

## Diuretics and Carbohydrate Metabolism: The Effects of Furosemide and Amiloride on Blood Glucose, Plasma Insulin and Cations in the Rat\*

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**Summary.** The effects of two unrelated diuretics, furosemide and amiloride on blood glucose, plasma insulin and glucose tolerance in the conscious rat are reported. Furosemide (1 mg/kg or 2 mg/kg) given intravenously caused an immediate but highly transient 23% and 53% fall in plasma insulin followed by a rise in blood glucose. The hyperglycaemic effect alone occurred in mild streptozotocin diabetic animals. Furosemide given with or 30 min before intravenous glucose (0.5 G/kg) caused glucose intolerance with diminished insulin response. None of these effects were observed in adrenalectomised animals. Amiloride (1, 5 or 10 mg/kg) given I.V. alone immediately increased plasma insulin (up to 213  $\mu$ U/ml) without affecting blood glucose. This effect was attenuated by

mild streptozotocin diabetes. Amiloride given with or 30 min before glucose increased the insulin area without affecting glucose disappearance. Chronic administration enhanced glucose disappearance with increased plasma insulin response, and caused hyperkalaemia. Chronic furosemide administration had no effects. Possible mechanisms for these effects are discussed.

**Key words:** Diuretics; glucose intolerance; furosemide; amiloride; plasma immunoreactive insulin; blood glucose; intravenous glucose tolerance; streptozotocin – diabetic rats; adrenalectomised rats; plasma sodium and potassium; red cell sodium and potassium; total body sodium and potassium.

### Introduction

Glucose intolerance following diuretic therapy has been reported in man and in experimental animals, although the mechanism of the effect, its incidence and clinical importance remain controversial. Benzothiadiazine diuretics are known to exacerbate hyperglycaemia in diabetic patients (for review, see [1]) and frequently to cause impaired carbohydrate tolerance in hypertensive patients [1]. Prospective studies in normal subjects [2] and non-diabetic hypertensive patients [3, 4] have shown that most patients who develop glucose intolerance have a diabetic predisposition, although prednisone-primed glucose tolerance tests or tolbutamide tests may reveal abnormalities in others [5, 6]. Animal studies have given conflicting results, showing hyperglycaemia [7, 8, 9], impaired glucose tolerance [10] or no effect [11, 12]. Acute diuretic induced hyperglycaemia using large doses of diuretics under ether anaesthesia has been described in the rodent [13, 14, 15], but few studies have examined sequential insulin changes in conscious animals. Further studies using small doses therefore seemed necessary, first to establish whether there was a consistent effect and second to examine the mechanisms involved.

Two diuretics were used, furosemide (Lasix) related to the benzothiadiazines, and amiloride (Midamor) a non-thiazide diuretic. Furosemide contains the thiazide

sulphamoyl anthranilic acid structure, but the thiadiazine ring is replaced by a furfuryl group (Fig. 1). It has been suggested that kaliuretic diuretics such as this may cause glucose intolerance secondary to potassium depletion, since potassium depletion is associated with diminished glucose tolerance [16]

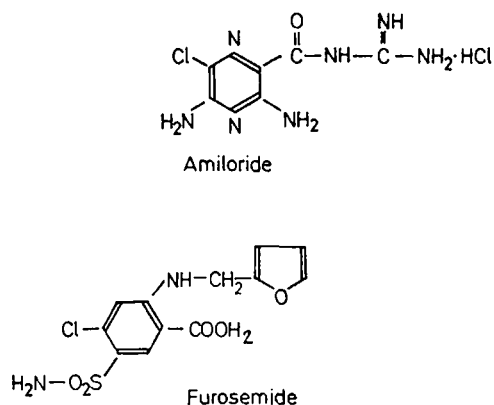


Fig. 1. The structures of amiloride and furosemide

whilst repletion improves tolerance [17]. Amiloride is a pyrazine-carbonylguanidine which is structurally unrelated to the thiazides, has a weaker natriuretic effect and does not cause potassium loss [18]. The guanidine group of amiloride is shared by arginine, a potent stimulus to insulin secretion (Fig. 1). It might therefore be predicted that furosemide and amiloride would have opposite effects on insulin secretion and glucose metabolism.

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Results confirming this hypothesis are presented below.

### Materials and Methods

**Special Chemicals:** Pure furosemide was a gift from Hoechst Pharmaceuticals, Ltd., and used at a final concentration of 1 and 2 mg/ml in normal saline, pH 7.0–7.4. Pure amiloride hydrochloride, a gift from Merck, Sharp and Dohme Ltd., was injected as a suspension in normal saline at concentrations of 1, 5 and 10 mg/ml. Anti-insulin serum was a gift from Dr. P.H. Wright;  $I^{125}$ -insulin was purchased from Abbott Laboratories, Chicago, U.S.A. Streptozotocin was kindly provided by the Upjohn Co., Kalamazoo, Michigan, U.S.A. Ultra pure water was obtained by passing distilled water through an Elgacan ion exchange column (Elga Products Ltd., Lane End, Bucks).

