A Possible Role for 5-Hydroxytryptamine as a Mediator for Calcitonin Actions on the Gastrointestinal Tract and Pancreas in Rats

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Administration of pig calcitonin (10 M.R.C. units/kg body wt.) produced an immediate decrease in 5-hydroxytryptamine content in the antrum–duodenum region and ileum. In contrast, the hormone treatment rapidly increased the concentration of 5-hydroxytryptamine in the blood and pancreas. Serum immunoreactive gastrin did not change over a period of 3 h after calcitonin administration. The results suggest that the release of 5-hydroxytryptamine probably mediates calcitonin actions on the gastrointestinal tract and pancreas.

Calcitonin has been shown to inhibit gastric acid secretion in mammals (Hesch *et al.*, 1971; Becker *et al.*, 1973; Yonaga *et al.*, 1976). Production of secretory diarrhoea due to calcitonin treatment, without affecting cyclic AMP or cyclic GMP concentrations, in the ileum has been confirmed (Walling *et al.*, 1977). An inhibition of pancreatic enzyme secretion has also been observed after calcitonin administration (Hotz *et al.*, 1977). The mechanism with which calcitonin exerts these actions is not known, since the doses used in the foregoing experiments do not lower blood calcium concentration. 5-Hydroxytryptamine (serotonin) has been shown to produce similar actions to those of calcitonin. It inhibits gastric acid secretion (Hano *et al.*, 1975; Jaffe *et al.*, 1977), produces secretory diarrhoea without altering cyclic AMP or cyclic GMP concentrations in the ileum (Donowitz & Charney, 1976) and inhibits the pancreatic enzyme secretion (Drapanas *et al.*, 1961).

The hypercalcaemia character of medullary thyroid carcinoma is accompanied by a high concentration of blood 5-hydroxytryptamine (Baylin, 1974). Furthermore, in hibernating bats, a high thyroidal calcitonin content is associated with an increased concentration of 5-hydroxytryptamine in the gland (Haymovits *et al.*, 1976). We have demonstrated that calcitonin administration increases the content of 5-hydroxytryptamine in the brain (Nakhla & Majumdar, 1978). Such results suggest that calcitonin plays a role in the mobilization of 5-hydroxytryptamine in the tissues.

The similarity in responsiveness of gastrointestinal and pancreatic tissues to calcitonin and 5-hydroxytryptamine, together with the observation that calcitonin could affect 5-hydroxytryptamine concentration in the tissues, raises the question of whether the observed changes in gastrointestinal and pancreatic functions after calcitonin administration

could in part be mediated by 5-hydroxytryptamine, a compound that is present in large amounts in the digestive tract (Resnick & Gray, 1962). Therefore the present work was undertaken to investigate (a) whether administration of calcitonin would change 5-hydroxytryptamine content in the different parts of the gut and pancreas and (b) whether any such change would be correlated to blood 5-hydroxytryptamine and serum immunoreactive gastrin concentrations.

Materials and Methods

Male Wistar rats weighing 150–200 g were maintained on a commercial laboratory diet and water *ad libitum* throughout the experimental period. The animals received a single intramuscular injection of either 10 M.R.C. units of pig calcitonin (10.25 M.R.C. units/mg; Armour Pharmaceutical Co., Eastbourne, Sussex, U.K.) dissolved in gelatin diluent/kg body wt. or an equivalent volume of gelatin diluent alone, and were killed by decapitation at different time intervals. The blood was collected in 0.06 ml of 15% tripotassium EDTA solution with 0.2 mg of potassium sorbate/ml and the concentration of 5-hydroxytryptamine was measured as described by Yuwiler *et al.* (1970). Antrum–duodenum section, ileum and pancreas were quickly dissected, rinsed thoroughly in cold 0.9% NaCl solution and frozen in liquid N₂. Frozen tissues were then kept at –20°C until analysis. 5-Hydroxytryptamine content in the various tissues was determined by the procedure described by Snyder *et al.* (1965).

Serum gastrin concentrations were measured radioimmunochemically as described by Stadil & Rehfeld (1973) by using the gastrin antisera no. 2604. Plasma Ca²⁺ concentration was measured by atomic-absorption spectrophotometry.

For the statistical analysis of the data, non-paired Student's *t* test was applied with $P < 0.05$ as the significance level.

Results

Effect of calcitonin on 5-hydroxytryptamine content in the antrum-duodenum region, ileum and pancreas

The dose of calcitonin applied in this study was chosen from dose- and time-response curves where it did not produce significant changes in plasma Ca^{2+} concentration in rats (results not shown).

The single injection of pig calcitonin (10 M.R.C. units/kg body wt.) produced an immediate and significant decrease in 5-hydroxytryptamine content in the antral-duodenal section, and 15 min after the injection 5-hydroxytryptamine concentration decreased by 16% ($P < 0.025$). The maximal decrease of

23% occurred 30 min after calcitonin injection, after which the concentration increased, returning essentially to the control value 6 h after the hormone treatment (Fig. 1).

