Chinese Journal of Physiology 42(3): 189-193, 1999

Calcium Rather than Calcitonin Is Dominant to Mediate Gastric Emptying in Thyroidectomized Rats

Full-Young Chang[1], Shou-Dong Lee[1], Ming-Luen Doong[2] and Paulus S. Wang[2]

[1]*Division of Gastroenterology*
Veterans General Hospital-Taipei
and
[2]*Department of Physiology*
National Yang-Ming University
Taipei 112, Taiwan, ROC

Abstract

Both calcium and calcitonin are important in mediating gastrointestinal motility. Present study tried to study what was the dominant role of calcitonin or calcium replacement on the gastric emptying in thyroidectomized animals. Adult Sprague-Dawley male rats received thyroidectomy or sham operation and then housed for two weeks until motility study, which was conducted using radiochromium to measure gastric emptying. Before motility study these rats were i.p. injected with saline or human calcitonin in the doses of 0.1, 1 and 10 μgM/kg, respectively. Another group of thyroidectomized rats received i.v. infusion of saline or $CaCl_2$ for 30 min before motility study. Among thyroidectomized rats, neither saline nor various doses of calcitonin treatment disturbed gastric emptying compared to this of sham operated rats. Thyroidectomy diminished plasma calcium level, however, additional calcitonin treatment did not restore the suppressed calcium level ($P<0.01$). Of rats following saline or $CaCl_2$ infusion, thyroidectomy did not change gastric emptying, whereas $CaCl_2$ infusion enhanced gastric emptying ($P<0.05$). In conclusion, exogenous calcium treatment further enhances gastric emptying in thyroidectomized rats, whereas calcitonin replacement has no effect on gastric emptying. We suggest that calcium rather than calcitonin is dominant to mediate gastric emptying.

Key Words: calcium, calcitonin, gastric emptying, gastrointestinal motility, gastrointestinal transit, thyroidectomy

Introduction

Intractable diarrhea is often encountered in patients with medullary thyroid carcinoma while surgical resection of this tumor immediately ameliorates diarrheic symptom (1, 28). Based on the observations shown i.v. infused calcitonin (CT) leading to jejunal and ileal secretions of water and electrolytes in normal humans, one of the pathophysiological mechanisms responsible for the diarrhea is thus suggested resulting from the overproduction of CT in medullary carcinoma (7, 27). Nevertheless, dysmotility may be the another pharmacological effect of CT on gastrointestinal (GI) tract. For example, the phenomenon of rapid colonic transit and the subsequently decreased water absorption is another mechanism known inducing diarrhea in patients of medullary carcinoma (26). It is of interest whether CT also leads to stomach dysmotility. Actually, CT, a family of 32-amino acid peptides secreted from C cells or parafollicular cells of mammalian thyroid, has the hypocalcemic effect (34). It has been indicated that CT delays human gastric emptying (GE), irrespective of peptic ulcer state (14, 15). Peripherally injected CT also inhibits rat GE while GI transit remains unchanged. On the other hand, centrally injected CT delays both stomach and small intestinal motor functions (10). All these

Corresponding author: Dr. Full-Young Chang, M.D., Division of Gastroenterology, Veterans General Hospital-Taipei, 201 Shih-Pai Road, Section 2, Taipei 112, Taiwan, ROC. Tel: 886-2-28757308, Fax: 886-2-28739318, E-mail: changfy@vghtpe.gov.tw
Received July 29, 1999; Revised August 3, 1999; Accepted August 18, 1999.

pharmacological effects on GI tract are undergone with an intact thyroid gland. Thyroidectomy does remove the secretion of CT. In addition, the calcium regulation exhibited as hypocalcemia is indeed resulted since the parathyroid gland is concomitantly removed. It is unknown what is the role of calcium or CT replacement on GE in animals with removed thyroid gland. Using the rat model present study tried to resolve whether CT or calcium replacement is important on GE in thyroidectomized (Tx) rats.

