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Salmon Calcitonin and Water and Electrolyte Transport in Rabbit Ileum[1] (38993)

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(Introduced by W. E. Lassiter)

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The intravenous infusion of synthetic salmon calcitonin (SCT) elicits the secretion of water and electrolytes in the human small intestine by mechanisms which remain to be elucidated (1). The development of an experimental model in a laboratory animal would facilitate the study of the mechanisms involved in calcitonin-induced secretion. Since the rabbit has served as a useful model for the study of intestinal secretion produced by other agents (2, 3), we have investigated the effect of SCT on water and electrolyte transport in the rabbit ileum.

Methods. The transport of water and electrolytes was measured with an *in vivo* perfusion technique (4, 5) and with the *in vitro* Ussing chamber technique (6) in male New Zealand white rabbits averaging 3 kg weight. Partially purified SCT[2] or synthetic SCT[3] administered either by iv infusion or by direct addition to the Ussing chamber solutions were used in these studies. Blood samples were obtained in five rabbits before and after SCT infusion for analysis of the serum calcium concentration by a modified colorimetric technique (7).

In vivo perfusion. Rabbits were anesthetized with sodium pentobarbital (20 mg/kg iv) and anesthesia was maintained with periodic injections of pentobarbital. After tracheostomy a midline abdominal incision was performed and 10–15 cm of distal ileum were cannulated *in situ*. Mannitol–Ringer's solution was recirculated from a closed 50-ml reservoir through the loop at 4 ml/min with a peristaltic pump (Harvard Apparatus, Millis, MA). The composition of the perfusion solution in millimoles/liter was: Na, 140; K, 5.2; Ca, 1.2; Mg, 1.2; Cl, 120; HCO_3, 25; HPO_4, 2.4; H_2PO_4, 0.4; and mannitol, 25. After bubbling with 95% O_2–5% CO_2 the pH was 7.4. Polyethylene glycol (PEG) was added as a nonabsorbable water marker in the form of polyethylene-C^{14}-glycol, 5 μCi/liter (New England Nuclear, Boston, MA) and PEG-4000 (1 g/liter). The perfusion protocol consisted of three 90-min periods. The first and third periods served as control periods before and after the iv infusion of SCT which was given during the second period. SCT diluted in 0.9% NaCl was infused via an ear vein at a dosage of 3 U/kg-hr. The first 30 min of each period were regarded as an equilibration interval. Every 30 min during the three periods a 2-ml aliquot was removed from the reservoir for the measurement of ^{14}C-PEG by liquid scintillation spectrometry (Searle Analytic, Inc., Des Plains, IL) and of sodium by flame photometry (Instrumentation Lab Inc., Boston, Massachusetts). Transport rates of water and sodium were calculated and expressed as μl or μequiv/hr–cm of ileal length.

In vitro transport. Distal ileum was removed from sacrificed rabbits, stripped of its muscular layers and mounted as a flat sheet for the measurement of unidirectional fluxes with ^{22}Na or ^{36}Cl. In some studies simultaneous measurements of bidirectional fluxes were performed with ^{22}Na and ^{24}Na. The measurements of Na or Cl fluxes, potential differences (P.D.), short circuit current (I_{sc}) and conductance (G) were performed by previously described techniques (6). The chamber solution for *in vitro* studies was similar in electrolyte com-

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[2] Generously provided by Dr. J. Bastian, Armour Pharmaceutical Company (Kankakee, Illinois), as AL1025 (K423-119). Potency of 590 MRC units/mg.

[3] Generously provided by Dr. J. Bastian, Armour Pharmaceutical Company (Kankakee, Illinois), as AL0977. Potency of 4334 MRC units/mg.

TABLE I. EFFECT OF SALMON CALCITONIN (SCT) ON NET H_2O AND Na TRANSPORT OF RABBIT ILEUM PERFUSED *In Vivo*

Net transport	Control period	SCT period	Control period
H_2O (μL/hr-cm)	128 ± 24	151 ± 23	135 ± 16
Na (μequiv/hr-cm)	14.2 ± 2.2	19.4 ± 3.2	14.5 ± 3.0

TABLE II. EFFECT OF SALMON CALCITONIN, ADMINISTERED *In Vivo* OR *In Vitro*, ON ELECTROLYTE TRANSPORT BY THE *In Vitro*, SHORT-CIRCUITED RABBIT ILEUM.[a]

		Salmon calcitonin	
	Control	*In vivo* injection 3.0 units/kg - hr	*In vitro* addition 0.02–1.0 U/ml
Na Flux (*n*)	(6)	(6)	(7)
m → s[c] (μequiv/hr-cm²)	10.8 ± 1.0	9.8 ± 0.8	11.1 ± 0.8
s → m[c]	10.4 ± 0.9	9.4 ± 0.3	10.1 ± 0.8
Net[c]	0.4 ± 0.5	0.4 ± 0.8	1.0 ± 0.3
P.D. (mV)	3.1 ± 0.3	3.2 ± 0.2	3.5 ± 0.3
I_{sc} (μA/cm²)	68.0 ± 5.0	65.0 ± 4.0	72.0 ± 4.0
G (mmho/cm²)	23.2 ± 2.3	20.8 ± 1.6	21.2 ± 1.2
Cl Flux (*n*)	(19)	(19)	(7)
m → s	6.5 ± 0.6	6.4 ± 0.4	7.9 ± 0.5
s → m	6.9 ± 0.6	6.6 ± 0.3	8.1 ± 1.0
Net	−0.4 ± 0.4	−0.2 ± 0.3	−0.2 ± 0.7
P.D.	3.2 ± 0.2	3.4 ± 0.2	2.8 ± 0.2
I_{sc}	65.0 ± 3.0	67.0 ± 3.0	65.0 ± 3.0
G	21.3 ± 1.2	20.7 ± 1.1	23.6 ± 1.0