**Analytical methods:** Insulin was assayed by a micro-modification of the double antibody radioimmunoassay of Soeldner and Slone [19] with ethylene diamine tetracetic acid (EDTA) present throughout the assay [20]. Purified rat insulin (kindly supplied by Novo Industri A/S Copenhagen) was used as the insulin standard. The assay was unaffected by furosemide and amiloride and allowed the measurement of  $1 \mu\text{U}$  insulin/ml in  $50 \mu\text{l}$  plasma samples, derived from EDTA treated blood. Glucose was measured in a neutralised perchloric acid extract of whole blood by a glucose oxidase method [21].

Sodium and potassium were measured by flame photometry using an E.E.L. integrating flame photometer with a lithium internal standard. Ammonium heparin plasma and red cell haemolysates were assayed after diluting 1:100 with lithium working diluent. No correction was made for plasma trapping. Whole body sodium and potassium values were obtained by assaying the nitric acid digest of the minced carcass.

**Animals:** Male white Wistar rats weighing 250 to 350 gm were used unless otherwise stated. Adrenalectomised Wistar rats (Scientific Products Farm, Ash, Canterbury), maintained on 0.9% saline, were used 5–10 days after adrenalectomy. Diabetic rats were obtained by intraperitoneal injection of 60 mg/kg or 75 mg/kg streptozotocin 48 h before the experiments.

**Experimental model:** Conscious overnight-starved rats were used 16–20 h after the insertion of small cannulae (0.4 mm internal diameter) into the left femoral artery and vein under light ether anaesthesia. The animals were kept in restraining cages with free access to water. Cannulae were kept patent with saline. Up to 0.35 ml arterial blood was withdrawn at each sample time, the volume being replaced with saline. Up to ten samples could be withdrawn without changes in glucose or insulin.

Immediately on withdrawing,  $100 \mu\text{l}$  of blood were added to 2 ml 2% perchloric acid for subsequent glucose assay and the remaining 0.2–0.25 ml were added to EDTA for insulin assay. Additional 0.4 ml blood samples were taken where applicable for plasma and red cell sodium and potassium assay.

**Acute experiments:** The diuretics were given as acute IV boluses either alone, with, or thirty minutes before a standard glucose dose of 0.5 g/kg body weight. The latter was given intravenously as a 50% solution (w/v) in 15 sec and subsequent timing of samples was made from the end of the glucose infusion.

**Chronic experiments:** Groups of animals were given daily intraperitoneal injections of either furosemide 2 mg/kg or 10 mg/kg amiloride. Control animals of similar weight were given an equivalent volume of intraperitoneal saline. After 30 days (furosemide) and 10 days (amiloride) intravenous glucose tolerance tests were performed, 24 h after the last dose of diuretic.

**Calculations:** The glucose disappearance rate (kg) was calculated using the absolute blood glucose values from 2.5–20 min [22]. Insulin areas were calculated from the areas under the insulin curves during the first five, ten, or thirty minutes of the test.  $\frac{\Delta I}{\Delta G}$  ratios were calculated from the increments of plasma insulin and blood glucose values from the zero value [23]. Statistical significance was assessed by the Student 't' test.

### Results

#### I. The effects of acute administration of furosemide on blood glucose and plasma insulin

a) **Normal animals:** A significant decrease in plasma insulin occurred with both 1 mg and 2 mg/kg furosemide (Fig. 2; Table 1). Plasma insulin fell by 23%

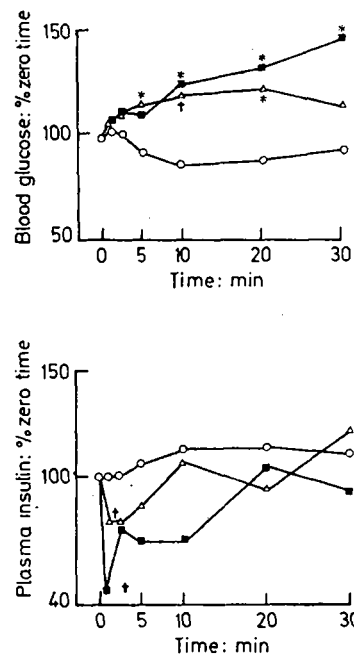


Fig. 2. The effects of acute administration of furosemide on blood glucose and plasma insulin in normal animals. Furosemide, 1 mg/kg, shown by  $\triangle-\triangle$  ( $n=5$ ), or 2 mg/kg shown by  $\blacksquare-\blacksquare$  ( $n=4$ ) was given as an acute intravenous injection after the time 0 sample had been drawn. Control animals, shown by  $\circ-\circ$  ( $n=6$ ) received an equivalent volume of saline. Each point represents the mean of the values obtained for each group. For clarity the standard error of the mean is omitted, but significant differences, compared with control values, are shown by \* ( $p < 0.05$ ) or † ( $p < 0.01$ ). Abscissa: time after injection. Ordinate: blood glucose and plasma insulin expressed as percentage of zero (pre-injection) value

at 1 min and 26% at 2.5 min with the lower dose ( $p < 0.025$ ), whereas with 2 mg/kg, there was a 53% decrease at one minute, the suppression persisting until 10 min after injection, when the plasma insulin was still 30% ( $p < 0.05$ ) below the fasting value. A small but significant rise in blood glucose occurred at twenty minutes with both groups and persisted to 30 min with

the higher dose. Absolute blood glucose and plasma insulin values showed some variation between groups. However, changes within groups were consistent.

