Physiol. Res. 49 (Suppl. 1): S57-S61, 2000

MINIREVIEW

Physiology and Genetics of Procalcitonin

P. MARUNA, K. NEDĚLNÍKOVÁ, R. GÜRLICH[1]

Department of Pathological Physiology, Third Internal Department and [1]First Surgical Department, First Faculty of Medicine , Charles University, Prague, Czech Republic

Received December 17, 1999
Accepted February 1, 2000

Summary

Procalcitonin (PCT), a protein of 116 amino-acids with molecular weight of 13 kDa, was discovered 25 years ago as a prohormone of calcitonin produced by C-cells of the thyroid gland and intracellularly cleaved by proteolytic enzymes into the active hormone. Circulating levels of PCT in healthy subjects are below detection limit. Since 1993 when its elevated level was found in patients with bacterial infection, PCT became an important protein in the detection and differential diagnostics of inflammatory states. The production of PCT during inflammation is linked with a bacterial endotoxin and with inflammatory cytokines (TNF, IL-6). PCT detectable in the plasma during inflammation is not produced in C-cells of the thyroid. The probable site of PCT production during inflammation are the neuroendocrine cells in the lungs or intestine. There is no evidence of plasma PCT binding to cellular receptors of calcitonin, and the role of PCT in calcium and phosphate metabolism during sepsis is still not clear. Other hypothetical roles of PCT (cytokine network regulation, PCT as an endogenous non-steroid antiinflammatory drug) are being considered.

Key words

Cytokines • Inflammation • Procalcitonin

Introduction

First indirect evidence of a third calciotropic hormone, later named calcitonin, was presented in 1962. The source of this new hormone was attributed to the thyroid gland following an experiment in which the thyroid of dogs was washed out with a hypercalcemic solution. The circulating levels of calcium rapidly dropped following this procedure, an effect that could not be explained by parathormone action only.

Intracellular precursors of calcitonin, preprocalcitonin and its cleavage products, were discovered by Moya *et al.* (1975). The exact structure of procalcitonin (PCT) is known since 1981. At first, it was difficult to understand clearly the physiological role of PCT. Circulating levels of PCT in healthy subjects are below the detection limit and are only enhanced in medullar carcinoma of the thyroid or in small cell lung carcinoma.

When its elevated levels were reported in patients with bacterial infection by Assicot *et al.* (1993), PCT became an important protein in the detection and differential diagnostics of inflammatory states. Despite intensive research, a number of uncertainties still exist concerning the metabolism of this "inflammatory" PCT and its physiological role.

Fax +420 2 293 894
E-mail physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

Biochemical characteristics

PCT is a protein consisting of 116 amino acids with a molecular weight of 13 kDa. The structure of PCT detected in plasma during inflammation is identical to that produced by thyroid C-cells as precursors of calcitonin. The sequence of calcitonin of 32 amino acids is to be found between the 60th and 91st position of the PCT chain (Fig. 1).

