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Hypercalcaemia and Glucagon-Mediated Urinary Electrolyte Excretion in Minipigs

P. O. Schwille and N. M. Samberger

Stoffwechsellabor, Abt. für Urologie, Chirurgische Klinik
der Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany (FRG)

Summary. The effects of glucagon (G) on glomerular filtration rate (GFR) and the urinary excretion of electrolytes were studied during sequentially increasing hypercalcaemia in minipigs. G has no specific effect on GFR. The observed increase in the excretion of electrolytes was probably due to increased amounts of calcium in the glomerular filtrate rather than to any specific hormonal effects. The results obtained in parathyroid suppression experiments (using i. v. infusion of calcium) suggest that the renal effects of G may somehow be related to intact thyroid-parathyroid activity and normal circulating levels of these hormones.

Key words: Hypercalcaemia, glucagon, urinary electrolytes, inulin clearance, minipig.

Glucagon (G) not only influences various stages of carbohydrate and lipid metabolism, but it also has definite effects on the body's handling of minerals (5, 4, 15). The latter became of clinical importance with Paloyan's (12) report that in primary hyperparathyroidism the serum calcium decreased in response to i. v. administration of G, and there was increased urinary excretion of sodium, potassium, chloride, ammonium and H-ions. As pancreatic secretion of G is stimulated by i. v. infusion of amino acids (16), it appeared necessary to consider whether G might be concerned in the aetiology of "idiopathic hypercalciuria".

Whether glomerular filtration rate (GFR) contributes to the glucagon mediated hyperelectrolyturia is still a matter of controversy. In earlier studies in dogs employing endogenous hormone stimulation (14), the GFR tended to fall when urinary calcium excretion reached its peak (40. to 50. min). In thyroparathyroidectomized animals there was no increase in urinary calcium, although the GFR did increase significantly.

These findings suggested that there might be an as yet undiscovered relationship between constituents of the diet, the kidneys and the functional state of pancreatic alpha cells, the thyroid and the parathyroids. The present work was undertaken to evaluate the renal effects of G after suppression of parathyroid activity by infusion of calcium. GFR and urinary phosphate levels were studied in particular as the latter is normally under the control of the parathyroid glands.

Material

The "Goettingen" minipig was chosen as a suitable experimental animal because in many ways its renal function is quite comparable to that of man (n = 6, 12 - 14 weeks, female, 11 - 20 kg b. w.). Owing to the lack of a standard diet for pigs, all the animals received normal feeds. They were fasted but received tap water ad libitum for 48 hours before the experiments, which were done during May-July, between 16.00 and 20.00 h.

Glucagon: crystallized extract from porcine pancreatic glands[1]. The half life of this material was 9 - 12 min (8). The use of a homologous preparation should give some advantages over its administration to a heterologous species. The duration of the clearance periods was adapted to half life of G.

Methods

Anesthesia: Neuroleptanalgesia ("Sedativum" and "Hypnotic"[2]); Diuresis: distilled water (250 ml/h) by an intragastric tube, i. v. infusion of Mannitol (20 per cent, 40 ml/h).

Inulin clearance: priming and maintenance infusion of 0.45 per cent inulin in Ringer solution with glucose (5 per cent) were given using a Unita-

1 Lilly Ltd., Giessen (W. Germany)
2 Janssen Ltd., Duesseldorf (W. Germany)

II-pump. The equilibration time was 40 min. Control values (baseline) correspond to the means of the first two clearance periods after achieving a steady state plasma level of inulin. Timed blood specimen were taken via a catheter in the femoral artery in the middle of each two periods, as well as each two periods preceding and following the infusion of G (0.01 mg/kg b.w. in 15 min).

Hypercalcaemia: 1st - periods I - V: 0.112 mg Ca^{++}/kg/min (Calcium gluconate[3]);
2nd - periods VI - X: 0.224 mg/kg/min
3nd - periods XI - XV: 0.448 mg/min

Analytical Methods

Inulin (3); inorganic phosphate (6); calcium by complexometric titration and ultrafiltration (13); magnesium by atomic absorption photometry; sodium and potassium by flame photometry; urinary-pH by glass electrode; H-ions by electrometric titration; ammonium (2), and osmolarity by a semi-micro-osmometer (Knauer, Berlin).

Calculation of free water clearance from $C_{H_2O} = V_U - C_{osm}$.

Total protein in serum was measured by the biuret method.

Statistical analysis: Student's t-test for unpaired data. All the values were shown to be normally distributed by the Kolmogoroff-Smirnow test.