[a] Mean ± SE.
[b] n = number animals.
[c] m → s = mucosal to serosal flux. s → m = serosal to mucosal flux. Net = m → s minus s → m.

position to the perfusion solution described above except that it also contained mannitol (10 mmoles/liter). Five 20-min flux periods were measured beginning 20 min after the addition of the isotope to the chamber solution. The flux value for each ileal tissue was obtained by averaging the five consecutive measurements.

In vitro transport of electrolytes was measured after SCT infusion or addition of SCT to the chamber solution. SCT was given iv via ear vein for 30–90 min in dosages of 1–10 units/kg·hr prior to sacrifice. A three unit dosage was used in most of the studies after this dosage was shown to have a hypocalcemic effect. Dosages ranging from 10 to 144 units/ml were added directly to the Ussing chamber solutions. Initially lyophilized SCT[1] was dissolved in the mannitol-Ringer's solution but in later studies using synthetic SCT[2] human serum albumin (0.1%) was added to the diluent and the chamber solutions.

The observed differences on the concentration of the total serum calcium and the calculated rates of ileal transport were tested for statistical significance by the Students' *t* test.

Results. The administration of SCT by iv infusion or by addition to the Ussing chamber solution had no detectable effect on water and electrolyte transport in the rabbit ileum (Tables I and II). Table I shows the net transport rates of water and sodium measured *in vivo* in the rabbit ileum before, during and after the iv infusion of SCT. There is no significant difference in the net transport rates of water or sodium among the three periods.

Table II shows the *in vitro* net and uni-

directional fluxes of Na and Cl together with simultaneous electrical measurements (P. D., I_{sc}, and G) of the rabbit ileum after administration of SCT by iv infusion prior to sacrifice (*in vivo*) or by direct addition to the Ussing chamber solution (*in vitro*). These values are compared to those observed in rabbit ileum obtained from animals given saline alone. No significant differences in the flux rates or the electrical measurements were detected when SCT treated tissues were compared to controls. The addition of large dosages (36–144 units/ml of bath solution) of synthetic SCT to the Ussing chamber was associated with a brief 25% increase in the P.D. and I_{sc}. This alteration lasted less than four minutes before returning to the control levels.

A significant fall in the serum calcium level occurred in rabbits given SCT infusion in a dosage of 3 U/kg-hr. The control value for the serum calcium level was 14.3 ± 0.02 mg/100 ml (mean ± SEM) and the mean value at the end of the SCT infusion was 11.7 ± 0.47 mg/100 ml ($P < .001$). In contrast, rabbits given saline infusion had a preinfusion level of serum calcium of 14.1 ± 0.28 mg/100 ml and the level after saline infusion was 13.6 ± 0.41 mg/100 ml.

Discussion. SCT had no effect on rabbit ileal transport of water and electrolytes when studied by *in vivo* perfusion or *in vitro* Ussing chamber techniques. The absence of an effect of SCT on ileal transport was observed over a range of SCT dosages given by iv infusion which exceeded by tenfold the dosage that induces intestinal secretion in humans (1). The addition of SCT to the Ussing chamber in amounts up to 144 units/ml of bath solution had no effect other than a transient electrical alteration. The transient nature of this alteration without any apparent change in the flux rates suggests that it is of little or no significance. The addition of protein to the chamber solution in order to decrease the binding of the peptide hormone to the glass and plastic surfaces did not change the negative results. The biological activity of the SCT was shown by its hypocalcemic action in five rabbits even though the SCT infusion did not influence ileal transport.

There are several possible explanations for the discrepancies between the observed results in humans and these negative results. First, the effects of SCT on the ileum may be so transient that the effect cannot be detected with these techniques. The significance of such a transient response would be dubious. Second, the effect may be mediated by another hormone, explaining the absence of an effect when SCT is given *in vitro*. If this were so, a response should have been observed with the *in vivo* perfusion studies.

Finally, the absence of an ileal effect in face of a skeletal effect, i.e., the hypocalcemia, may be a species-dependent phenomenon. The calcitonins have been reported to increase cyclic AMP content in renal and skeletal tissue (10), which are generally accepted as target organs for these hormones. An increase in mucosal content of cyclic AMP or adenylate cyclase activity has been observed in other models of intestinal secretion (8). However, Schwartz, Kimberg, and Sheerin *et al.* did not observe an increase in cyclic AMP content of mucosa after the *in vitro* incubation of rabbit ileum with SCT (9).

Summary. The administration of SCT, natural and synthetic, had no apparent effect on the ileal water and electrolyte transport in the rabbit. The failure of SCT to influence ileal transport of water and electrolytes in the rabbit, as it does in man, may be due to differences in the rabbit intestinal response to a foreign peptide hormone.

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