The decrease in ileal 5-hydroxytryptamine content after calcitonin treatment also became evident within 15 min and remained significantly lower than in the initial control throughout the 6 h experimental period. The maximal decrease of 40% was observed 3 h after the hormone treatment (Fig. 1).

In contrast with what has been observed with antral-duodenal section and ileum, calcitonin administration produced an immediate increment in 5-hydroxytryptamine concentration in the pancreas. At 15 min after the hormone treatment pancreatic 5-hydroxytryptamine was increased by 36%, remaining essentially at that value for the next 15 min, and then decreased to the control value, without further changes for the rest of the experimental period (Fig. 1).

Fig. 1. Time-course changes in the content of 5-hydroxytryptamine in the antrum-duodenum region (■), ileum (○) and pancreas (●) after calcitonin injection

Groups of six rats were injected intramuscularly with pig calcitonin (10 M.R.C. units/kg body wt.) and killed at different times after the injection. Antrum-duodenum region, ileum and pancreas were dissected and the content of 5-hydroxytryptamine was determined. The points on the curves represent the means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$, **** $P < 0.005$ compared with the zero-time control.

Effect of calcitonin on the concentration of blood 5-hydroxytryptamine and serum immunoreactive gastrin

To determine whether the decrease in gut 5-hydroxytryptamine contents would result in higher blood 5-hydroxytryptamine, the concentration of the compound in the blood was measured after calcitonin administration. Indeed, the results shown in Fig. 2 revealed that, after calcitonin administration, the

Fig. 2. Effect of calcitonin on the concentration of blood 5-hydroxytryptamine (●) and serum immunoreactive gastrin (○)

Groups of rats were killed at different times after an intramuscular injection of pig calcitonin (10 M.R.C. units/kg). Blood ($n = 8$) or serum ($n = 4-6$) samples were collected individually and used for the determination of 5-hydroxytryptamine or immunoreactive gastrin. Each point on the curve represents the mean $\pm$ S.E.M. * $P < 0.05$ compared with the zero-time control. Different groups of rats were used for the assay of 5-hydroxytryptamine and immunoreactive gastrin.

concentration of 5-hydroxytryptamine in the blood remained elevated (25–30%) for 30 min after the treatment when compared with the control values.

Administration of pig calcitonin did not produce any significant change in the concentration of serum immunoreactive gastrin, measured over a period of 3 h (Fig. 2).

To determine whether diurnal variation might influence the concentration of 5-hydroxytryptamine in the tissue or in the blood, control rats were killed at different times after injection of gelatin diluent only. No significant variations in 5-hydroxytryptamine concentration in the tissues or in the blood were observed (results not shown).

Discussion

The results of the present investigation show that administration of calcitonin produces a rapid decrease in 5-hydroxytryptamine content in the antrum–duodenum region and ileum, with a concomitant rise in the concentration of the compound in the blood and pancreas. The response of the pancreas to calcitonin treatment is similar to what has been observed with the brain (Nakhla & Majumdar, 1978). The observation that calcitonin enhances the concentration of 5-hydroxytryptamine in the pancreas and brain, whereas it decreases the content of the compound in the gut tissues, indicates that calcitonin has differential effectiveness on tissue 5-hydroxytryptamine. Moreover, the decrease in 5-hydroxytryptamine contents in the gut tissues simultaneous with the increase in its concentration in the blood suggests that calcitonin causes a release of 5-hydroxytryptamine from these tissues.

It has been shown that calcitonin inhibits gastric acid secretion without altering plasma Ca^{2+} concentration (Hesch *et al.*, 1971; Becker *et al.*, 1973; Yonaga *et al.*, 1976). The mechanism by which calcitonin inhibits gastric secretion is not fully understood, but it is generally believed that it could be achieved by two separate mechanisms: first, through influencing the secretory activity of the parietal cells, by changing humoral, vascular or nervous influences (Bieberdorf *et al.*, 1974); secondly, through the decrease of serum gastrin concentration, by inhibiting the secretion or activating the catabolism of gastrin (Becker *et al.*, 1974).

5-Hydroxytryptamine is present in substantial concentrations in the mucosa of the pyloric antrum and duodenum (Resnick & Gray, 1962). Its release into the peripheral as well as the portal vein, induced by acidification of the duodenum is found to inhibit gastric secretion (Koren *et al.*, 1976; Jaffe *et al.*, 1977).

The maximal inhibition of gastric secretion in response to calcitonin occurs at 1 h after the hormone treatment (Becker *et al.*, 1973; Ziegler *et al.*, 1974;

Yonaga *et al.*, 1976). On the other hand, an immediate inhibition in gastric secretion occurs after 5-hydroxytryptamine administration (Black *et al.*, 1958; Jaffe *et al.*, 1977). The present results show that calcitonin administration produced the maximal release of antral–duodenal 5-hydroxytryptamine after 0.5 h of the treatment (Fig. 1), whereas it did not change significantly the basal concentration of serum immunoreactive gastrin over a period of 3 h in the rats (Fig. 2). Furthermore 5-hydroxytryptamine administration had no effect on serum immunoreactive gastrin concentration in rats (A. P. N. Majumdar & A. M. Nakhla, unpublished work). These results therefore suggest the possibility that calcitonin inhibits gastric secretion through the release of antral–duodenal 5-hydroxytryptamine, which in turn influences the secretory activity of the parietal cells.