3 Sandoz Corp., Nuernberg (W. Germany)

Results

Tables 1 and 2 show the principal effects of various degrees of hypercalcaemia. It should be noted that urinary sodium and volume increased whilst plasma sodium decreased: the baseline and final values of serum-Ca were 9.60 ± 0.79 and 19.60 ±

Table 1. Glomerular filtration rate (GFR), free water clearance (C_{H_2O}), urinary volume (V_U) and pH during i.v. infusion of Calcium gluconicum and Glucagon (+: 15 min). a = baseline values

periods	GFR/m² ml/min	C_{H_2O} ml/min	V_U ml/min	pH
a)	49.6 ± 29.6	1.0 ± 0.5	0.79 ± 0.30	5.2 ± 0.4
I	44.6 ± 18.4	1.0 ± 0.6	2.21 ± 1.41	5.3 ± 0.5
II	46.6 ± 23.8	0.6 ± 0.5	2.33 ± 1.52	5.4 ± 0.5
III+	61.6 ± 44.0	0.6 ± 0.6	3.29 ± 1.21	5.8 ± 0.7
IV	44.2 ± 26.4	0.4 ± 0.6	2.66 ± 1.09	5.6 ± 0.8
V	41.5 ± 23.3	0.5 ± 0.7	2.01 ± 0.80	5.1 ± 0.7
VI	41.2 ± 20.7	0.8 ± 0.3	2.15 ± 1.04	5.0 ± 0.6
VII	40.3 ± 24.0	0.9 ± 0.4	2.06 ± 1.10	4.9 ± 0.3
VIII+	47.6 ± 36.4	0.7 ± 0.7	3.07 ± 2.37	5.4 ± 0.8
IX	51.0 ± 26.5	0.2 ± 0.9	4.18 ± 2.23	5.4 ± 0.6
X	39.6 ± 19.9	0.3 ± 0.7	2.93 ± 1.81	4.7 ± 0.2
XI	48.7 ± 22.0	0.03 ± 1.1	3.38 ± 1.22	4.8 ± 0.3
XII	40.3 ± 19.4	-0.3 ± 1.0	3.73 ± 1.65	4.8 ± 0.4
XIII+	47.9 ± 17.9	-0.4 ± 0.9	4.52 ± 2.91	5.0 ± 0.6
XIV	42.8 ± 17.0	-1.0 ± 1.1	5.47 ± 2.21	5.0 ± 0.6
XV	36.9 ± 16.7	-0.3 ± 0.7	3.64 ± 1.16	4.6 ± 0.3
$p < 0.005$		(I:XIV		
$p < 0.0125$			(a:VII)	(a:XV)
$p < 0.025$		(VIII:XIII	(a:I)	
		(X:XIV	(a:VIII)	
$p < 0.05$		(I:IV)	(III:V)	
		(III:VIII)	(VII:IX)	
		(IX:XIV)	(XI:XIV)	

Table 2. Urinary electrolytes during infusion of Calcium gluconicum and Glucagon ($^+$: 15 min). a = baseline values

periods	U_{Ca} x 100 / GFR ug/min	U_{Na} x 100 / GFR uval/min	U_K x 100 / GFR uval/min	U_{Mg} x 100 / GFR ug/min	U_{PO_4} x 100 / GFR ug/min
a)	418.5 ± 249.0	295.5 ± 105.8	32.6 ± 13.0	221.2 ± 163.7	1253.8 ± 1746
I	1366.8 ± 598.9	518.4 ± 377.7	53.4 ± 25.8	364.9 ± 278.2	1424.7 ± 1537
II	1754.5 ± 1641.1	602.0 ± 494.0	60.6 ± 49.8	366.8 ± 352.1	1870.0 ± 2701
III$^+$	1611.8 ± 1156.3	738.5 ± 617.8	75.4 ± 46.0	362.1 ± 335.1	1656.8 ± 1704
IV	1243.0 ± 674.4	581.7 ± 451.2	61.2 ± 19.7	208.7 ± 134.5	1317.3 ± 1233
V	1038.3 ± 580.1	387.6 ± 343.5	46.7 ± 20.3	144.5 ± 133.7	1036.4 ± 983
VI	1469.6 ± 672.0	348.1 ± 204.1	49.7 ± 17.0	142.4 ± 111.6	832.8 ± 725
VII	2026.6 ± 1084.1	362.6 ± 225.8	58.9 ± 16.2	201.1 ± 150.9	728.8 ± 840
VIII$^+$	2952.5 ± 2810.0	848.0 ± 952.4	115.3 ± 110.6	328.5 ± 393.4	1635.5 ± 2613
IX	3119.5 ± 2738.3	724.8 ± 293.3	87.2 ± 30.0	171.5 ± 94.3	861.0 ± 949
X	2381.2 ± 1097.4	693.6 ± 416.8	80.8 ± 28.4	203.1 ± 91.5	712.3 ± 799
XI	3223.3 ± 1548.6	852.0 ± 788.6	73.3 ± 20.0	252.6 ± 99.6	626.6 ± 750
XII	4018.0 ± 2833.1	980.7 ± 628.2	119.7 ± 54.3	415.6 ± 192.5	664.9 ± 871
XIII$^+$	3142.3 ± 1112.7	832.2 ± 548.7	133.2 ± 71.4	325.8 ± 146.8	590.2 ± 1022
XIV	4070.2 ± 1915.1	1228.3 ± 961.1	155.1 ± 71.2	411.6 ± 163.6	691.5 ± 940
XV	4015.8 ± 1718.3	916.2 ± 546.5	138.0 ± 53.8	322.7 ± 181.8	576.5 ± 890
$p < 0.01$				(VI:XII)	
$p < 0.0125$			(VI:IX) (XI:XIV)		
$p < 0.025$		(a:XIV) (a:XII) (VI:IX)			
$p < 0.05$		(VII:IX)			