No significant changes in plasma insulin or blood glucose occurred in animals injected with saline alone.

b) *Diabetic animals*: No significant change occurred in insulin secretion in the "mild" diabetic group after furosemide 2 mg/kg and although fasting values were within the normal range a significant rise in blood glucose of 27% occurred after 20 min (Table 1). No change in blood glucose or plasma insulin occurred in the ketotic hyperglycaemic animals (Table 1).

animals, animals given furosemide 1 mg/kg and 2 mg/kg simultaneously with glucose, and animals pretreated with furosemide 1 mg/kg 30 min before glucose is shown in Fig. 3.

Control animals showed a glucose disappearance rate of  $3.7 \pm 0.27$  %/min (Table 2). Glucose disappearance was markedly decreased in the animals given furosemide either with or before glucose (Table 2), although the 2.5 min blood glucose value in the low dose group and the 5 min value in all the furosemide groups were significantly lower than in control animals (Fig. 3). However, the initial insulin response was

Table 1. Blood glucose and plasma insulin after acute administration of furosemide ( $\pm$  S.E.M.)

Time (min)	Furosemide 2 mg/kg			Furosemide 1 mg/kg		
	Saline control	Normal	Diabetic mild	Diabetic moderate	Adrenalectomised	Normal
a) Blood glucose mg/100 ml						
-10	78 $\pm$ 8	103 $\pm$ 8	80 $\pm$ 17	262 $\pm$ 47	39 $\pm$ 2	103 $\pm$ 6
0	82 $\pm$ 2	99 $\pm$ 10	82 $\pm$ 8	321 $\pm$ 33	38 $\pm$ 3	97 $\pm$ 5
1	93 $\pm$ 6	97 $\pm$ 4	81 $\pm$ 7	320 $\pm$ 28	36 $\pm$ 4	102 $\pm$ 4
2.5	91 $\pm$ 9	99 $\pm$ 5	83 $\pm$ 13	316 $\pm$ 27	40 $\pm$ 4	107 $\pm$ 3
5	78 $\pm$ 7	98 $\pm$ 6	94 $\pm$ 22	281 $\pm$ 25	38 $\pm$ 3	110 $\pm$ 4
10	73 $\pm$ 7	112 $\pm$ 2	100 $\pm$ 24	306 $\pm$ 24	36 $\pm$ 4	117 $\pm$ 3 <sup>c</sup>
20	75 $\pm$ 11	120 $\pm$ 3 <sup>b</sup>	109 $\pm$ 5 <sup>a</sup>	335 $\pm$ 28	40 $\pm$ 4	120 $\pm$ 6 <sup>c</sup>
30	80 $\pm$ 12	123 $\pm$ 5 <sup>b</sup>	103 $\pm$ 11	326 $\pm$ 22	38 $\pm$ 5	109 $\pm$ 9
	n = 5	4	3	4	11	5
b) Plasma insulin $\mu$ U/ml						
-10	16 $\pm$ 3	19 $\pm$ 5	14 $\pm$ 4	6.0 $\pm$ 0.8	3.0 $\pm$ 0.5	34 $\pm$ 8
0	17 $\pm$ 4	17 $\pm$ 3	13 $\pm$ 4	5.4 $\pm$ 0.8	2.8 $\pm$ 0.5	31 $\pm$ 6
1	15 $\pm$ 3	8 $\pm$ 2 <sup>c</sup>	12 $\pm$ 3	5.9 $\pm$ 0.9	3.2 $\pm$ 0.4	24 $\pm$ 4 <sup>b</sup>
2.5	15 $\pm$ 2	13 $\pm$ 4	11 $\pm$ 3	6.5 $\pm$ 1.5	3.3 $\pm$ 0.4	23 $\pm$ 4 <sup>c</sup>
5	18 $\pm$ 2	12 $\pm$ 2 <sup>c</sup>	12 $\pm$ 3	5.5 $\pm$ 1.3	2.6 $\pm$ 0.3	22 $\pm$ 6
10	17 $\pm$ 3	12 $\pm$ 3 <sup>b</sup>	12 $\pm$ 4	3.6 $\pm$ 0.5	2.6 $\pm$ 0.5	31 $\pm$ 6
20	16 $\pm$ 2	18 $\pm$ 4	16 $\pm$ 4	5.2 $\pm$ 0.7	3.0 $\pm$ 0.4	29 $\pm$ 6
30	16 $\pm$ 2	16 $\pm$ 5	14 $\pm$ 2	4.5 $\pm$ 0.8	3.0 $\pm$ 0.4	34 $\pm$ 4
	n = 5	4	3	4	6	5

<sup>a</sup>  $p < 0.05$  compared with control values;

<sup>b</sup>  $p < 0.05$  for paired differences with zero value

<sup>c</sup>  $p < 0.01$  for paired differences with zero value

Mild diabetic animals received 60 mg/kg streptozotocin; moderate diabetic animals received 75 mg/kg streptozotocin.

c) *Adrenalectomised animals*: Mean fasting blood glucose and plasma insulin were significantly lower than in control animals (Table 1). Furosemide 2 mg/kg did not affect either parameter, implying that the decrease in plasma insulin and hyperglycaemia may have been mediated in normal animals by an adrenergic or glucocorticoid effect. Small changes in insulin would not have been detected by the methods used. However, a 30 to 50% change would have been discerned.

