Renal Effects of Calcitonin and Gelatin Infusions in Anesthetized Rats (37694)

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The pharmacologic effects of calcitonin on the kidney appear to be controversial. Many data suggest that it may be a natriuretic agent (1, 3–6, 8, 9, 11–13, 16, 18), whereas other data indicate no effect on sodium excretion (7). Still others point to an equivocal natriuresis at one dosage level and no effect at higher or lower doses (1). It has been reported to increase (1, 13) or have no effect on (11, 16) potassium excretion. When effects on water and solute excretion were found, they were attributed to tubular effects. However changes in filtered load could not be ruled out since glomerular filtration rate was not measured (1, 2, 4, 6, 11–13, 16–18).

The current experiments were designed to evaluate the effects of the infusion of salmon calcitonin[1] and its vehicle, gelatin, on glomerular filtration rate (GFR), urine flow rate (V), sodium ($U_{Na}V$) and potassium (U_KV) excretion and plasma sodium (P_{Na}) and potassium (P_K) in intact, anesthetized, nonfluid nor solute-loaded rats. To improve the interpretability of the results each animal was used as its own control.

Methods. Fifty-six adult Sprague-Dawley rats of both sexes whose average weight was 342 ± 9 (SEM) g were maintained, three or four per cage, in a constant temperature room, before the experiments. They were allowed free access to Purina Lab Chow pellets and tap water. Anesthesia was induced with sodium pentobarbital, 45 mg/kg, via a tail vein. Supplements were given as required during the experiments. A tracheostomy was performed.

Cannulations with polyethylene tubing (PE-50) of a jugular vein for fluid administration and a carotid artery for blood sampling were accomplished. A ureter was exposed through a midline abdominal incision and cannulated with polyethylene tubing. After completion of the surgery, all wounds were closed with metal wound clips.

Two preliminary experiments were done in which only urine flow rate and sodium excretion were measured. In the first of these, 13 rats were given intravenous isotonic saline at 0.05 ml/min during a 60 min equilibration period and during each of two 30 min urine collection periods. While the infusion rate stayed the same, each animal then received a priming injection of 0.5 ml of 1% gelatin, after which gelatin was added to the infusate in a concentration of 0.132%. Two to four 30 min urine collections were observed during the next 3 to 4 hr. In the second preliminary experiment, nine animals received an infusion of 0.132% gelatin in isotonic saline at 0.05 ml/min during an equilibration period and two 30 min urine collection periods. Then each rat received a priming injection of 0.5 ml of 1% gelatin in isotonic saline containing 5 units of salmon calcitonin. The original infusion was continued at the same rate but calcitonin was added to it so as to deliver 0.067 units/min. Again two to four 30 min collection periods were observed with the new infusate.

In all subsequent experiments, in addition to data on urine flow rate and sodium excre-

[1] Natural Salmon Calcitonin (lot K423-119, 590 MRC units/mg solids) and Calcitonin Vehicle (16% gelatin diluent solution, lots K423-135 and K527-054) were very kindly supplied by Dr. J. W. Bastian, Head, Department of Pharmacology, Armour Pharmaceutical Co., Kankakee, IL.

tion, inulin clearance, plasma sodium and potassium concentrations and urine potassium excretion were measured. Each animal received a priming dose of 2.1 mg/kg body weight of inulin in isotonic saline as well as sufficient inulin in all infusates to deliver 0.2 mg/min. All infusions were given at 0.05 ml/min in isotonic saline. For each protocol a 1 hr equilibration period was first observed after the first infusion was begun. Then two 30 min clearance periods were observed. After the infusate was changed a second 30 to 45 min equilibration period was observed after which two to six 30 min clearances were done. Blood was sampled at the midpoint of each clearance period. The contents of the infusates were as follows: (a) (6 rats) isotonic saline for all clearance periods, (b) (15 rats) isotonic saline for first two clearances and 0.067% gelatin in isotonic saline subsequently, (c) (6 rats) isotonic saline first, followed by 0.067% gelatin in saline to which enough calcitonin was added to give 0.133 units/min, and (d) (7 rats) 0.067% gelatin in saline first then the same infusate to which calcitonin was added to give 0.133 units/min.

Urine was collected in preweighed plastic tubes and the volume was determined gravimetrically to the nearest milliliter. Blood was collected in ice-cold plastic syringes and centrifuged. Sodium and potassium in urine and plasma were determined on an Instrumentation Laboratories flame photometer using an internal lithium standard. Inulin in urine and trichloroacetic acid filtrates was determined by a micro-adaptation of the diphenylamine method of Harrison (10). Inulin clearance was calculated and expressed as glomerular filtration rate. All urinary data are for one kidney. All results are expressed as mean ± standard error of the mean (SEM). As appropriate, the statistical significance of the differences between means was determined using paired and unpaired t tests.

