight) frusemide doses could be observed in any of our experiments, although the difference in hyperglycaemia was profound.

Taken together, the present results indicate that the plasma concentration rather than the diuretic effect of frusemide determines the degree of hyperglycaemia and that this action is mediated by an effect on extrarenal organs.

Since probenecid decreases the clearance and increases the half-life of frusemide in man (Homeida *et al.*, 1977; Honari *et al.*, 1977; Chennavasin *et al.*, 1979) and as the two drugs are quite often given together in clinical practice, the extent to which probenecid affects the diabetogenic action of frusemide in human patients remains to be examined.

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