Calcitonin administration affects the electrolytes and fluid transport across the ileum causing secretory diarrhoea (Walling *et al.*, 1977). On the other hand, the elevated blood 5-hydroxytryptamine concentration is associated with the same type of diarrhoea (Donowitz & Charney, 1976). Since the results of the present experiments show that calcitonin stimulates the release of ileal 5-hydroxytryptamine throughout the 6 h experimental period (Fig. 1), it seems that the release of 5-hydroxytryptamine probably mediates the action of calcitonin in the ileum.

Calcitonin and 5-hydroxytryptamine have been shown to inhibit the exocrine pancreatic enzyme secretion by two different mechanisms. Calcitonin interferes with hormone-mediated stimulation of the acinar cells without affecting cholinergic mechanisms (Hotz *et al.*, 1977), whereas 5-hydroxytryptamine exerts its action through the vagal pathway (Drapanas *et al.*, 1961). On the basis of these findings and the present result of enhanced concentration of pancreatic 5-hydroxytryptamine, the latter observation does not appear to be a mediator for the action of calcitonin on enzyme secretion. Nevertheless, Drapanas *et al.* (1961) obtained a decrease in pancreatic enzyme secretion in response to the release of 5-hydroxytryptamine from the gastrointestinal tract. It is therefore possible to postulate that the release of antral–duodenal 5-hydroxytryptamine could at least in part contribute to the inhibitory effect of calcitonin on enzyme secretion. Whether the increased content of pancreatic 5-hydroxytryptamine is due to the accumulation or the stimulated synthesis of the compound in the tissue remains to be elucidated.

In conclusion the present results suggest that the release of 5-hydroxytryptamine from the tissue plays a mediated role for calcitonin actions on the gastrointestinal tract and pancreas.

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References

- Baylin, S. B. (1974) *Surg. Clin. North Am.* **54**, 309–323
- Becker, H. D., Konturek, S. J., Reeder, D. D. & Thompson, J. C. (1973) *Am. J. Physiol.* **225**, 277–280
- Becker, H. D., Reeder, D. D., Scurry, M. T. & Thompson, J. C. (1974) *Am. J. Surg.* **127**, 71–75
- Bieberdorf, F. A., Gray, T. K., Walsh, J. H. & Fordtran, J. S. (1974) *Gastroenterology* **66**, 343–346
- Black, J. W., Fisher, E. W. & Smith, A. N. (1958) *J. Physiol. (London)* **141**, 27–34
- Donowitz, M. & Charney, A. N. (1976) *Gastroenterology* **70**, 880
- Drapanas, T., Pollack, E. L. & Shim, W. K. T. (1961) *Arch. Surg.* **83**, 150–154
- Hano, J., Bugajski, J., Danek, L. & Wantuch, C. (1975) *Arch. Int. Pharmacodyn.* **216**, 28–39
- Haymovits, A., Gershon, M. D. & Nunez, E. A. (1976) *Proc. Soc. Exp. Biol. Med.* **153**, 383–387
- Hesch, R. D., Hufner, M., Hasenhager, B. & Creutzfeldt, W. (1971) *Horm. Metab. Res.* **3**, 140
- Hotz, J., Goebell, H. & Ziegler, R. (1977) *Gut* **18**, 615–622
- Jaffe, B. M., Kopen, D. F. & Lazan, D. W. (1977) *Surgery* **82**, 156–163
- Koren, E., Wapnick, S., Solowiejczyk, M., Pfeifer, Y. & Sulman, F. G. (1976) *Int. Surg.* **61**, 370–371
- Nakhla, A. M. & Majumdar, A. P. N. (1978) *Biochem. J.* **170**, 445–448
- Resnick, R. H. & Gray, S. J. (1962) *Gastroenterology* **42**, 48–55
- Snyder, S. H., Axelrod, J. & Zweig, M. (1965) *Biochem. Pharmacol.* **14**, 831–835
- Stadil, F. & Rehfeld, J. F. (1973) *Scand. J. Gastroenterol.* **8**, 101–112
- Walling, M. W., Brasitus, T. A. & Kimberg, D. V. (1977) *Gastroenterology* **73**, 89–94
- Yonaga, T., Nakamura, K. & Matsuura, K. (1976) *Proc. Jpn. Acad.* **52**, 532–535
- Yuwiler, A., Plotkin, S., Geller, E. & Ritvo, E. R. (1970) *Biochem. Med.* **3**, 426–431
- Ziegler, R., Minne, H., Hotz, J. & Goebell, H. (1974) *Digestion* **11**, 157–160