## II. The effects of acute administration of furosemide on intravenous glucose tolerance

a) *Normal animals*: The response of blood glucose and plasma insulin to I.V. glucose (0.5 g/kg) in control

much lower in the furosemide-treated animals (Table 2). This was not significant in the pretreated group.  $\frac{\Delta I}{\Delta G}$  values were also lower, particularly in the 2 mg/kg group confirming that the plasma insulin at the early times was inappropriately low for the plasma glucose (Table 2). The thirty minute insulin area was decreased in all furosemide treated groups.

b) *Adrenalectomised animals*: There was no significant difference in any parameter between control adrenalectomised animals given glucose alone, and animals given glucose together with furosemide 2 mg/kg ( $n = 3$  for each group). Mean kg for control animals was 2.30%/min and for the furosemide group 2.33%/min.

Table 2. The effects of acute administration of furosemide and amiloride on intravenous glucose tolerance (all values  $\pm$  S.E.M.)

Saline control	Furosemide		Amiloride				
	1 mg/kg	2 mg/kg	1 mg 30 min before glucose	5 mg/kg	10 mg/kg	5 mg/kg 30 min before glucose	
a) Glucose disappearance rate (kg) (%/min)	3.71 $\pm$ 0.27	2.14 $\pm$ 0.21 <sup>b</sup>	2.03 $\pm$ 0.32 <sup>b</sup>	2.34 $\pm$ 0.47 <sup>b</sup>	2.92 $\pm$ 0.31	3.46 $\pm$ 0.34	3.27 $\pm$ 0.69
b) Ten minute insulin area ( $\mu$ U/ml/10 min)	827 $\pm$ 89	476 $\pm$ 123 <sup>a</sup>	453 $\pm$ 68 <sup>a</sup>	550 $\pm$ 104	1618 $\pm$ 434	1753 $\pm$ 405	1896 $\pm$ 560
c) Thirty minute insulin area ( $\mu$ U/ml/30 min)	1189 $\pm$ 73	805 $\pm$ 108 <sup>a</sup>	692 $\pm$ 63 <sup>b</sup>	924 $\pm$ 25 <sup>a</sup>	2194 $\pm$ 460 <sup>b</sup>	3157 $\pm$ 599 <sup>b</sup>	2407 $\pm$ 471 <sup>a</sup>
d) $\frac{\Delta I}{\Delta G}$							
1 min	1.43 $\pm$ 0.22	0.78 $\pm$ 0.24	0.77 $\pm$ 0.13 <sup>a</sup>	0.70 $\pm$ 0.06 <sup>b</sup>	2.14 $\pm$ 0.69	1.80 $\pm$ 0.51	1.62 $\pm$ 0.28
2.5 min	1.08 $\pm$ 0.09	0.94 $\pm$ 0.16	0.63 $\pm$ 0.17 <sup>a</sup>	0.83 $\pm$ 0.10	3.36 $\pm$ 1.63	1.92 $\pm$ 0.57	2.08 $\pm$ 0.55
5 min	0.86 $\pm$ 0.26	0.42 $\pm$ 0.14	0.74 $\pm$ 0.27	0.38 $\pm$ 0.09	2.17 $\pm$ 0.80	2.52 $\pm$ 1.00	2.12 $\pm$ 0.97
10 min	0.74 $\pm$ 0.17	0.63 $\pm$ 0.16	0.43 $\pm$ 0.11	0.40 $\pm$ 0.09	2.04 $\pm$ 1.20	1.71 $\pm$ 0.66	0.96 $\pm$ 0.50
n = 5	5	5	5	5	4	5	4

<sup>a</sup>  $p < 0.05$   
<sup>b</sup>  $p < 0.01$  compared with control values

