# Calcitonin: Effect on protein synthesis in different rat tissues[1]

A. M. Nakhla

*Department of Biochemistry, University of Cairo, Faculty of Agriculture, Giza (Egypt), 3 January 1979*

*Summary.* Protein synthesis was inhibited in the pancreas whereas it was enhanced in the kidney and intestine (jejunum-ileum) after a single injection of porcine calcitonin (20 MRC units/kg b.wt). The incorporation of [$^3$H]leucine into total protein in the brain, heart, liver and stomach did not change after the hormone treatment.

The hypocalcaemia-producing hormone, calcitonin, has been shown to cause several other actions in the various tissues of the body. It stimulates the activity of acetylcholinesterase in the cerebral cortex[2], inhibits gastric and pancreatic secretions[3,4], affects the electrolytes and fluid transport across the small intestine[5,6], and stimulates renal adenylate cyclase activity[7]. Recently we have found that whereas calcitonin treatment enhances 5-hydroxytryptamine content in the brain and pancreas, it decreases the concentration of the compound in the antrum-duodenum and ileum regions[8]. The present work was undertaken to determine whether these actions could be related to the change in the capacity of the various tissues to synthesize protein.

*Materials and methods.* Adult male Wistar rats, weighing 200–250 g, were fasted 24 h and had access to water. They were injected i.m. with either porcine calcitonin (20 MRC units/kg b.wt; Armour Pharmaceutical Co., Eastbourne, Sussex, England) in gelatin vehicle or an equivalent volume of gelatin vehicle alone. L-[4,5-$^3$H]leucine (10 µCi/100 g; 120 Ci/mmole; Radiochemical Center, Amersham, England) was injected i.p. 30 min before sacrifice. The rats were killed by decapitation 1 h after the hormone treatment. Cerebral cortex, heart, liver, pancreas, stomach fundus (oxyntic gland area), a part from the intestine (the combined jejunum and ileum) and kidney cortex were quickly dissected out, washed in 0.9% cold saline solution and were immediately frozen in liquid nitrogen. The tissues were kept at − 20 °C until analyzed.

Protein specific activity was determined according to the procedure described by Wannemacher et al.[9]. The radioactivity was counted in 10 ml of Insta-Gel (Packard Instrument Co., Downers Grove, Ill., USA) in LKB-Wallac scintillation spectrometer at a 30% efficiency, and the incorporation of [$^3$H]leucine into tri-chloroacetic acid (TCA)-insoluble total protein is expressed as cpm/mg protein in the tissue[10]. Protein concentration in the various tissues was measured by the method of Lowry et al.[11].

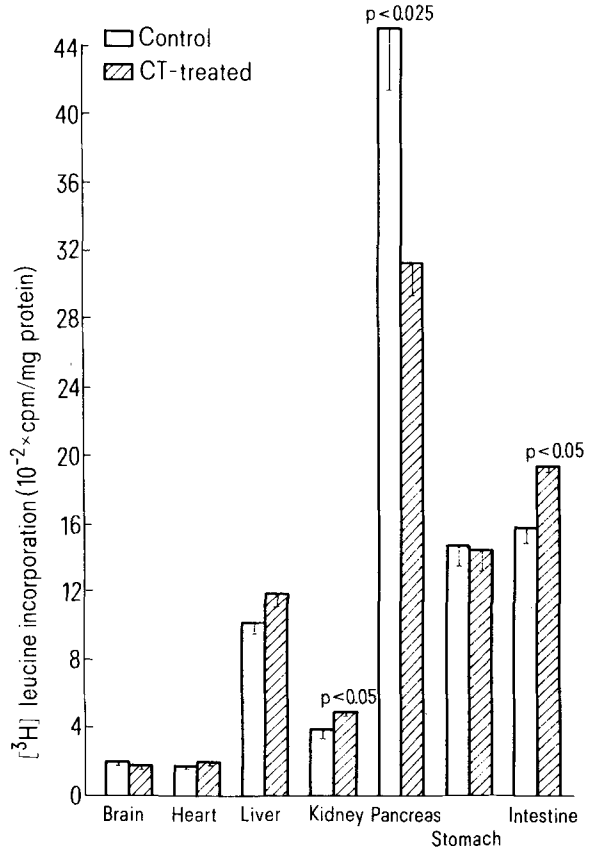
For the statistical evaluation of the data, Student's t-test for non-paired values was employed with $p < 0.05$ as the significance level.