Materials and Methods

Animal Preparations and Gastric Emptying Measurement

Adult Sprague-Dawley male rats, 3-4 months old, weighing 300-400 g were obtained from the Animal Room of National Yang-Ming University. All studied animals were housed under the controlled conditions of light (06:00-20:00), humidity and temperature (22±1°C). Standard laboratory chow and water were available *ad libitum*. Rats received Tx treatment in the following procedure. Under a transient anesthesia using ether, the thyroid glands were carefully removed and then the Tx-rats soon recovered and housed for two weeks until motility study. Prior to the motility experiment, these animals were deprived of food but allowed free access to tap water for 18 hr. Those rats received sham operation served as controls. Sham operated (n=6) and Tx (n=7) rats were intraperitoneally injected with normal saline before the immediate oral feeding of motility marker. While another three groups of Tx rats were intraperitoneally injected with human CT (Sigma, St. Louis, USA) in the doses of 0.1 (n=7), 1 (n=7) and 10 μgM/kg (n=6), respectively. The feeding of radiochromium motility marker was achieved in the conscious state with a temporarily placed orogastric catheter (ID: 1.67 mm, OD: 2.42 mm, PE-205, Clay-Adams, Parsippany, NJ, USA) and motility measurement was undergone 15 min later after the successfully feeding via orogastric catheter. GE was assessed by the propulsion of the non-absorbable motility marker within the GI tract (3). The main composition of feeding solution was $Na^{51}CrO_4$ (1 mCi=37 MBq, Dupont, NEN Research Products, Boston, MA, USA) with a radioactivity 0.5 μgCi/ml. It was diluted with saline. The feeding amount for each animal was adjusted to 1 ml/kg. The rats were sacrificed by the guillotine 15 min since the oral feeding of motility marker. Blood from decapitated neck was collected to measure plasma calcium level. The stomach and small intestine were quickly exposed by a laparotomy. Both ends of stomach and ileocecal junction of small intestine were ligated. Then the whole stomach and small intestine were carefully removed outside and divided. Finally, stomach and small intestine were placed into the counting tubes to count their radioactivities with a gamma counter (10/880 Plus, ICN Biomedicals, Costa Mesa, CA, USA) for one minute. GE is the percentage of radioactivity of intestine divided by the total radioactivity recovered from both stomach and intestine (3).

A separate group of Tx rats were employed receiving either calcium or saline i.v. infusion to assess the calcium effect on GE. These rats were housed two weeks following either Tx or sham operation using similar procedures did by the former group. The right jugular vein and left femoral vein were catheterized respectively with silastic tubing connection (PE-50, OD: 0.965 mm, ID: 0.58 mm, Clay Adams) under pentobarbital anesthesia (30 mg/kg) on the day before motility study. Sham operated (n=7) and Tx (n=7) rats received i.v saline infusion via right jugular vein in the rate of 1 ml/300 gm/30 min for 30 min immediately before the feeding of radiochromium marker. In addition, another Tx rats (n=7) just received i.v infusion of 1% $CaCl_2$ (1 gm/100 ml) via right jugular vein in the rate of 1 ml/300 gm/30 min for 30 min until the orogastric feeding of radiochromium marker. All studied rats were sacrificed 15 min following the successful feeding of radiochromium marker. GE was measured using similar procedure. The blood samples were intermittently collected via the implanted femoral vein before, during and at sacrifice, respectively to measure plasma calcium levels by an electrolyte analyzer (EFOX 5053, Eppendorf, Hamburg, Germany).

Statistical Analysis

All values were expressed as mean±SE, numerical data were analyzed by using a one-way analysis of variance (ANOVA) with post Dunnett's test. A p value less than 0.05 was considered to be significant.

Results

When the rats received sham operation and an intraperitoneal saline treatment, the GE seen at 15 min was 69.3±5.6%. Thyroidectomy plus i.p saline injection in rats did not disturb GE (62.5±7.1). On the other hand, the GEs of Tx rats received i.p. CT treatment in the doses of 0.1, 1 and 10 μgM/kg were 73.9±2.9%, 71.2±3.6% and 67.4±4.6%, respectively. Their differences compared to these of sham operation or thyroidectomy plus saline treated rats were not significant. Plasma calcium level of sham operated rats was 2.09±0.03 mmol/L. Thyroidectomy elicited a lower plasma calcium level while i.p. CT treatment