Results. The data from the preliminary experiments are shown in Table I. Increases in urine flow rate and sodium excretion were observed when the calcitonin vehicle, gelatin, first as a bolus of 0.5 ml of a 1% solution plus an infusion of a 0.132% solution at 0.05 ml/min, was added to the saline infusion. When calcitonin (a 5 unit bolus plus an infusion of 0.067 units/min) was added to an infusion of gelatin, a significant increase in urine sodium excretion was observed but not an increase in urine flow rate.

Table II shows the detailed paired data resulting from the different infusions. In each pair the value on the left is the mean of data generated during the first two clearance periods and before the infusate was changed. The value on the right is the mean of data collected during the two to six clearance periods after the infusate was changed. The first data pair, where the infusate was isotonic saline throughout, shows that there was no difference in any value during this time.

The second pair of data confirm the preliminary experiments in that when gelatin was added to the saline infusion, a significant rise in urine flow rate and sodium excretion resulted. Further, this also resulted in a rise in glomerular filtration rate. The fourth pair of data indicates that neither urine flow rate nor sodium excretion increase

TABLE I. Effect of Saline, Gelatin and Calcitonin on Urine Flow Rate and Sodium Excretion.[a]

Infusion period:	1	2	1	2
Infusate:	Saline $n=13$	Gelatin	Gelatin $n=9$	Gelatin + calcitonin
Urine flow rate (μliter/min kg body wt)	80.7 ± 15.3	154.9 ± 21.9[b]	171.9 ± 34.2	209.8 ± 41.9
Urine Na excretion (μEq/min kg body wt)	19.8 ± 3.2	29.6 ± 3.0[b]	32.4 ± 3.6	51.6 ± 6.5[c]

[a] Data are mean ± standard error. Significance of differences analyzed by paired t tests.
[b] Compared to mean of first infusion period: $p < 0.05$; [c] $p < 0.01$.

TABLE II. Complete Data Resulting from Infusion of Saline, Gelatin and Calcitonin.[a]

Infusion period:	1	2	1	2	1	2	1	2
Infusate:	Saline	Saline	Saline	Gelatin	Saline	Gelatin[b] calcitonin	Gelatin	Gelatin[b] calcitonin
Plasma K (mEq/liter)	3.88 ± 0.10	3.98 ± 0.17	3.89 ± 0.16	3.65 ± 0.06	4.14 ± 0.10	3.68 ± 0.19	4.11 ± 0.17	3.35 ± 0.17[c]
Plasma Na (mEq/liter)	145.5 ± 0.7	145.4 ± 1.3	145.6 ± 1.6	145.4 ± 1.2	143.5 ± 0.9	142.0 ± 0.8	146.2 ± 1.6	146.1 ± 2.0
GFR (ml/min kg)	5.23 ± 0.30	5.06 ± 0.20	3.34 ± 0.28	3.89 ± 0.39[b]	3.71 ± 0.25	4.68 ± 0.34[c]	5.75 ± 0.79	6.56 ± 1.11
Urine flow rate (μliter/min kg)	48.0 ±11.3	50.8 ±14.5	13.7 ± 0.9	21.9 ± 2.7[b]	19.6 ± 3.0	51.6 ± 9.5[c]	31.7 ± 6.4	49.6 ± 9.1
Urine Na excretion (μEq/min kg)	8.80 ± 2.15	11.83 ± 3.64	1.40 ± 0.27	4.36 ± 0.95[b]	1.48 ± 0.35	16.27 ± 3.27[d]	6.39 ± 1.82	14.37 ± 2.59
Urine K excretion (μEq/min kg)	4.80 ± 0.57	4.45 ± 0.37	3.36 ± 1.31	4.97 ± 2.10	2.83 ± 0.45	2.29 ± 0.40	4.00 ± 0.69	2.89 ± 0.51

[a] Data are mean ± standard error. Number of rats for data pairs are 6, 15, 6, 7, respectively. Except for plasma Na and K, values are for one kidney only. Significance of difference analyzed by paired t test. When no symbol appears next to a data pair, $p > 0.05$.

[b] Compared to mean of first infusion period: $p < 0.05$; [c] $p < 0.02$; [d] $p < 0.01$.

as a result of adding calcitonin to the gelatin infusion. The preliminary experiments showed that with this pair of infusions adding calcitonin resulted in an increase in sodium excretion. Actually the p value for sodium excretion in the more detailed experiments very nearly reached the 95% confidence limits. The facts that GFR, urine flow rate, and sodium excretion rate increase after gelatin, whether calcitonin is added or not, can be seen by comparing the third pair of data with the second and fourth.

