THE EFFECTS OF SYNTHETIC HUMAN AND SALMON CALCITONINS ON ELECTROLYTE EXCRETION IN THE RAT

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SUMMARY

1. The effects on renal electrolyte excretion of human and salmon synthetic calcitonins were studied in the rat.
2. As little as 50 ng of salmon calcitonin produced an increase in sodium excretion, but human calcitonin in doses up to 15 μg had only a slight effect.
3. Small doses of both hormones decreased urinary calcium and magnesium. Larger doses of salmon calcitonin produced less depression of calcium and magnesium excretion even in the presence of a greater fall in plasma calcium.
4. The mechanisms responsible for these effects and the possible therapeutic implications are discussed.

Key words: calcitonin, salmon calcitonin, human calcitonin, urinary calcium, urinary magnesium.

Calcitonin lowers plasma calcium by inhibiting bone resorption. It also affects the renal excretion of electrolytes in experimental animals and man.

Most studies of the renal effects of calcitonin have been performed with extracted porcine calcitonin preparations. In the rat porcine calcitonin increased phosphate and sodium excretion and diminished magnesium excretion (Robinson, Martin & MacIntyre, 1966; Milhaud & Moukhtar, 1966; Rasmussen, Anast & Arnaud, 1967; Pechet, Bobadilla, Caroll & Hesse, 1967). Conflicting results of the effect of porcine calcitonin on calcium excretion have been reported (Pechet et al., 1967; Rasmussen et al., 1967). Synthetic porcine calcitonin also increased sodium and phosphate excretion and decreased magnesium excretion in parathyroidectomized rats, confirming that these effects were not due to the stimulation of parathyroid hormone or caused by impurities in extracted preparations (Pors Nielsen, Buchanan-Lee, Matthews, Moseley & Williams, 1971).

There have been few studies of the renal effects of other species of calcitonin. Human calcitonin increased phosphate excretion in patients with mild osteoporosis (Woodhouse, Reiner, 1971).

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Kalu, Galante, Leese, Foster, Joplin & MacIntyre, 1970). Bijvoet, van der Sluys Veer, de Vries & van Koppen (1971) found human calcitonin to have effects similar to porcine calcitonin in increasing sodium, calcium and phosphate excretion in patients with osteoporosis and Paget's disease.

In the rat extracted salmon calcitonin produced a marked increase in sodium excretion while increasing phosphate excretion only slightly (Keeler, Walker & Copp, 1970). Urinary magnesium fell, whereas calcium excretion decreased with small doses but was increased by a large dose. Aldred, Kleszynski & Bastian (1970) found salmon calcitonin to have a much greater effect on electrolyte excretion in the rat than porcine calcitonin.

The present study was designed to compare the effects of human and salmon synthetic calcitonins on electrolyte excretion. It was hoped to resolve some of the contradictions in the literature and to compare the ratio of the effects of the two calcitonins on bone and the kidney.

**METHODS**

A wide dose range of synthetic human and salmon calcitonins was administered by intravenous infusion to conscious normal rats. In our bioassay system (Kumar & Sturtridge, 1968), the particular batch of salmon calcitonin used in this study had a specific biological activity of 3800 MRC units/mg and that of human calcitonin was 100 MRC units/mg.

Starved male Wistar rats (weight range 90–100 g) were given an intragastric water load equivalent to 2% of body weight. The animals were restrained in individual cages and infused via a needle inserted in a lateral tail vein. A solution containing 150 mM-glucose, 10.0 mEq of Na\(^+\)/l, 3.0 mEq of K\(^+\)/l, 2.0 mEq of Ca\(^{2+}\)/l and 1.0 mEq of Mg\(^{2+}\)/l was administered at a rate of 3.0 ml/h. After a 3 h equilibration period human calcitonin (dose range 250 ng to 1500 pg/rat) and salmon calcitonin (dose range 10.0 ng to 1.0 pg/rat) were infused over 1 h. Each dose of hormone was dissolved in 3.0 ml of the infusion fluid containing 0.1% crystalline bovine albumin at pH 4.5. Control animals received 3.0 ml of the albumin-containing infusion fluid. For each animal a single urine sample was collected for 3 h from the onset of the hormone infusion. Plasma was obtained by aortic puncture under ether anaesthesia at the end of the urine collection period, i.e. 2 h after the completion of the hormone infusion.

Similar experiments were conducted to study the effect of performic acid oxidation of the calcitonin molecules on their renal and hypocalcaemic activities. Performic acid oxidation was carried out as described by Hirs (1956). The effects of performic acid-oxidized salmon calcitonin (1.0 pg/rat) and performic acid-oxidized human calcitonin (10.0 pg/rat) were compared with equal and lower doses of the unaltered hormones.

The urine samples were acidified with 0.1 M-hydrochloric acid (0.05 ml/ml of urine). Creatinine and inorganic phosphate were measured by the Technicon Autoanalyser (Wootton, 1964), and Na\(^+\) by emission flame photometry. Mg\(^{2+}\) was determined by atomic-absorption spectrophotometry and Ca\(^{2+}\) by emission spectrophotometry (MacIntyre, 1961). Blood samples were heparinized and separated immediately. Plasma calcium and magnesium were determined as above and inorganic phosphate by the micro method described by Bauminger & Walthers (1966).