6.51 mg/100 ml; of Na 142.4 ± 2.4 and 133.2 ± 7.5 mequiv/l, respectively. Probability: $p < 0.01$ (Ca), $p < 0.05$ (Na). The clearance of total solutes (C_{osm}) increased as the free water clearance (C_{H_2O}) and urinary volume (V_U) decreased. There is no evidence that G affected this pattern of reaction.

Discussion

The tendency for a marked natriuresis found in this study must be interpreted with regard to tubular transport mechanisms for both calcium and sodium. Parathyroid hormone, which normally diminishes tubular reabsorption of sodium (7), would disappear during the calcium infusion. Walser (17) postulated a common proximal tubule carrier mechanism for both of these cations so that they compete with each other for transport. In addition, calcium may also be handled in a bidirectional manner (11). In the present experiment the calciuria exceeded the natriuresis but there was still no evidence of a active secretory process for calcium (i.e. $C_{Ca} < GFR$), whether or not G was infused. On the contrary, a week positive correlation was established between filtered load (F_{Ca}) and urinary calcium (U_{Ca}, Fig. 1). Hypoparathyroidism may be compatible with elevated urinary magnesium (U_{Mg}), but other factors must be responsible for the observed variability of urinary rates of inorganic phosphate (U_{P04}) excretion:
1st: lack of a standardised diet for pigs;
2nd: variation in individual response to the infused G;
3nd: differential skeletal avidity for phosphate etc. (turn over, age?).

The quantitative contribution of GFR to rates of urinary excretion of electrolytes is open to question: the initial increase observed during the first stage of the hypercalcaemia opposed the decrease observed later (Fig. 2), suggesting that the calcium ion itself might be the decisive factor rather than G alone. It might be suggested that G would stimulate GFR and that exogenous calcium might inhibit this effect, but there is little evidence to support either of these hypotheses. It seems likely that the renal effects of G (GFR, tubular electrolyte handling) are basically dependent on a functionally intact thyroid-parathyroid system. Calcitonin from the thyroidal-C-cells can be stimulated by both calcium and G (8) but whatever its role in a system gathering hypercalcaemia, hyperglucagonaemia and suppressed

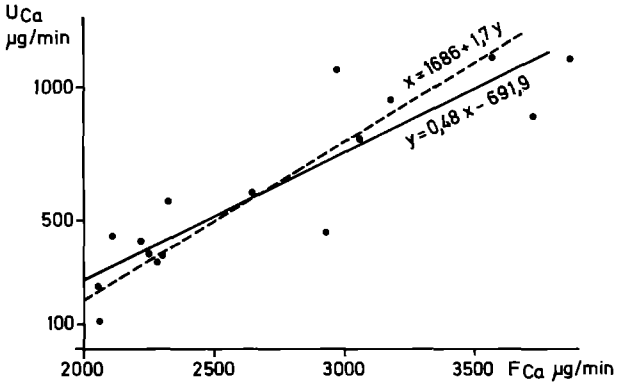

Fig. 1. Hypercalcaemia in minipigs. Filtered load and urinary calcium ($r = 0.64$, $p < 0.01$). Mean values, 16 periods, $n = 6$.

parathyroids (i. e. malignancy, endocrine adenomatosis) it is as yet unknown.

