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Differential Effects of Salmon, Porcine, and Human Calcitonin on Gastric Secretion and Gastric Emptying in Rats (38327)

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(Introduced by R. J. Schlueter)

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Recently calcitonin (CT) has been shown to have marked actions on gastrointestinal (G. I.) function in addition to its established effects on bone and kidney. On the basis of studies in man and animals, CT has been shown to inhibit: gastric acid and volume secretion (1–5), pancreatic exocrine secretion (3, 6), contraction of the lower esophageal sphincter (7) and gallbladder motility (1, 3, 6, 8).

Recently Hüfner *et al.* (3) reported inhibition of gastric acid secretion in man with porcine, salmon, and human CT. Little attention, however, has been given to more precise quantitative comparisons of the relative actions of these three species of CT on G. I. function. In the present studies we have compared the actions of porcine, salmon, and human CT on gastric secretion and gastric emptying in rats, using as the basis for comparison their hypocalcemic actions in the same test species.

Materials and Methods. A. Calcitonin preparations. The porcine calcitonin (PCT) used in these studies was of natural origin and was prepared by Dr. R. J. Schlueter, Armour Pharmaceutical Company, Kankakee, Illinois.

Synthetic salmon calcitonin (SCT) was obtained from Sandoz Ltd., Basle, Switzerland and Armour Pharmaceutical Company, Kankakee, Illinois.

Synthetic human calcitonin (HCT) was prepared by Dr. J. L. Hughes, Armour Pharmaceutical Company, Kankakee, Illinois.

To determine possible qualitative differences among the three species of CT regarding G. I. action, they were first equated for hypocalcemic potency and then, on this basis, tested for their relative actions on the G. I. endpoints. The relative hypocalcemic potencies of the three CT preparations were determined by rat bioassay versus a single standard preparation (MRC Porcine Research Standard B). The term "MRC" units is, therefore, properly applied only to the porcine preparation. Accordingly, potencies of SCT and HCT are reported herein as "units/mg." Hypocalcemic potencies of the CT preparations were determined 1 hr after subcutaneous injection in CFE[1] male rats weighing 180–200 g (9, 10). Measured activities of the preparations per mg solids (± SE) were: porcine calcitonin, 62 ± 6 MRC units; salmon calcitonin, 4400 ± 550 units, and human calcitonin, 408 ± 32 units.

B. Effects on gastric acid secretion. Adult male Holtzman[2] rats weighing 190–210 g were placed in individual metal cages and starved for 24 hr. Drinking water was permitted *ad lib.* during the starvation period. Each animal was etherized, the abdomen opened by a midline incision, the pylorus ligated (11), and the incision closed with wound clips. To assure adequate hydration and to maximize uniformity of results between rats, an injection of 25 ml of air was made sc at the midpoint of the dorsum while the rats were still under ether anesthesia, and a 5-ml volume of physiological saline injected into the formed pouch. The pouch was then deflated. At a separate site the animals were immediately treated sc (0.1 ml/100g) with either gelatin vehicle (16% gelatin with 0.5% phenol at pH 6.0) or CT in the same vehicle, and the rats were returned to their respective individual cages. Four hr after pyloric ligation all animals were sacrificed with ether. The stomachs were removed after ligating the esophagus in each case to prevent loss of accumulated gastric fluid. The excised stomachs were washed to remove any blood and opened along the greater curvature.

[1] Carworth Farms, Portage, Michigan.

[2] Holtzman Company, Madison, Wisconsin.

Gastric contents were collected and centrifuged to remove solids. Fluid volumes were measured and total acidities were determined by titration with 0.1 *N* NaOH to pH 7.0. Volume outputs were calculated and expressed as milliliters/animal. Statistical analyses were carried out using Student's *t* test. Relative potency estimates were calculated (12) using a 3 × 3 factorial design comparing the three lowest dose levels of PCT, SCT, and HCT.

C. Effects on gastric emptying. Young female Sprague–Dawley[3] rats weighing 95–115 g were placed in individual cages and starved for 24 hr. Drinking water was permitted *ad lib.* until time of the experiment. Treated animals were given CT, sc, using the same volume dose of the same gelatin vehicle described above. Control rats were given the gelatin vehicle alone. One milliliter of Evan's blue dye was given by oral intubation as an aqueous solution at a concentration of 10 mg/ml. The times of dye administration and sacrifice in relation to the time of CT administration were varied in the different experiments. At specified times after dye administration the animals were sacrificed with ether and the stomachs were removed and individually minced in 15 ml of distilled water (made basic by addition of 2 drops of 10 *N* KOH). This procedure allowed for total solubilization of the Evan's blue dye including any which had been adsorbed by the gastric mucosa. The resultant liquids were then centrifuged and the supernatants were diluted 1:10 with distilled H_2O. The percentage light transmission (475 nm) was determined for each sample using a Spectronic-20. A standard curve was established and the percentage of dye retained in the stomach was calculated. Statistical significance was determined by Student's *t* test. Relative potency estimates of PCT, SCT, and HCT were calculated (12).