Where indicated the test groups were compared with the control group by means of a paired Student's t test. In other instances the linear regression of the response on the log dose of the hormones was calculated.
RESULTS

Fig. 1 shows the effects of the two hormones on the plasma concentrations of calcium, phosphate and magnesium measured 2 h after the completion of the hormone infusion. As little as 10.0 ng of the salmon hormone produced a significant fall in plasma calcium ($P < 0.02$). Considerably higher doses of human calcitonin were required to produce a similar hypocalcaemic response. Both calcitonins produced a marked fall in plasma phosphate, salmon calcitonin being effective at lower doses than human calcitonin. Plasma magnesium was not altered by either hormone.

Fig. 2 shows the effects of salmon and human calcitonins on the renal excretion of calcium, magnesium and phosphate. Small doses of either hormone decreased calcium and magnesium excretion. However, with increasing doses of salmon calcitonin, calcium and magnesium excretion increased towards the amount of excretion seen in the control animals. Neither hormone significantly altered phosphate excretion.

The effects on urine volume and the excretion of sodium and creatinine are shown in Fig. 3. The infusion of salmon calcitonin was followed by a marked increase in sodium excretion. The minimum effective dose was only 50.0 ng/rat ($P < 0.05$). The natriuresis was accompanied by an increase in urine volume. Under these experimental conditions human calcitonin in doses up to 15.0 $\mu$g/rat failed to produce a significant increase in sodium excretion. The regression of sodium excretion on the log dose of the calcitonins is given in Fig. 4.

Endogenous creatinine excretion increased slightly after both salmon and human calcitonin. Fig. 5 shows the regression of creatinine excretion on the log dose of the hormones.

Fig. 6 demonstrates the effects of performic acid oxidation on the renal and hypocalcaemic activities of the calcitonins. Performic acid oxidation abolished both the hypocalcaemic activity and the effects of the hormones on renal electrolyte excretion.

DISCUSSION

Compared in the rat and related to the porcine MRC Standard B, the hypocalcaemic activity of salmon calcitonin is 30-40 times more potent than that of human calcitonin (Galante, Horton, Joplin, Woodhouse & MacIntyre, 1971). Salmon calcitonin is also highly effective in inhibiting the release of $^{45}$Ca from cultured mice calvaria (Reynolds, Minkin & Parsons, 1970). It is of great interest therefore to compare the action of the two calcitonins on renal electrolyte excretion.

In the present study a decrease in urinary calcium excretion was seen after both human and salmon calcitonin infusion. This results primarily from the action of the hormones on bone. By inhibiting bone resorption calcitonin decreases the plasma calcium concentration and hence the filtered calcium load.

Magnesium excretion also decreased after both hormones. The mechanism responsible for this effect is not certain. The plasma magnesium concentration was not affected by either calcitonin. Since there is evidence that calcium and magnesium share a common pathway for tubular reabsorption (Alcock & MacIntyre, 1962) the decrease in magnesium excretion may be due to a decrease in the filtered load of calcium, permitting increased tubular reabsorption of magnesium.

With increasing doses of salmon calcitonin there was a progressive rise in urinary calcium and magnesium even in the presence of a greater fall in plasma calcium. This indicates that
FIG. 1. Effects of (a) salmon and (b) human calcitonin on plasma calcium, phosphate and magnesium measured 2 h after the infusion of the hormones or control solution (no calcitonin dose). Mean ± SEM, n=5-8 animals.

FIG. 2. Effects of (a) salmon and (b) human calcitonin on the urinary excretion of calcium, magnesium and phosphate. Mean ± SEM, n=5-8 animals.
Calcitonin and electrolyte excretion

Fig. 3. Effects of (a) salmon and (b) human calcitonin on urine volume and sodium and endogenous creatinine excretion. Mean ± SEM, n=5–8 animals.

Fig. 4. Regression of sodium excretion on the dose of salmon and human calcitonin. For salmon calcitonin: $y=79.6x+6.97$, $r=0.88$, $P<0.001$. For human calcitonin: $y=7.14x+86.8$, $r=0.20$, $P<0.2$. 
Fig. 5. Regression of creatinine excretion on the dose of salmon and human calcitonin. For salmon calcitonin: \( y = 1.39x + 12.8, \ r = 0.60, \ P < 0.001 \). For human calcitonin: \( y = 0.79x + 12.1, \ r = 0.49, \ P < 0.005 \).

Fig. 6. Effect of performic acid oxidation of salmon and human calcitonin. Mean \( \pm \) SEM, \( n = 5–8 \) animals.
Calcitonin and electrolyte excretion

salmon calcitonin, at least at higher doses, acts on the kidney to increase calcium and magnesium excretion. It is not certain if this is a specific action of the hormone or whether it is dependent on the increase in sodium excretion. A similar effect was not observed with human calcitonin demonstrating a greater renal action of the salmon hormone.