Other peptide hormones resemble G (e. g. calcitonin, parathyroid hormone) in producing their physiological effects by stimulating the intracellular formation of 3' - 5' - cyclic adenosine monophosphate (cAMP) in target tissues. Exact interpretation of the mechanism of their renal tubular actions is much more complicated and uncertain. Urinary cAMP during G infusion is derived from hepatic gluconeogenesis and subsequent glomerular filtration and not from renal tubular cells. Whether increased cAMP concentrations in post glomerular capillaries could be responsible for diminished reabsorption has also been studied using G and the stop flow technique (unpublished data). The results indicate that haemodynamic factors may be of greater importance than cAMP levels.

Another point of interest is the relationship between total serum protein and the level of ultrafiltrable calcium, which is normally governed by the law of mass action. Fig. 3 shows that there is a weak negative correlation between total protein and the concentration of ultrafiltrable calcium. This might represent diminished synthesis or increased

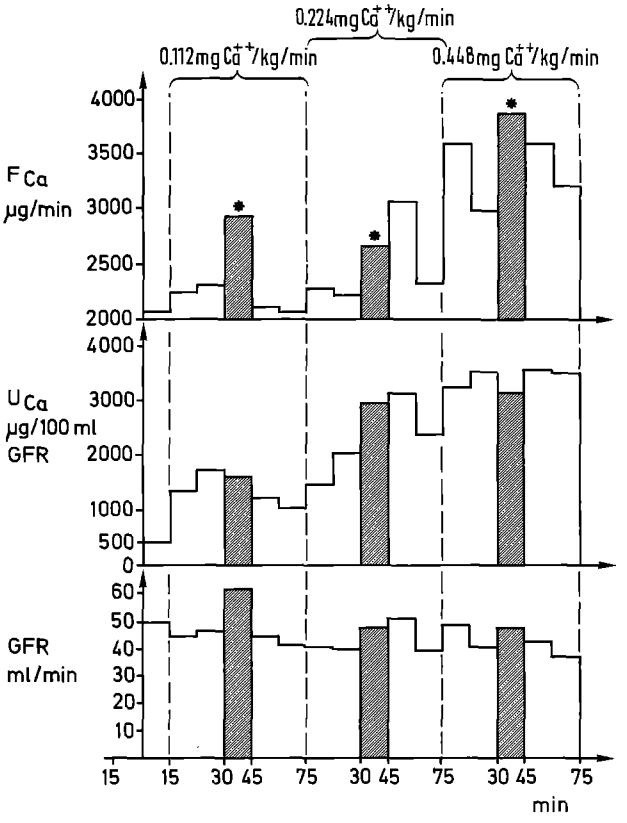

Fig. 2. The pattern of glomerular filtration rate (GFR), filtered load (F_{Ca}) and urinary Calcium (U_{Ca}) with increasing amounts of i. v. Calcium and additional Glucagon (*). Mean values ($n = 6$).

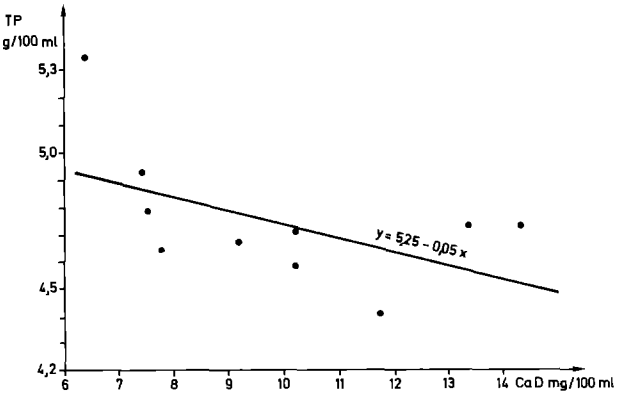

Fig. 3. Interrelations of serum ultrafiltrable calcium (Ca_D) and total protein (TP) during increasing amounts of i. v. calcium and additional Glucagon ($r = -0.53$, $p > 0.05$).

breakdown of proteins. The phenomenon of G-mediated hyperelectrolyturia could conceivably involve a disturbance of the binding capacity for calcium of the serum proteins as a major cause of the observed hypercalciuria.

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Dr. P. O. Schwille
Stoffwechsellabor
Chirurgische Universitätsklinik
Abt. für Urologie,
Krankenhausstr. 12
D-8520 Erlangen
Federal Republic of Germany

Responsible for the text: Prof. Dr. F. Truss, Göttingen, and Professor Dr. W. Vahlensieck, Bonn.
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