The data on plasma potassium suggest that gelatin, when given for a sufficient period of time, will bring about a decrease. When gelatin, with or without calcitonin, was infused during the experimental periods only, the decrease in plasma potassium was not significant. However when gelatin was infused during the control period as well, plasma potassium fell significantly. The effect of calcitonin on plasma potassium, if any, cannot be determined from these experiments.

In Figs. 1 and 2, GFR is plotted against urine flow rate (V) and urine sodium excretion ($U_{Na}V$), respectively. The data are those generated when saline was the first infusate and the second was either gelatin or gelatin plus calcitonin. The control values are the averages of those during saline infusions. These were divided into each subsequent experimental value. The figures suggest that the diuresis and natriuresis were independent of GFR, although when GFR rose as a result of the second infusion, both urine volume and sodium excretion nearly always rose.

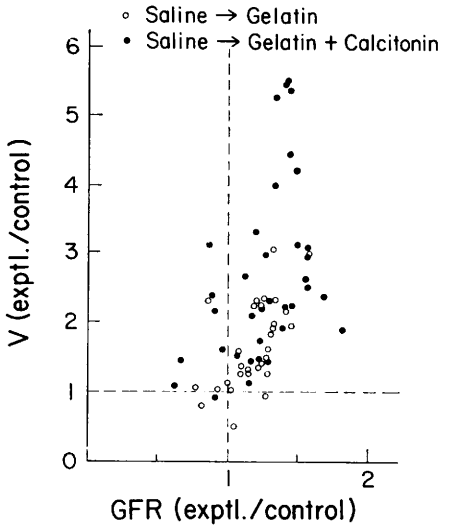

FIG. 1. Ratio of urine flow rate during control (saline) and experimental (gelatin or gelatin + calcitonin) infusions *versus* ratio of GFR at the same time. Each point indicates data from a single rat. The broken lines each indicate identity. Points above and to the right of these indicate increases in both parameters during the experimental period.

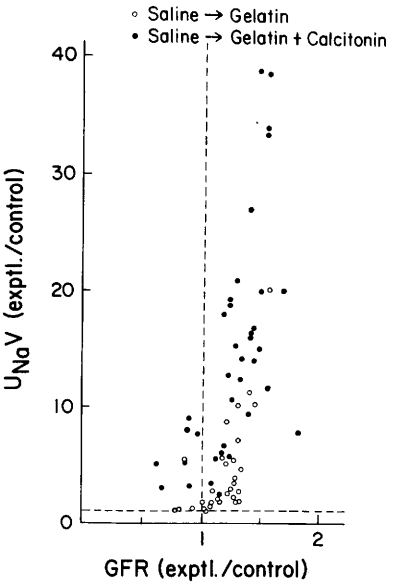

FIG. 2. Ratio of urine sodium excretion during control (saline) and experimental (gelatin or gelatin + calcitonin) infusions *versus* ratio of GFR at the same time. Each point indicates data from a single rat. The broken lines each indicate identity. Points above and to the right of these indicate increases in both parameters during the experimental period.

Finally sufficient blood for calcium determination was available before and after calcitonin infusion in 16 animals. The control serum calcium value was 9.12 ± 0.31 (SE) mg/100 ml while that after calcitonin infusion was 8.07 ± 0.28 mg/ml. By paired t test the difference between these was highly significant ($p < 0.005$).

Discussion. In order to evaluate the renal effects of calcitonin, it is necessary to separate its effects from those of the vehicle in which calcitonin is given. In our experiments, the vehicle—gelatin—not only increased the GFR but also was diuretic and natriuretic. The addition of calcitonin caused no further

increase in these parameters with the possible, but undramatic, exception of sodium excretion. There was a slight, but significant, increase in sodium excretion in the preliminary series of experiments, and in the complete series, the 95% confidence limit for increased sodium excretion was nearly reached. However, it could be argued that any natriuresis exhibited by rats receiving gelatin plus calcitonin (compared to their sodium excretion rates when they were receiving only gelatin) was the simple result of the fact of their having received a larger total amount of gelatin during the course of the experiments. Our conclusion is that calcitonin does not affect the GFR and urine flow rate, and may not affect the sodium excretion rate of anesthetized rats. In support of previous findings (11, 16), potassium excretion appears to be unaffected by calcitonin also. Clark and Kenny (7) report nearly identical findings for calcitonin administration to anesthetized dogs.

This conclusion is in contrast with a very widely held view. Diuretic and/or natriuretic effects have been reported in rats (1, 2, 11, 13), sheep (4), and humans (3, 5, 6, 8, 18). This apparent discrepancy can be resolved by consideration of several possibilities, as outlined below.