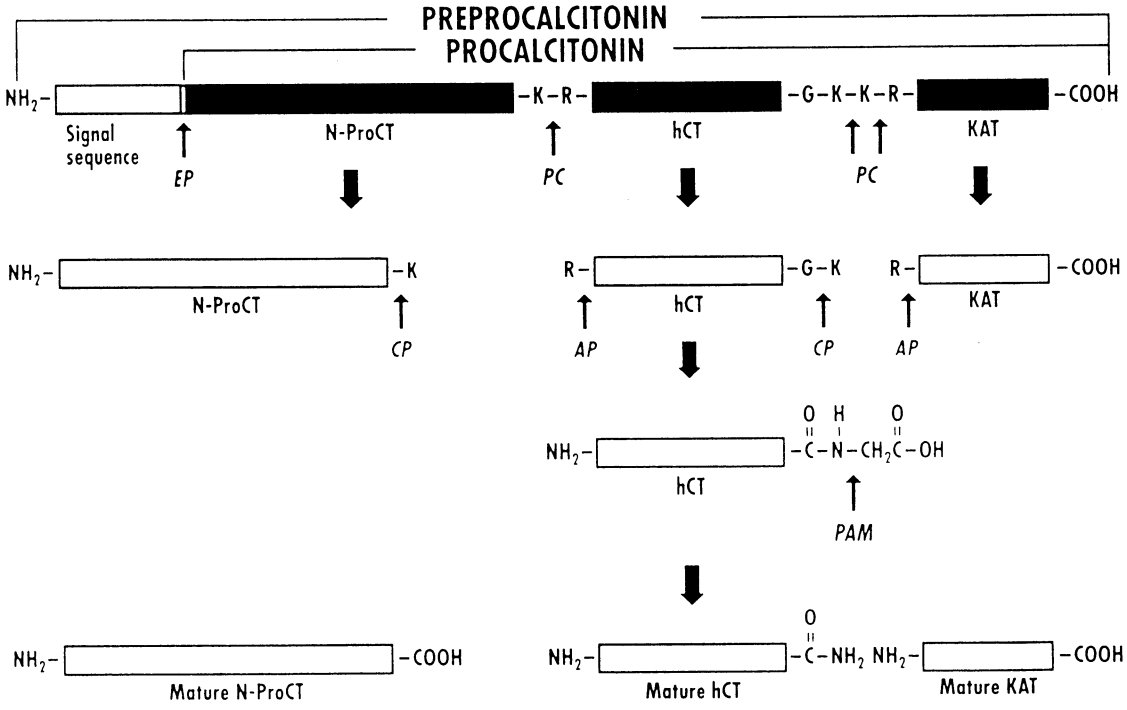

Fig. 1. *Cleavage of procalcitonin (from Meisner 1996). AP = aminopeptidase, CP = carboxypeptidase, CT = calcitonin, EP = endopeptidase, KAT = katacalcin, PAM = peptidyl glycine amidating monooxydase, PC = prohormone convertase*

The original product of CALC-I gene, responsible for the production of PCT in C-cells of the thyroid, and most likely also during inflammation, is the 141 amino acid chain of preprocalcitonin. The signaling sequence at the N-terminus with its hydrophobic properties allows binding to the endoplasmatic reticulum where it is cleaved by endopetidase, giving rise to PCT. Inside the C-cells, PCT is cleaved at its N-terminal sequence and the C-terminal fragment (catacalcin) to produce calcitonin.

Calcitonin is released into the blood stream after its secondary and tertiary structures had been formed. Two cysteine remnants in position 1 and 7 form a disulfidic bridge and the C-terminus proline is hydroxylated. Both structures, essential for binding of calcitonin to its receptor, are therefore not a part of the PCT structure and are only formed after the calcitonin had been cleaved from PCT. Practically all of PCT formed in C-cells is converted into calcitonin, so that no PCT enters the circulation and its levels in healthy subjects are below the detection level. There are no enzymes in the plasma that could break down the circulating PCT. This means that if PCT somehow escapes intracellular proteolysis and is secreted into the circulation, it remains there unchanged with a half-life of 25-30 h, in comparison to the 4-5 min of calcitonin half-life.

PCT gene

Gene for the calcitonin precursor, known as CALC-I, is localized on the short arm of human chromosome 11. It comprises of 6 exones, the fourth carrying the sequence of calcitonin, the fifth the sequence of calcitonin gene-related peptide I (CGRP-I) and the sixth is non-coding. The CGRP-I sequence is discarded after transcription from mRNA (before translation).

Three known genes from the calcitonin "family" are found in the neighborhood of the CALC-I gene,

namely CALC-II through CALC-IV. It is supposed that they evolved through duplication and sequence divergence from one primordial gene. Out of the three related genes, only CALC-II codes the prohormone that contains the full sequence for calcitonin. This is, however, discarded during intracellular processing and this translation produces the CGRP-II molecule, acting mainly as a neurotransmitter. The gene for CALC-III is a pseudogene which is not coding any protein. CALC-IV is coding the protein amyline, expressed primarily in the beta cells of pancreatic Langerhans islets.