In young rats the rate of bone turnover is exceedingly high and hence the fall in plasma calcium after calcitonin infusion is marked. The results of these experiments indicate that under conditions of less rapid bone turnover salmon calcitonin could cause a net increase in calcium excretion.

Urinary phosphate excretion was not significantly altered by either hormone. However, the fact that phosphate excretion did not decrease as a result of the fall in plasma phosphate implies an effect on phosphate clearance of both calcitonins. In parathyroidectomized rats we have demonstrated an increase in phosphate excretion after both human and salmon calcitonins. The effect of salmon calcitonin was considerably greater than that of human calcitonin (C. C. Williams, E. W. Matthews, J. M. Moseley & I. MacIntyre, unpublished work).

The excretion of calcium, magnesium and phosphate after calcitonin infusion is influenced to a considerable extent by the action of the hormone on bone. It is therefore difficult to compare the renal activity of salmon and human calcitonin on the basis of these electrolytes. Sodium excretion is better suited for this purpose. Salmon calcitonin has a marked natriuretic effect in the rat. Only 50 ng of calcitonin/animal was required to produce a significant increase in sodium excretion. There was a further dose-dependent increase in sodium excretion throughout the dose range studied. The natriuresis was accompanied by an increase in urine volume. In comparison, human calcitonin in doses up to 15.0 μg/rat failed to increase sodium excretion significantly. However, in a study in which half-hourly urine samples were obtained human calcitonin has been shown to have a slight natriuretic effect in the rat (C. C. Williams, E. W. Matthews, J. M. Moseley, I. M. Moseley & I. MacIntyre, unpublished work). It is likely that the length of the urine collection period in the present study obscures such a response.

Based on the consideration that 50 ng of salmon calcitonin produced a much greater increase in sodium excretion than 15.0 μg of human calcitonin, it would appear that the renal activity of salmon calcitonin in the rat relative to that of human calcitonin is even greater than the relative hypocalcaemic potencies of the two hormones.

The mechanism by which calcitonin alters renal electrolyte excretion has not been studied in detail. In rats (Milhaud & Moukhtar, 1966) and in the dog and pig (Russell & Fleisch, 1968) no change in the glomerular filtration rate followed the administration of porcine calcitonin. Conversely, Salako, Smith & Smith (1970) reported a marked increase in the clearance of both inulin and p-aminohippurate in the rabbit. In man a slight increase in the glomerular filtration
rate has also been reported after porcine calcitonin (Ardaillou, Fillastre, Milhaud, Rousselet, Delaunay & Richet, 1969).

In the present study endogenous creatinine excretion was increased by the infusion of salmon calcitonin. This suggests that salmon calcitonin may increase sodium excretion through an increase in the glomerular filtration rate. However, endogenous creatinine excretion also increased after infusion of human calcitonin, and at doses of the two hormones which caused a similar rise in creatinine excretion, sodium excretion was increased by salmon calcitonin but not by human calcitonin. It is therefore unlikely that an increase in the glomerular filtration rate is solely responsible for the natriuretic effect of salmon calcitonin. The precise mechanism of the renal action of calcitonin is obscure and needs further investigation.

The reason for the greater potency of salmon calcitonin is not understood. All species of calcitonin for which the amino acid sequence has been determined are polypeptides of thirty-two amino acids. All have a 1-7 disulphide linkage at the amino terminus and proline amide at the carboxyl terminus. However, among the species of calcitonin there are many differences in the amino acid sequences. Fig. 7 shows that human and salmon calcitonin share the same amino acids in only sixteen of the thirty-two positions. It is therefore possible that differences in structure render salmon calcitonin better suited to fit the receptor sites. The relatively greater renal action of salmon calcitonin may indicate that the receptors in bone and kidney are not identical.

However, the greater potency of salmon calcitonin may not depend on differences in hormone–receptor interaction. Salmon calcitonin is more resistant to inactivation by plasma than porcine calcitonin (Copp, Brooks, Low, Newsome, O'Dor, Parkes, Walker & Watts, 1970). de Luise, Martin & Melick (1970) have also shown salmon calcitonin to be more resistant to inactivation and degradation by rat liver. It is possible therefore that the greater activity of salmon calcitonin depends, at least in part, on its greater stability and the persistence of the hormone in the plasma.

The effect of the calcitonins on bone, once initiated, persists beyond the disappearance of the hormone from the plasma. In comparison the effect on the kidney is much shorter. The renal action of the hormone may therefore be more dependent on the circulating concentration of calcitonin. If so the greater stability of salmon calcitonin would favour its action on electrolyte excretion relative both to its hypocalcaemic activity and to the renal action of the other calcitonins.

It is uncertain if the results of this study are applicable to man. Whereas the calciuric action of salmon calcitonin may be useful in the treatment of hypercalcaemia, it may be disadvantageous under other circumstances. The chronic administration of salmon calcitonin to patients with Paget's disease or osteoporosis may result in a negative calcium balance which might further complicate the metabolic bone disorder. The role of calcitonin in sodium metabolism in man requires further study.

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