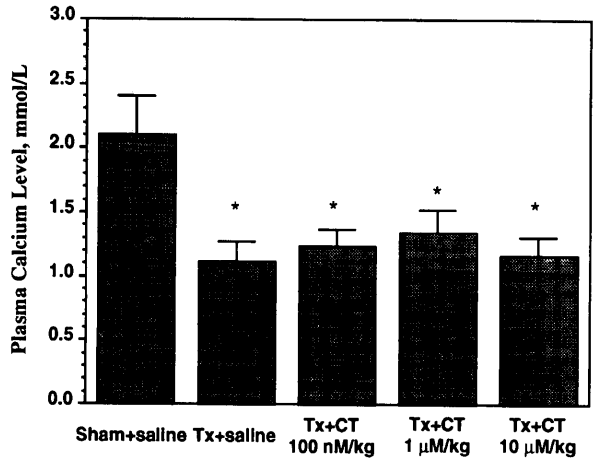

Fig. 1. Plasma calcium levels of studied rats measured during sacrifice. Removal of thyroid gland diminished plasma calcium level in thyroidectomized (Tx) rats compared to this sham operated rats. Additional calcitonin (CT) i.p. treatments did not alter the already suppressed calcium levels. (*: compared with sham treated rats, P <0.01). Vertical bars above columns are SE.

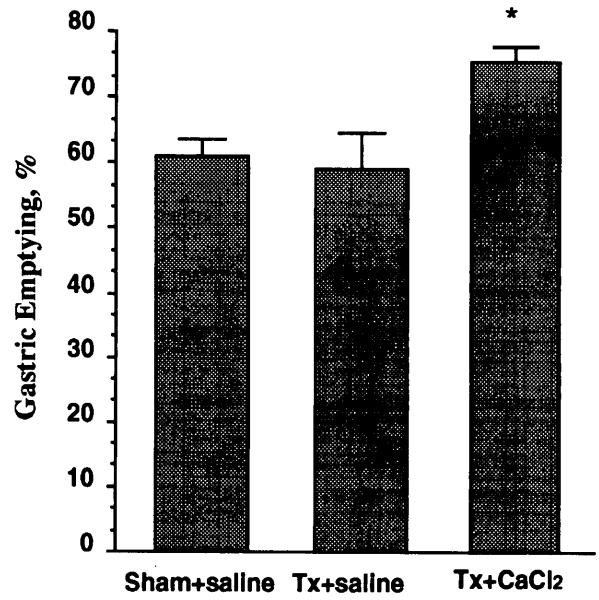

Fig. 2. The effect of saline or CaCl2 infusion on the gastric emptying in thyroidectomized (Tx) of sham operated rats. Saline or $CaCl_2$ was already infused for 30 min before feeding motility marker. Thyroidectomy did not alter gastric emptying, whereas $CaCl_2$ infusion enhanced gastric emptying (*: compared with sham treated rats, P<0.05). Vertical bars above columns are SE.

in any doses did not restore the already suppressed calcium levels (P<0.01, Fig. 1).

In the separate group of rats received either saline or $CaCl_2$ infusion, the GE (60.6±2.4%) of sham operated rats following saline infusion was not different from this of Tx rats received saline infusion. In contrast, GE of Tx rats received $CaCl_2$ infusion was significantly enhanced (P<0.05, Fig 2). Plasma calcium level of sham operated rats before saline infusion was 2.15±0.09 mmol/L. Saline infusion in these rats did not alter calcium level throughout the whole infusion period even at sacrifice. Both Tx groups had suppressed plasma calcium levels in comparison with this of sham operated rats (P<0.01). Infusion of $CaCl_2$ immediately restored hypocalcemia (P<0.01) while this elevation remained elevated at sacrifice (P<0.05). The characteristic alteration of calcium level was not encountered in Tx rats received saline infusion (Fig. 3).

Discussion

Present study mainly indicated that GE in rats following thyroidectomy remained unchanged. Thyroidectomy has several pathophysiological impacts on hormone production. Most importantly, it removes thyroid gland in turn resulting in hypothyroidism. Hypothyroidism *per se* displays various GI motor dysfunctions, e.g. constipation and adynamic ileus (21, 31). The decreased small intestinal slow-wave frequency in hypothyroidism is responsible for one of mechanisms mediating GI disturbance (4). Among human stomach the effect of hypothyroidism on GE is debatable. Some hypothyroidism patients are reported to have bezoars owing to delayed GE (9). Scintigraphy study confirms the impaired solid GE in hypothyroidism patients (17). The pathogeneses of impaired GI motor function in these patients include autonomic neuropathy, disturbed conduction in myoneural junction, intestinal ischemia and chronic inflammatory changes in stomach (16, 17, 27). However, a wide variation of individual GE data was found in hypothyroid patients before and after thyroxine treatment while their half GE times remained delayed (16). In contrast, solid GEs studied in euthyroid and hypothyroid subjects are similar (8). Moreover, literature also illustrates the unchanged liquid GE in hypothyroidism patients while their GE remained similar after thyroxine replacement until the euthyroid state (6). Our study shown unchanged liquid GE in Tx rats was somewhat similar to the latter study.