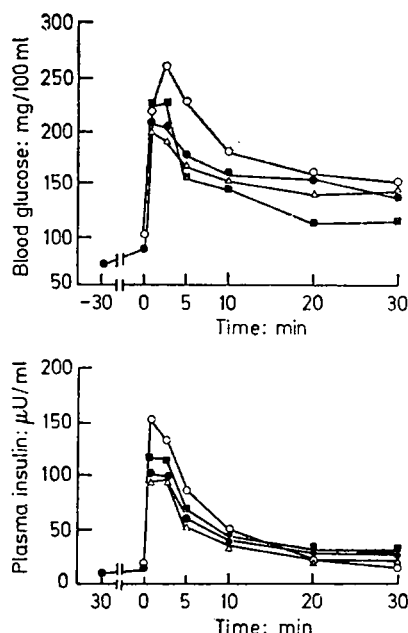


Fig. 3. The effects of acute administration of furosemide on blood glucose and plasma insulin during the intravenous glucose tolerance test. Furosemide 1 mg/kg, shown by  $\triangle$ - $\triangle$  ( $n = 5$ ) or 2 mg/kg shown by  $\blacksquare$ - $\blacksquare$  ( $n = 5$ ) was given by intravenous injection simultaneously with glucose (0.5 g/kg) after time zero sample had been drawn. Control animals ( $o-o$ ) ( $n = 5$ ) received glucose alone. Furosemide 1 mg/kg, shown by  $\bullet-\bullet$  ( $n = 5$ ) was given 30 min before glucose. Each point represents the mean of the values obtained for each group

c) *Chronic administration of furosemide:* Intravenous glucose tolerance tests were performed after the daily intraperitoneal injection of furosemide 2 mg/kg for 30 days, and on a control group which had received

an equivalent daily injection of saline. No significant differences were found.

### III. Cation changes after furosemide administration

a) *During the glucose tolerance test:* When furosemide was given 30 min before glucose, a significant fall in plasma and red cell potassium had occurred at the zero sample time. Red cell potassium had returned to control values at the end of the test, although plasma potassium was still decreased (Table 3). The group receiving furosemide simultaneously with glucose showed no significant change.

b) *After chronic administration:* There was no significant difference in plasma, red cell or total body cation values. Total body sodium was  $100 \pm 8$  mg/100 G body weight compared with  $123 \pm 6$  for controls,  $p$ : N.S. Total body potassium was  $224 \pm 17$  mg/100 G body weight compared with  $255 \pm 10$  for controls,  $p$ : N.S.

### IV. The effects of acute administration of amiloride on blood glucose and plasma insulin

a) *Normal animals:* Amiloride was injected as a suspension in normal saline at 1 mg, 5 mg and 10 mg/kg (4, 20 and 40  $\mu$ M/kg respectively). A significant dose related increase in insulin secretion occurred one minute after injection in all groups, which persisted to 2.5 min after injection in the high dose group (Fig. 4). The one minute insulin values represent a 2, 5 and 7 fold increase in insulin concentration respectively. Interestingly, no significant change in blood glucose occurred.

b) *Diabetic animals:* In the milder diabetic group (60 mg/kg streptozotocin) amiloride, 10 mg/kg, caused

a significant rise in plasma insulin, but this was only an increase of  $25 \mu\text{U/ml}$ , compared with  $213 \mu\text{U/ml}$  for the same dose in normal animals (Table 4). No change in plasma insulin occurred in the ketotic hyperglycaemic group. As both diabetic groups contained very small numbers the results only show a trend and do not allow statistical comparison.

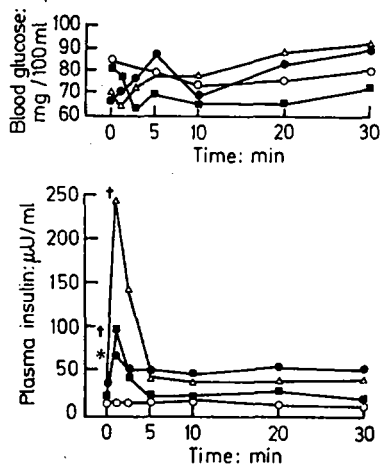


Fig. 4. The effects of acute administration of amiloride on blood glucose and plasma insulin in normal animals. Amiloride  $1 \text{ mg/kg}$  ( $n = 3$ ), shown by  $\bullet-\bullet$ ,  $5 \text{ mg/kg}$  ( $n = 6$ ), shown by  $\blacksquare-\blacksquare$  or  $10 \text{ mg/kg}$  ( $n = 5$ ) shown by  $\triangle-\triangle$ , was given as an acute intravenous injection after the time 0 sample had been drawn. Control animals, shown by  $\circ-\circ$  ( $n = 5$ ) received saline alone. Each point represents the mean of the values for each group. For clarity, the standard error of the mean is omitted, but significant differences compared with control values are shown by  $*$  ( $p < 0.05$ ) or  $\dagger$  ( $p < 0.01$ )

c) *Adrenalectomised animals*: A significant increase in insulin secretion occurred 1 min after the injection of  $10 \text{ mg/kg}$  amiloride (Table 4). There was marked attenuation of the absolute response compared with normal animals, but it represents a seventeen fold increase in plasma insulin compared with a nine fold increase in the normal group.