First, it is possible that in many previous studies the diuretic and/or natriuretic effects were due to administration of the vehicle rather than calcitonin. In many studies this possibility was not controlled for adequately, and there is some evidence that every single vehicle used does have renal effects. We, and others (2) have found effects of gelatin injections on urine flow rate. Oxypolygelatin has been found to cause sustained increases in GFR, renal plasma flow, sodium excretion rate, and urine flow (15). Bovine serum albumin was used as a vehicle in two studies (4, 13), and though the data of Nielson *et al.* (13) are difficult to interpret, it seems that this vehicle caused a doubling of sodium excretion after only 1 hr. A glycine acetate buffer has also been used as a vehicle (11); Pitts (14) showed many years ago that glycine administration dramatically increases both renal plasma flow and GFR, the consequences of which would certainly be natriuresis and diuresis.

Second, even when natriuretic and diuretic effects were observed, these effects in many cases appeared to be extremely variable and labile even in the hands of the same investigators. As an example, diuresis was observed upon administration of 1, 5, and 10 units/kg of porcine calcitonin in one report (2) but later (1), 2 units/kg had no effect on, 20 units/kg decreased, and 40 units/kg increased, urine flow rates. Whatever the cause of the variability in response, it is conceivable that on the average, there was no effect of calcitonin on urine flow in these studies.

Third and finally, it is indeed possible that in some circumstances, calcitonin may be natriuretic and diuretic. It should be noted that these effects may be expressed only in certain species and only under specific conditions within the species: conscious *versus* anesthetized, saline or water loaded *versus* normally hydrated animals, thyroid-parathyroidectomized *versus* normal animals, administration of porcine *versus* salmon calcitonin preparations, and so on.

Summary. Pentobarbital-anesthetized rats of both sexes received intravenous infusions of isotonic saline, with or without gelatin and/or salmon calcitonin. Each animal served as its own control. Diuresis, natriuresis and increased GFR followed the addition of gelatin to saline infusions. Similar effects were observed when gelatin plus calcitonin were added to the saline infusion. Calcitonin may have increased sodium excretion, but did not affect urine flow rate or GFR when added to infusions of gelatin. No effect on potassium excretion was seen. Therefore gelatin is diuretic and natriuretic and calcitonin may be natriuretic but not diuretic when infused into the anesthetized rat. The changes in urine flow rate and sodium excretion were not caused by changes in GFR, suggesting altered tubular function as the cause.

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1. Aldred, J. P., Kleszynski, R. R., and Bastian, J. W., Proc. Soc. Exp. Biol. Med. 134, 1175 (1970).

2. Aldred, J. P., Stubbs, R. K., Hermann, W. R., Zeedyk, R. A., and Bastian, J. W., Acta Endocrinol. Copenhagen **65**, 737 (1970).

3. Ardaillon, R., Fillastre, J. P., Milhaud, G., Rousselet, F., Delaunay, F., and Richet, G., Proc. Soc. Exp. Biol. Med. **131**, 56 (1969).

4. Barlet, J. P., J. Endocrinol. **55**, 153 (1972).

5. Bijvoet, O. L. M., Van der Sluys Veer, J., and Jansen, A. P., Lancet **1**, 876 (1968).

6. Bijvoet, O. L. M., Van der Sluys Veer, J., De Vries, H., and Van Koppen, A. T. J., N. Engl. J. Med. **284**, 681 (1971).

7. Clark, J. D., and Kenny, A. D., Endocrinology **84**, 1199 (1969).

8. Cochran, M., Peacock, M., Sachs, G., and Nordin, B. E. C., Brit. Med. J. **1**, 135 (1970).

9. Haas, H. G., Dambacher, M. A., Guncaga, J., and Lauffenburger, T., J. Clin. Invest. **50**, 2689 (1971).

10. Harrison, H. E., Proc. Soc. Exp. Biol. Med. **49**, 111 (1942).

11. Keeler, R., Walker, V., and Copp, D. H., Can. J. Physiol. Pharmacol. **48**, 838 (1970).

12. Kenny, A. D., and Heiskell, C. A., Proc. Soc. Exp. Biol. Med. **120**, 269 (1965).

13. Nielsen, S. P., Buchanan-Lee, B., Matthews, E. W., Moseley, J. M., and Williams, C. C., J. Endocrinology **51**, 455 (1971).

14. Pitts, R. F., Amer. J. Physiol. **142**, 355 (1944).

15. Raïsz, L. G., J. Lab. Clin. Med. **40**, 880 (1952).

16. Rasmussen, H., Anast, C., and Arnaud, C., J. Clin. Invest. **46**, 746 (1967).

17. Robinson, C. J., Martin, T. J., and MacIntyre, I., Lancet **2**, 83 (1966).

18. Singer, F. R., Woodhouse, N. J. Y., Parkinson, D. K., and Joplin, G. F., Clin. Sci. **37**, 181 (1969).

19. Smith. H. W., "The Kidney. Structure and Function in Health and Disease," p. 568. Oxford Univ. Press, New York (1951).

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