To the best of our knowledge, at least the role of CT to mediate GI motility in hypothyroidism subjects has not been mentioned or studied. Thyroidectomy also removes the CT secretion from its original thyroid C cells and thus the regulation of calcium is in turn to be disturbed (23, 34). Finally thyroidectomy removes parathyroid gland which mainly maintains the extracellular calcium concentration and is necessary in the physiological homeostasis concerning calcium metabolism. Hence hypocalcemia is invariably resulted as obtained in our study (24). Hypoparathyroid patients have obvious small

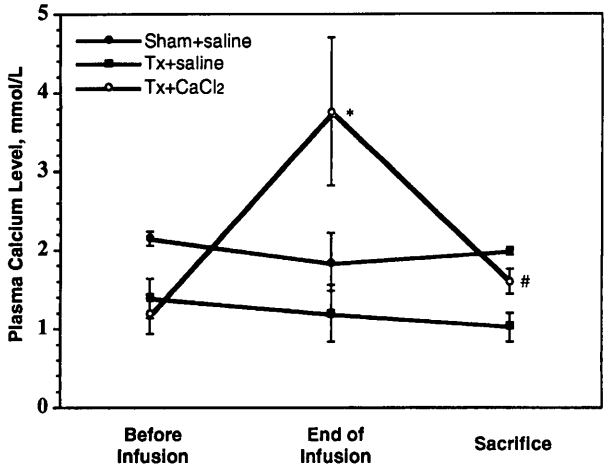

Fig. 3. Plasma calcium levels of studied rats measured before infusion, at the end of infusion and after sacrifice. Saline infusion did not change the plasma calcium level of sham treated rats. Hypocalcemia seen in the thyroidectomized (Tx) rats remained unchanged after saline infusion. Instead, $CaCl_2$ infusion quickly restored the hypocalcemia of Tx rats even to hypercalcemia at the end of infusion (*: compared with this before infusion, $P<0.01$, #: compared with this before infusion, $P<0.05$). Vertical bars are SE.

intestinal and colonic hypomotility disorders such as distension, nausea, vomiting, constipation and steatorrhea (24, 30). For the GE it is unknown whether GE is impaired. Instead, familial polyendocrine failure syndrome patients including hypoparathyroidism have varying degrees of gastric atrophy with anti-parietal cell antibodies, it is probably that GE may be disturbed (32). Our study confirmed the existence of hypocalcemia after thyroidectomy while exogenous CT replacement did not restore the thyroidectomy elicited hypocalcemia. Similarly, an i.v. bolus CT treatment does not change human serum calcium level (11, 15, 18). It is obvious that exogenous CT replacement is ineffective in the regulation of thyroidectomy elicited hypocalcemia. Some GI upsets such as nausea, abdominal cramp and diarrhea are usually encountered in patients receiving CT treatment (20). Likewise, the observations showing delayed solid GE in humans has been one of CT elicited side effects (12, 13, 15, 29). The major peptides or mechanisms likely involving the inhibited human GE during CT treatment include transiently elevated gastrin level, hyperglycemia with reactive hyperinsulinemia, and the inhibited vagal activity. In addition, CT also suppresses the serum motilin level (5,29). It looks that these CT related effects are responsible to delay human GE. We did not observe such an inhibitory effect on GE in thyroidectomy plus CT treated rats. It is most likely that species difference may account for a variety of pharmacological effects after systemic CT administration (25, 34). For instance, the employed human-CT in our study often has a less pharmacological potency compared with those of eel- or salmon-preparation (23). Eel-CT inhibits stomach migrating motor complex phase III and suppresses calcium level while the duodenal but not the ileal contraction is inhibited, irrespective of the unchanged motilin level (23). Alternatively, the salmon-CT in human study induces intestinal phase III activity with reduced duration of migrating motor complex but these effects are not observed in the stomach (5). We suggest that human-CT conducted in our rat study is less effective to display a pharmacological influence on GE. It is of interest whether a previous thyroidectomy may restore the CT inhibited GE. Based on our observation shown no influence of thyroidectomy on GE, this suggestion of thyroidectomy restoration is unlikely existed.