#### V. The effects of acute administration of amiloride on intravenous glucose tolerance

a) *Normal animals*: The response of blood glucose and plasma insulin to I.V. glucose ( $0.5 \text{ g/kg}$ ) in control animals, animals given amiloride  $5 \text{ mg/kg}$  and  $10 \text{ mg/kg}$  simultaneously with glucose, and animals pretreated with amiloride,  $5 \text{ mg/kg}$ , 30 min before glucose is shown in Fig. 5. Absolute blood glucose values in the amiloride treated groups were not significantly different from control values. Glucose disappearance rate was not affected. However, all the amiloride treated groups showed a highly significant increase in total 30 min insulin area (Table 2).

b) *The effects of chronic administration of amiloride on intravenous glucose tolerance*: Intravenous glucose tolerance tests were performed after the daily intra-

peritoneal injection of amiloride  $10 \text{ mg/kg}$  for 10 days. Fasting blood glucose was significantly elevated in the experimental group with a slight elevation in plasma insulin (Fig. 6). Glucose disappearance was markedly enhanced ( $\text{kg } 2.96 \pm 0.14$  for controls,  $4.16 \pm 0.17$  for the amiloride treated group;  $p < 0.005$ ), and was associated with a significant elevation in peak initial in-

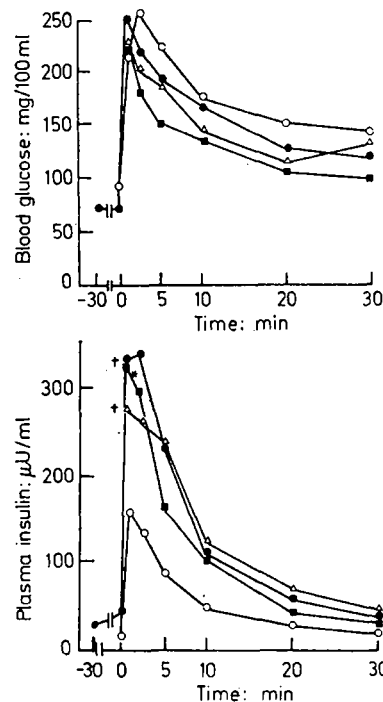


Fig. 5. The effects of acute administration of amiloride on blood glucose and plasma insulin during the intravenous glucose tolerance test. Amiloride  $5 \text{ mg/kg}$ , shown by  $\blacksquare-\blacksquare$  ( $n = 4$ ) or  $10 \text{ mg/kg}$ , shown by  $\triangle-\triangle$  ( $n = 5$ ) was given by intravenous injection simultaneously with glucose ( $0.5 \text{ g/kg}$ ) after the time zero sample had been drawn. Control animals, shown by  $\circ-\circ$  ( $n = 5$ ) received glucose alone. Amiloride  $5 \text{ mg/kg}$  shown by  $\bullet-\bullet$  ( $n = 4$ ) was given 30 min before glucose. Each point represents the mean of the values for each group. For clarity significant differences in plasma insulin compared with control values are shown only for the 1 min values  $*$  ( $p < 0.05$ ,  $\dagger p < 0.01$ ). The plasma insulin values in all the amiloride treated groups at 2.5 min were also significantly increased ( $p < 0.05$ )

sulin response and 5 min insulin area. ( $481 \pm 23 \mu\text{U ml}^{-1} 5 \text{ min}^{-1}$  for controls,  $845 \pm 124 \mu\text{U ml}^{-1} 5 \text{ min}^{-1}$  for the amiloride group;  $p < 0.025$ ).

#### VI. Cation changes after amiloride administration

a) *During the glucose tolerance test after acute administration of amiloride*: No significant change in red cell or plasma cation values between control IVGTT and the high dose amiloride IVGTT occurred during the test.

b) *After chronic administration*: Cation values are shown in Table 5. In contrast with furosemide there

is a highly significant increase in plasma potassium in the experimental group (7.0 mEq/l compared with 4.2 mEq/l  $p < 0.01$ ), associated with increased total body potassium.

chronically, could not show any effect. Foy and Furman [15] found hyperglycaemia, glucose intolerance and impaired insulin response in the mouse. Most of these studies can be criticized because of the use of very

Table 3. The effects of acute administration of furosemide on red cell and plasma sodium and potassium during the intravenous glucose tolerance test ( $\pm$  S.E.M.)

Time (min)	Red cell ( $\mu$ g/g)		n	Plasma (mEq/l)		n
	Sodium	Potassium		Sodium	Potassium	
Control						
0	471 $\pm$ 16	3496 $\pm$ 360	36	137 $\pm$ 1	4.8 $\pm$ 0.1	36
30	547 $\pm$ 88	3213 $\pm$ 153	6	138 $\pm$ 1	4.6 $\pm$ 0.2	6
Furosemide						
2 mg/kg						
30	482 $\pm$ 87	3292 $\pm$ 98	5	139 $\pm$ 2	4.0 $\pm$ 0.1	5
1 mg/kg 30 min before glucose						
0	541 $\pm$ 110	3156 $\pm$ 112 <sup>a</sup>	5	136 $\pm$ 2	4.0 $\pm$ 0.2 <sup>b</sup>	5
30	459 $\pm$ 39	3139 $\pm$ 77	5	139 $\pm$ 2	3.8 $\pm$ 0.1 <sup>a</sup>	5

<sup>a</sup>  $p < 0.05$   
<sup>b</sup>  $p < 0.01$  compared with control values

Table 4. Blood glucose and plasma insulin after acute administration of amiloride ( $\pm$  S.E.M.)