*Results and discussion.* The dose of calcitonin used in this study was chosen from an earlier work where it was found to produce a maximum decrease (30–35%) in plasma calcium concentration after 1 h treatment[2]. On the other hand, calcitonin has been shown to exert its maximal observed effects during the 1st h of the treatment[2,3,4,8].

The present experimental results demonstrate that a single injection of calcitonin (20 MRC units/kg) inhibits the protein synthesis in the pancreas by 31%, whereas it enhances the synthesis of the protein in the kidney cortex and intestine (jejunum-ileum) by 27% and 22% respectively 1 h after the hormone treatment. The incorporation of [$^3$H]leucine into TCA-insoluble total protein in the cerebral cortex, heart, liver and stomach fundus did not change following calcitonin injection (figure).

To determine whether the reduction in pancreatic protein synthesis is due to the decreased ability of the tissue to synthesize protein or to the reduced supply of the precursor amino acid, the radioactivity of the TCA-soluble fraction was measured. It was found that TCA-soluble radioactivity of the treated pancreas is higher than the non-treated ones (data not shown), indicating that the reduction in protein synthesis is due to the decreased utilization of the precursor amino acid. Since it was found that calcitonin inhibits the pancreatic exocrine enzyme secretion[4], the present results suggest that the inhibition of enzyme secretion following the hormone injection could in part be due to the decreased ability of the tissue to synthesize protein. Melson et al.[7] reported that calcitonin stimulates the activity of adenylate cyclase in the kidney cortex. According to the present results, it is possible that the stimulated enzyme activity following the hormone treatment is due to the increased synthesis of the protein in the kidney cortex. Calcitonin has been shown to affect the electrolytes and fluid transport across the jejunum and ileum[5,6]. Whether or not these actions should be attributed to the observed increase in intestinal protein synthesis after calcitonin treatment, require further studies before firm conclusions can be drawn.

The observation that calcitonin inhibits the protein synthesis in the pancreas, enhances it in the kidney and intestine




Effect of calcitonin on the incorporation of [$^3$H]leucine into TCA-insoluble total protein in the brain, heart, liver, kidney, pancreas, stomach and intestine. Values represent the mean ± SEM (n = 4–6). Experimental details are as mentioned in the practical part.

and had no effect on the brain, heart, liver and stomach, may indicate a tissue specificity of the hormone.
In conclusion, the results of the present investigation suggest that, unlike in brain, heart, liver and stomach, the changes in pancreatic, renal and intestinal functions following calcitonin treatment may in part be due to the change in the capacity of these tissues to synthesize protein.

1 Acknowledgment. This work has been achieved at the Institute of Medical Biochemistry, University of Aarhus, Aarhus, Denmark.
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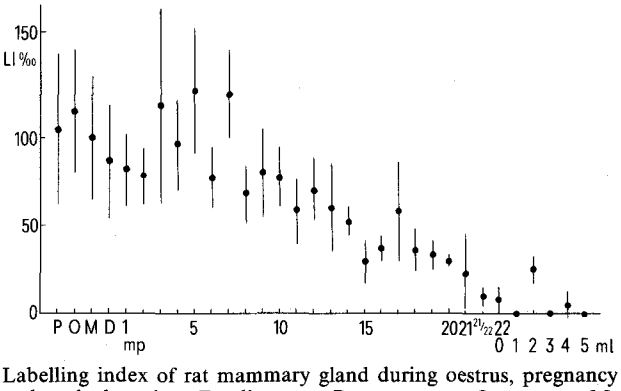
## Variations of the labelling index in vitro of rat mammary gland in pregnancy and early lactation