Results. Gastric acid secretion. Effects of PCT, SCT, and HCT on gastric secretion are shown in Table I and Fig. IA. Volumes and total acidities of the gastric contents were diminished by all three preparations. For each of the three species of calcitonin considered separately, results for each test group were significantly different from those for the vehicle-treated control

[3] A. R. S. Sprague–Dawley, Madison, Wisconsin.

TABLE I. Effects of Porcine, Salmon and Human Calcitonin on Volume and Total Acidity of Gastric Secretion in Rats.

Treatment	Calcitonin (units/kg)	Mean volume/rat (ml ± SE)	Total acidity μeq/liter/100 gm ± SE
Vehicle control	—	7.88 ± .62	358 ± 36
PCT	0.5	4.74 ± .45**	203 ± 20*
PCT	1.0	4.41 ± .26**	155 ± 23**
PCT	2.0	3.16 ± .43**	92 ± 9**
PCT	4.0	2.85 ± .31**	103 ± 16**
PCT	8.0	3.20 ± .49**	81 ± 28**
Vehicle control	—	9.01 ± .92	335 ± 41
SCT	0.5	4.98 ± .25*	195 ± 33*
SCT	1.0	4.68 ± .34*	167 ± 30*
SCT	2.0	3.40 ± .43**	104 ± 16**
SCT	4.0	2.88 ± .61**	85 ± 17**
SCT	8.0	2.82 ± .68**	61 ± 7**
Vehicle control	—	9.06 ± .39	499 ± 25
HCT	2.0	5.54 ± .39**	269 ± 23**
HCT	4.0	5.34 ± .64**	247 ± 42**
HCT	8.0	4.86 ± .64**	219 ± 42**
HCT	16.0	4.78 ± .49**	238 ± 36**
HCT	32.0	4.50 ± .55**	172 ± 38**

* $P = < 0.01$ vs vehicle control.
** $P = < 0.001$ vs vehicle control.
N = 10 rats/dose level.
SE = standard error of the mean.

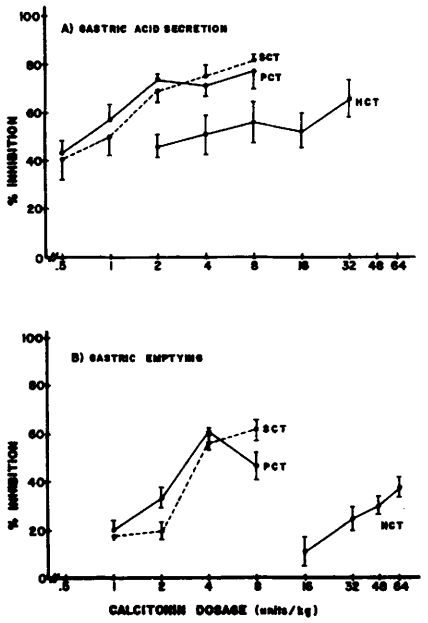

FIG. 1. Inhibition of gastric acid secretion and gastric emptying by porcine, salmon, and human calcitonin. Limits shown are ± 1.0 SEM.

group ($P < 0.01$ or $P < 0.001$). Similar dose-response curves were obtained with PCT and SCT, but HCT, at equivalent hypocalcemic dosages, was markedly less effective in inhibiting gastric secretion. The relative potency of SCT compared to PCT was 0.80 ± 0.63. The relative potency of HCT was approximately 0.06, although exact potency limits could not be calculated.

Gastric emptying. The comparative effects of PCT, SCT, and HCT are shown in Table II and Fig. IB. In these experiments the CT preparations were administered 15 min prior to test meal. The animals were sacrificed 60 min after receiving the test meal. Similar dose-response curves were obtained with PCT and SCT, but HCT was markedly less potent in inhibiting gastric emptying in rats. The relative potencies of SCT and HCT compared to PCT were 0.91 ± 0.13 and 0.04 ± 0.02, respectively.

In a separate study the duration of the effect of SCT on gastric emptying was investigated. This was carried out by altering either the time from SCT to test meal administration or the time from test meal administration to sacrifice. The results are shown in Table III. Significant inhibitory effects were demonstrable when: (a) SCT was administered 15 min prior to test meal administration and the animals were sacrificed 30, 60, or 120 min after meal administration, or (b) SCT was administered 30, 90, or 180 min prior to test meal administration.

Discussion. Results of the present studies demonstrate that CT markedly decreases gastric acid secretion, a finding in agreement with reports of other investigators (1–6).

Our interest in possible effects of calcitonin on gastric emptying stemmed from the observation of one of us (G. R. B.) that SCT prevented lethality in rats orally treated with an otherwise lethal dose of phenylbutazone. Necropsy revealed that most of the administered dose of phenylbutazone appeared to have been retained in the stomachs of the CT-treated animals. Subsequent studies using the charcoal meal technique clearly showed an inhibition of gastric emptying with SCT. A desire to improve quantitation of this phenomenon prompted development of the Evan's blue method described. In quantitative studies in rats using the latter technique we have shown inhibition of gastric emptying with PCT, SCT, and HCT. These results are in agreement with Garel *et al.*, who reported a reduction of gastric motility with SCT in rats (13). However, Segerström has reported a negative effect on gastric emptying in rats with PCT

TABLE II. Effects of Porcine, Salmon, and Human Calcitonin on Gastric Emptying in Rats.