Amiloride 10 mg/kg					
Time (min)	Saline control	Normal	Diabetic		Adrenalectomised
			mild	moderate	
a) Blood glucose (mg/100 ml)					
-10	78 $\pm$ 4	68 $\pm$ 4	70 $\pm$ 8	320 $\pm$ 30	43 $\pm$ 5
0	82 $\pm$ 2	72 $\pm$ 6	68 $\pm$ 17	330 $\pm$ 35	41 $\pm$ 6
1	93 $\pm$ 6	65 $\pm$ 2	83 $\pm$ 15	315 $\pm$ 31	56 $\pm$ 9
2.5	91 $\pm$ 9	73 $\pm$ 5	68 $\pm$ 5	361 $\pm$ 58	46 $\pm$ 7
5	78 $\pm$ 7	79 $\pm$ 7	61 $\pm$ 5	341 $\pm$ 48	42 $\pm$ 7
10	73 $\pm$ 7	79 $\pm$ 5	67 $\pm$ 12	362 $\pm$ 78	42 $\pm$ 7
20	75 $\pm$ 11	89 $\pm$ 5	72 $\pm$ 7	349 $\pm$ 76	41 $\pm$ 7
30	80 $\pm$ 12	92 $\pm$ 6	90 $\pm$ 4	377 $\pm$ 58	43 $\pm$ 9
	n = 5	5	2	3	3
b) Plasma insulin ( $\mu$ U/ml)					
-10	16 $\pm$ 3	27 $\pm$ 6	11 $\pm$ 3	6 $\pm$ 1	3 $\pm$ 1
0	17 $\pm$ 4	33 $\pm$ 3	10 $\pm$ 1	7 $\pm$ 1	2 $\pm$ 0.4
1	15 $\pm$ 3	246 $\pm$ 18 <sup>b</sup>	35 $\pm$ 1	5 $\pm$ 2	34 $\pm$ 21 <sup>c</sup>
2.5	15 $\pm$ 2	142 $\pm$ 16 <sup>b</sup>	22 $\pm$ 1	6 $\pm$ 1	23 $\pm$ 14 <sup>c</sup>
5	18 $\pm$ 2	47 $\pm$ 12 <sup>a</sup>	18 $\pm$ 6	5 $\pm$ 1	5 $\pm$ 1
10	17 $\pm$ 3	41 $\pm$ 7 <sup>a</sup>	15 $\pm$ 5	6 $\pm$ 1	3 $\pm$ 1
20	16 $\pm$ 2	42 $\pm$ 3 <sup>a</sup>	13 $\pm$ 4	4 $\pm$ 1	3 $\pm$ 1
30	16 $\pm$ 2	46 $\pm$ 5 <sup>a</sup>	13 $\pm$ 5	5 $\pm$ 1	4 $\pm$ 1
	n = 5	5	2	3	3

<sup>a</sup>  $p < 0.05$   
<sup>b</sup>  $p < 0.01$  compared with controls  
<sup>c</sup>  $p < 0.01$  for paired differences with zero value;  $p$  values significant but not stated for mild diabetic group as  $n$  only 2.

Mild diabetic animals received 60 mg/kg streptozotocin; moderate diabetic animals received 75 mg/kg streptozotocin.

### Discussion

Previous studies in the rat have shown hyperglycaemia and impaired glucose tolerance after acute administration of furosemide (50–200 mg/kg) [9, 14, 24], while chronic administration (100 mg/kg/day) resulted in glucose intolerance [25]. In contrast, Senft *et al.* [12] using up to 200 mg/kg acutely or 200 mg/kg

large doses and the use of anaesthesia, which in the rat is known to profoundly affect blood glucose and plasma insulin [26, 27, 28]. In addition, early sequential changes in insulin and glucose following diuretic administration have not been studied.

Our studies in the conscious rat have shown that low doses of furosemide suppress plasma insulin instantly with subsequent hyperglycaemia. The rate of

fall of plasma insulin is of particular interest in that the normal half-life of insulin quoted in the literature is of the order of 3–4.8 min [29]. Our data suggest a half-life of about one minute. This disappearance rate could have been artificially enhanced by an extremely rapid shift of fluid into the intravascular compartment.

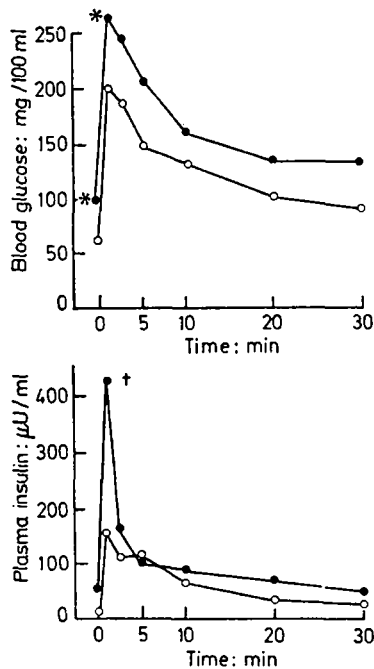


Fig. 6. The effects of chronic administration of amiloride on blood glucose and plasma insulin during the intravenous glucose tolerance test. Animals received amiloride 10 mg/kg (shown by ●—●) or an equivalent volume of saline (shown by ○—○) by daily intraperitoneal injection for 10 days. Intravenous glucose (0.5 g/kg) was given 24 h after the last injection. Each point represents the mean of the values obtained for each group ( $n = 4$  for both groups). Significant differences compared with control values are shown by \* ( $p < 0.05$ ) or † ( $p < 0.01$ )

secretion since it persisted in diabetic animals without a change in plasma insulin.