J. van Marle, A. Lind and J. van Weeren-Kramer[1]

*Pharmacological Laboratory, University of Amsterdam, Polderweg 104, NL-Amsterdam KP 1093 (The Netherlands), 26 January 1979*

*Summary.* The labelling index of rat mammary gland during oestrus, pregnancy and early lactation was studied in vitro. The implications concerning the existence of a critical cell division are discussed.

The size of the mammary gland increases during pregnancy, on the one hand by an increase in the number of cells, on the other hand by differentiation and increase in cell size as part of this differentiation. Both systems, proliferation and differentiation, are determined by hormones[2,3]. It has been assumed that a critical division must take place before differentiation can start[4]. The cell(s) formed after this division would not divide further, but they would differentiate.
The aim of this investigation was to study the changes taking place in the population of cells still capable of division during pregnancy and at the beginning of lactation.
*Material and methods.* Rat mammary gland explants taken at various stages of the oestrus, from day 0 to day 22 of pregnancy and from the first 5 days of lactation were cultured in a tissue culture system for 24 h. For each experiment (i.e. per day) 2 Wistar rats (180 g at the moment of prooestrus) were used. From each rat 6 explants were cultured.
A medium consisting of 50% serum of non-pregnant rats, 50% synthetic medium (t16) and insulin (50 µg/ml) was used[5]. 4 h before the experiments were finished 5 µCi/ml $^3$H-thymidine was added to the medium. For histological examination the explants were fixed in Bouin and stained with haematoxylin-phloxin. Autoradiographs were prepared according to Rogers[6]. The labelling index (LI) was determined by counting at least 2000 nuclei in each explant.
*Results and discussion.* During the various stages of the oestrus cycle the LI does not change significantly (figure). This LI, approximately 100‰, is maintained during the first 8 days of pregnancy. Thereafter the LI slowly decreases until it has reached 7‰ on day 22. In the first 5 days of lactation the LI fluctuates considerably. It is known that the culture medium ensures a good maintenance of the explants during the 24-h culture period, whereas it does not stimulate growth[7]. Therefore, it may be assumed that the course of the measured LI reflects the change in the population of dividing cells in vivo.
The constant LI during the 1st part of pregnancy may indicate that the chance of cell division remains equal for each cell in the population during this period. In other words there is no proliferating subpopulation. Bresciani[8] described a cell cycle time for the mammary gland in oestrogen stimulated mice of only 13 h. If this figure holds for rats too, then an existing subpopulation of dividing cells should result in an increased LI, at least during the first 7 days of pregnancy, in which this subpopulation divides about 12 times. If after each division 1 of the daughter cells does not divide anymore, a decreased LI on account of the increasing number of non-dividing cells would be the result. Furthermore, no morphological indications exist for such a population of stem cells[3]. In contrast to LI the mitotic index increases during the 1 half of pregnancy[9] and shows significant changes during the oestrus cycle (a peak in dioestrus)[10]. These 2 findings taken together show that the generation period of the cells of the mammary gland has been shortened, possibly due to the effect of oestrogens which are known to shorten the generation period. Not only the $G_1$ phase is shortened but also the S phase (from 27 to 9 h)[8].
The decrease in LI starting at day 8 indicates that the population of dividing cells diminishes. This decrease of




Labelling index of rat mammary gland during oestrus, pregnancy and early lactation. D: dioestrus, P: prooestrus, O: oestrus, M: metoestrus. mp 1-22: mammary glands from day 1 to day 22 of pregnancy. ml 1-5: mammary glands from day 1 to 5 of lactation. Each point shows the mean ± SD. 12 determinations per group.