Treatment	Calcitonin (units/kg)	Milligrams dye retained in stomach ± SE
Vehicle control	—	2.02 ± .46
PCT	1.0	3.61 ± .36*
PCT	2.0	4.66 ± .42**
PCT	4.0	6.39 ± .14**
PCT	8.0	5.71 ± .59**
Vehicle control	—	2.31 ± .21
SCT	1.0	3.64 ± .46*
SCT	2.0	3.82 ± .37*
SCT	4.0	6.64 ± .32**
SCT	8.0	7.01 ± .41**
Vehicle control	—	1.95 ± .18
HCT	16.0	2.80 ± .61
HCT	32.0	3.91 ± .49*
HCT	48.0	4.36 ± .38**
HCT	64.0	4.96 ± .45**

* $P = < 0.05$ vs vehicle control.
** $P = < 0.001$ vs vehicle control.
N = 10 rats/dose level.
SE = standard error of the mean.

TABLE III. Effects of Salmon Calcitonin on Gastric Emptying in Rats as a Function of Dose and Time of Administration

Treatment	Calcitonin (units/kg)	*N*	Time from calcitonin admin. to Evan's blue admin. (min)	Time from Evan's blue admin. to sacrifice (min)	Average mg dye retained in stomach ± SE	Percent of administered dye retained in stomach
Experiment A						
Vehicle control	—	8	15	30	2.29 ± .21	23
SCT	4.0	8	15	30	6.48 ± .29*	65
Vehicle control	—	8	15	60	2.55 ± .29	26
SCT	4.0	8	15	60	6.96 ± .27*	70
Vehicle control	—	8	15	120	1.63 ± .10	16
SCT	4.0	8	15	120	5.24 ± .66*	52
Experiment B						
Vehicle control	—	8	30	60	2.90 ± .33	29
SCT	4.0	8	30	60	6.39 ± .46*	64
Vehicle control	—	8	90	60	1.81 ± .11	18
SCT	4.0	8	90	60	5.83 ± .49*	58
Vehicle control	—	8	180	60	1.86 ± .20	19
SCT	4.0	8	180	60	5.39 ± .76*	54

* $P = < 0.001$ vs vehicle control.
N = number of rats/group.
SE = standard error of the mean.

(14). This discrepancy remains unexplained.

On the basis of the results of the gastric-emptying studies, the effects of a single sc dose of SCT were still nearly fully manifest 4 hr later.

Interestingly, in each of the experiments on gastric emptying, mucosal adsorption of a portion of the administered dye was visually evident in all vehicle-treated rats but was essentially absent in all CT-treated animals.

In both the gastric secretion and gastric emptying studies, HCT was markedly less potent than was PCT or SCT even though the comparisons were made on the basis of equal hypocalcemic activities. It should be noted that different strains of rats were utilized for the hypocalcemic, gastric-secretion, and gastric-emptying studies, and that no experiments were conducted to determine the influence of this factor on the responses studied. It seems unlikely, however, that the qualitative differences noted for HCT vs PCT and SCT are explainable on this basis.

Previous work in our laboratory elucidated a marked qualitative difference between PCT and SCT in their actions on bone and kidney. Specifically, in rat studies, when equated for hypocalcemic effect, SCT was found to be greatly more natriuretic than PCT (15). This finding has been confirmed in additional rat studies by Keeler *et al.* (16) and MacIntyre (17). In sheep, Barlet (18) has likewise found SCT more natriuretic than PCT. HCT has been shown more similar to PCT than to SCT regarding natriuretic action in rats (17) and sheep (18).

Taking into account the natriuretic and the gastric effects of the calcitonins, and evaluated on the basis of equivalent hypocalcemic activities, rat studies show the following qualitative differences for the three species of calcitonin: PCT has weak natriuretic plus strong gastric effects; SCT has strong natriuretic plus strong gastric effects; and HCT has weak natriuretic plus weak gastric effects.

In view of the fact that the inhibitory effects of the CT preparations on gastric secretion and gastric emptying did not parallel their hypocalcemic potencies, it seems clear that the gastric effects could not be mediated by changes in serum calcium. Additional data (unpublished) from our laboratory supporting this concept has shown that serum calcium levels in both Sprague–Dawley and Holtzman rats are nearly maximally depressed by all three CT preparations at the dosages tested. This is in agreement with the conclusion of others oased on different criteria (2–5). At this time the mechanism of the inhibitory action of CT on gastric function re-

mains unexplained.

Summary. The effects of PCT, SCT, and HCT were studied on gastric acid secretion in pylorus-ligated rats and gastric emptying in normal rats. Volume and acidity of gastric secretion were inhibited in a dose-dependent manner by all three preparations; however, HCT was markedly less potent than SCT or PCT when comparisons were based on equal hypocalcemic potencies. Similar results were obtained, including the lesser effect of HCT, in studies on inhibition of gastric emptying involving the administration of an Evan's blue test meal. These gastric effects of calcitonin appear not to be mediated by changes in serum calcium.

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