Possible hepatic mechanisms include catecholamine stimulation of glycogenolysis or inhibition of phosphodiesterase by furosemide, as suggested by Senft [12]. This hypothesis would explain the lack of effect on blood glucose in the ketotic hyperglycaemic animals in whom hepatic glycogen stores would be decreased. Decreased peripheral utilisation of glucose caused by furosemide has also been reported by Weller and Borondy [25].

A change in glucose space is implied by the decreased absolute blood glucose values during the IVGTT with furosemide. A decreased glucose stimulus at the  $\beta$  cell could then result in an apparent decrease in insulin secretion. However, the  $\frac{\Delta I}{\Delta G}$  ratio shows that the plasma insulin response was still reduced.

Insulin release could have been suppressed by a direct effect on the pancreas, through an effect on electrolyte exchange which is of major importance in insulin release [30]. However, red cell and plasma sodium and potassium showed minimal changes in the present study, although this does not preclude effects on the  $\beta$  cell which need not be reflected in changes in the plasma, nor be analogous to red cell effects.

The absence of any effect on glucose tolerance after chronic administration and the absence of significant changes in tissue cations supports the observation in man that chronic administration of furosemide does not affect glucose tolerance [3].

In contrast, amiloride is a potent insulin secretagogue, with or without exogenous glucose. When given acutely alone, it caused a dose related increase in plasma insulin. Between 20 and 40  $\mu\text{M}/\text{kg}$  gave a similar peak insulin response to 2800  $\mu\text{M}/\text{kg}$  of glucose. The insulinogenic effect of a glucose load was potentiated both by the concurrent injection of amiloride,

Table 5. Red cell, plasma and total body sodium and potassium after chronic administration of amiloride ( $\pm$  S.E.M.)

	Red cell ( $\mu\text{g}/\text{g}$ )			Plasma (mEq/l)			Total body (mg/100 g B.W.)		
	Sodium	Potassium	$n$	Sodium	Potassium	$n$	Sodium	Potassium	$n$
Control group	425 $\pm$ 22	4134 $\pm$ 55	4	147 $\pm$ 1	4.2 $\pm$ 0.1	4	90 $\pm$ 8	181 $\pm$ 6	4
Amiloride treated group	491 $\pm$ 40	3734 $\pm$ 139 <sup>a</sup>	4	141 $\pm$ 3	7.0 $\pm$ 0.3 <sup>b</sup>	4	78 $\pm$ 4	237 $\pm$ 17 <sup>a</sup>	6

<sup>a</sup>  $p < 0.05$   
<sup>b</sup>  $p < 0.01$  compared with controls

Adrenalectomy abolishes the furosemide effects, implying that in normal animals they are secondary to catecholamine release. Foy and Furman [15] have suggested this mechanism in the mouse. However, the hyperglycaemia is not due to a decrease in insulin

and by pretreatment. Chronic administration of amiloride also enhanced glucose stimulated insulin secretion.

Studies in man have given conflicting results on the effect of amiloride on blood glucose [31, 32, 33, 34, 35]

without investigating its effect on plasma insulin. No effect on glucose tolerance was reported by Pyörälä and Rantanen [36] while McNay *et al.* [37] showed increased glucose disappearance and I/G ratios.

The lack of early changes in blood glucose suggests direct  $\beta$  cell stimulation by amiloride. The diuretic promotes natriuresis and decreases potassium and hydrogen ion secretion by an effect on the renal tubule [18]. In frog skin [38] and toad bladder [39] amiloride inhibits sodium entry on the mucosal side of the cell. Inhibition of the sodium pump is not involved [18].

A structural feature common to amiloride and other compounds affecting apical cell sodium transport in amphibia is the presence of a guanidine group, which has a close structural relationship to the triply hydrated sodium ion [40]. An effect of amiloride on active potassium [41] and sodium [34, 42] transport in the human red cell has also been described. It is possible that the action of amiloride reflects a direct effect on  $\beta$  cell sodium and/or potassium exchange.

Other guanidine derivatives, including arginine, stimulate insulin secretion *in vivo* and *in vitro* [43, 44, 45, 46]. Amiloride shows similarities with arginine in that they both have synergistic effects with glucose, and both inhibit sodium transport in the toad bladder (Alberti, K.G.M.M. — unpublished observations). Thus it is possible that the stimulation of insulin secretion by all these compounds is due to the ionic effects of the guanidine group.

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