Our study pointed out that thyroidectomy with concomitant hypocalcemia was not associated with disturbed GE. In contrast, hypercalcemia, although showing gradual decline, remained to be observed from the end of calcium infusion until the rat sacrifice. This hypercalcemic state was associated with enhanced GE. Our study probably suggests that calcium is an important mediator to enhance GE in Tx rats. Intracellular free calcium ion movement is essential in mediating the functions of muscle contractile proteins and the subsequent intracellular mechanisms, also its influx involving the voltage-dependent calcium channels to generate action potentials (22). On the smooth muscle cells, contractile activity is modulated by the levels of free intracellular calcium. At higher levels, contractile proteins interact and the muscle contraction ensuing (33). Any significant change in the calcium homeostasis would therefore induce severe physiological responses including GI motor disturbance (2). Our study showing enhanced GE after calcium infusion probably confirmed the hypercalcemic effect on GI motility. Since exogenous CT did not has similar effect on GE and restored calcium level in Tx rats. We suggest that direct calcium influence on rat GE is dominant than this of CT. In conclusion, thyroidectomy has no definite effect on GE, however, exogenous calcium rather than CT replacement enhanced GE suggests that calcium is dominant in mediating GE in Tx rats.

Acknowledgments

This work was supported by Veterans General Hospital-Taipei, Taiwan, Republic of China (grant no: VGH 87-20).

References

1. Bernier, J.J., Rambaud, C., Catan, D. and Prost, A. Diarrhoea

associated with medullary carcinoma of the thyroid. *Gut* 10: 980-985, 1969.
2. Bielefeldt, K. and Conklin, J.L. Intestinal motility during hypoxia and reoxygenation in vitro. *Dig. Dis. Sci.* 42: 878-884, 1997.
3. Chang, F.Y., Chen, C.Y., Chen, T.S., Lee, S.D., Doong, M.L. and Wang, P.S. Variation of capsaicin-sensitive motor activities along the rat gastronitestinal tract. *Chin. J. Physiol.* 42: 41-45, 1999.
4. Christensen, J., Clifton, J.A. and Schedl, H.P. Variations in the frequency of the human duodenal basic electrical rhythm in health and disease. *Gastroenterology* 51: 200-206, 1966.
5. Demol, P., Hotz, J., Muller, M.K., Trompeter, R., Muttathil, J. and Goebell, H. Effect of calcitonin on the interdigestive motility and on gastric and pancreatic secretion in humans. *Arch. Int. Physiol. Biochim.* 94: 331-338, 1986.
6. Dubois, A. and Goldman, M. Gastric secretion and emptying in hypothyroidism. *Dig. Dis. Sci.* 29: 407-410, 1984.
7. Gray, T.K., Brannan, P., Juan, D., Morawski, S.G. and Fordtran, J. S. Ion transport changes during calcitonin-induced intestinal secretion in man. *Gastroenterology* 71: 392-398, 1976.
8. Hays, J.H., Silverman, E., Potter, B.B. and Shakir, K.M.M. Normal gastric inhibitory polypeptide response to oral glucose in hypothyroidism. *J. Endocrinol.* 140: 309-312, 1994.
9. Kaplan LR. Hypothyroidism presenting as a gastric phytobezoar. *Am. J. Gastroenterol.* 74: 168-169, 1980.
10. Jenz, H.J. Calcitonin and CGRP inhibit gastrointestinal transit via distinct neuronal pathways. *Am. J. Physiol.* 254: G920-924, 1988.
11. Jonderko, K. Effect of calcitonin on gastric emptying in patients with an active duodenal ulcer. *Gut* 30: 430-435, 1989.
12. Jonderko, G., Golab, T. and Jonderko, K. Calcitonin suppresses gastric emptying of a radiolabelled solid meal in humans. *Br. J. Clin. Pharmac.* 24: 103-105, 1987.
13. Jonderko, G., Golab, T. and Jonderko, K. Effect of calcitonin on gastric emptying. *Digestion* 40: 191-196, 1988.
14. Jonderko, G., Jonderko, K.and Golab, T. Effect of calcitonin on gastric emptying and on postprandial gastrin and insulin release in patients with type I gastric ulcer. *Neth. J. Med.* 37: 11-16, 1990.
15. Jonderko, K., Jonderko, G. and Golab, T. Effect of calcitonin on gastric emptying and on serum insulin and gastrin concentrations after ingestion of a mixed solid-liquid meal in humans. *J. Clin. Gastroenterol.* 12: 22-28, 1990.
16. Jonderko, G., Jonderko, K., Marcicz, C. and Golab, T. Gastric emptying in hypothyreosis. *Isr. J. Med. Sci.* 33: 198-203, 1997.
17. Kahraman, H., Kaya, N., Demircali, A., Bernay I and Tanyeri, F. Gastric emptying in patients with primary hypothyroidism. *Eur. J. Gastroterol. Hepatol.* 9: 901-904; 1997.
18. Kurosawa, M., Matsuo, Y., Ohno, H. and Sato, A.L. Vagal nerve contribution to the calcitonin-induced changes in intrapyloric pressure in anesthetized rats. *Neurosci. Lett.* 86: 322-327, 1988.
19. Lake-Bakaar, G. Hypothyroidism and functional bowel disease. *Am. J. Med.* 88: 312-313, 1990.
20. Lang, R., Milkman, M., Jensen, P.S. and Vignery, A.M. Chronic treatment of Paget's disease of bone with synthetic human calcitonin. *Yale J. Biol. Med.* 54: 355-365, 1981.
21. Miller, L., Gorman, C., Liang, V. and Go, W. Gut-thyroid interrelationships. *Gastroenterology* 75: 901-911, 1978.
22. Murphy, R.A. Contraction in smooth muscle cells: general introduction. *Annu. Rev. Physiol.* 51: 275-283, 1989.
23. Nakamura, H., Asano, T., Haruta, K. and Takeda, K. Gastrointestinal motor inhibition by exogenous human, salmon, and eel calcitonin in conscious dogs. *Can. J. Physiol. Pharmacol.* 73: 43-49, 1995.
24. Potts, J.T. Jr. Diseases of the parathyroid gland and other hyper- and hypocalcemic disorders. In: Harrison's Principles of Internal Medicine, edited by Isselbacher, K.J., 13th ed., New York: McGraw-Hill, 1994: pp. 2151-2171.
25. Primi, M.P. and Bueno, L. Centrally mediated stimulation of jejunal water and electrolyte secretion by calcitonin in dogs. *Am. J. Physiol.* 250: G172-176, 1986.
26. Rambaud, J.C., Jian, R., Flourie, B., Hautefeuille, M., Salmeron, M., Thuillier, F., Ruskone, A., Florent, C., Chaoui, F. and Bernier, J.J. Pathophysiological study of diarrhoea in a patient with medullary thyroid cacinoma. Evidence against a secretory mechanism and for the role of shortened colonic transit time. *Gut* 29: 537-543, 1988.
27. Shafer, R.B., Prentiss, R. and Bond, J.H. Gastrointestinal transit in thyroid disease. *Gastroenterology* 86: 852-855, 1984.
28. Steinfeld, C.M., Moertel, C.G. and Wollner, L.B. Diarrhea and medullary carcinoma of the thyroid. *Cancer* 31: 1237-1239, 1973.
29. Stevenson, J.C., Adrian, T.E., Christofides, N.D. and Bloom, S.R. Effect of calcitonin on gastrointestinal regulatory peptides in man. *Clin. Endocrinol.* 22: 655-660, 1985.
30. Taybi, H. and Keele, D. Hypoparathyroidism: a review of the literature and report of two cases in sisters, one with steatorrhea and intestinal pseudoobstruction. *AJR. Radium Ther. Nucl. Med.* 88: 432-442, 1962.
31. Wartofsky, L. Diseases of the thyroid. In: Harrison's Principles of Internal Medicine, edited by Isselbacher, K.J., 13th ed., New York: McGraw-Hill, 1994, pp. 1930-1952.
32. Weber, J.R. and Ryan, J.C. Effects on the gut of systemic disease and other extraintestinal conditions. In: Sleisenger & Fordtrans's Gastrointestinal and Liver Disease, edited by Feldman, M., Scharschmidt, B.F. and Sleisenger, M.H. 6th ed., Philadephia: W. B. Saunders, 1998, pp. 411-438.
33. Weisbrodt, N.W. The regulation of gastrointestinal motility. In: Motility Disorders of the Gastrointestinal Tract, Principles and Practice, edited by Anuras, S., New Yorker: Raven Press, 1992, pp. 27-48.
34. Young, A.A., Wang, M.W., Gedulin, B., Rink, T.J., Pittner, R. and Beaumont, K. Diabetogenic effects of salmon calcitonin are attributable to amylin-like activity. *Metabolism* 44: 1581-1589, 1995.