Regulation of procalcitonin

Regulations of thyroid and "inflammatory" PCT are fundamentally different. C-cells of the thyroid gland react to elevated calcium levels as well as to a number of other stimuli, such as glucocorticoids, CGRP, glucagon, gastrin or β-adrenergic stimulation. Somatostatin and vitamin D suppress calcitonin production. Neither hypercalcemia nor any other of above listed stimuli leads to a release of PCT during inflammation. The production of PCT during inflammation is linked to the bacterial endotoxin and to inflammatory cytokines. This was demonstrated by the rapid rise of PCT levels after the injection of endotoxin, the most potent stimulator of PCT release into circulation (Dandona *et al.* 1994), or after the administration of TNF, tumor necrosis factor. Their effects are most likely mediated by the cascade of cytokines.

After the administration of endotoxin, TNF rises first, reaching its peak in 90 min, followed by interleukin-6 (IL-6) with its peak at 180 min. PCT only reacts after 3-6 h, peaking at 6-8 h. Owing to their long half-life, PCT levels culminate 12-48 h after the administration of endotoxin. Levels of these cytokines follow the same pattern in patients with acute bacterial infection. PCT rises after the elevation of TNF and IL-6, but before the rise in the C reactive protein.

The administration of TNF, IL-1, IL-2 and IL-6 also leads to an increase in PCT. The mechanisms responsible for such an action are not known. Based on the information about calcitonin formation, it was hypothesized that cytokines block the proteolysis of PCT to calcitonin in the endoplasmatic reticulum. However, the site of production of circulating PCT is different and the possibilities of its regulation are more diverse.

In contrast to cytokines such as TNF and IL-6, the rise of which is not specific for certain types of inflammation, PCT rises selectively in bacterial inflammatory processes. Therefore, several modulating factors must be involved in the production and release of PCT.

Source of "inflammatory" procalcitonin

Seven years after the demonstration of its role as an inflammatory marker, we can still only speculate on the site of PCT production and modes of its regulation.

PCT detectable in the plasma during inflammation is not produced in C-cells of the thyroid (Russwurm *et al.* 1999). The dynamic changes during initial stages of inflammation are the same in thyroidectomized individuals. Cells of neuroendocrine origin express all proteins related to calcitonin (CGRP-I and II and amyline), derived from the same family of genes CALC-I through IV. This origin is presumed for "inflammatory" PCT. Even if we do not rule out more members to the CALC gene family, we can speculate that 'inflammatory' PCT is coded by the same gene as PCT in the C-cells of the thyroid, the CALC-I gene. Contrary to C-cells, it is not cleaved by proteolytic enzymes intracellularly.

The probable site of PCT production in inflammation are the neuroendocrine cells in the lungs or intestine. The expression of mRNA coding PCT has been demonstrated *in vitro* in monocytes stimulated by endotoxin or inflammatory cytokines (Oberhoffer *et al.* 1999). The production of PCT in circulating blood cells has, however, not been confirmed in another study in which elevated levels of cytokines but not PCT could be detected after the administration of endotoxin to isolated blood (Monneret *et al.* 1999).

In an experiment where blood samples were withdrawn at different sites of patients after cardiac surgery with extracorporal circulation, it was presumed that PCT is produced in the splanchnic area. The level of PCT was significantly higher in samples from hepatic veins than from anywhere else (Silomon *et al.* 1999). Nevertheless, these authors argue that the result can be biased due to the very specific situation of patients during the operation, when the mucosal barrier of their intestine could have been damaged and the endotoxin could have entered the portal blood circulation.

Physiological importance of procalcitonin

The present studies have not provided evidence that plasma PCT binds to cellular receptors for calcitonin with significant affinity, or that specific PCT receptors do exist. Although it is highly unlikely that the human

organism would waste proteosynthetic activity during inflammation on a protein with no function, we do not hitherto know anything about the physiological role of plasmatic PCT. Current hypotheses, based on animal models or on *in vitro* experiments deal with the role of PCT in the following areas:

- metabolism of calcium
- cytokine network and modulation of NO synthesis
- pain relieving effects

The hypothetical role of PCT in calcium and phosphate metabolism during sepsis is based on the structural similarities of PCT and calcitonin. PCT includes the amino acid sequence of calcitonin. But the similarity of the primary structure of the protein chains is less important than the marked differences in secondary and tertiary structure. The shape of calcitonin is determined by a disulphide bridge between cystein remnants at position 1 and 7. This creates a circle of seven amino acids at the N-terminus that, together with hydroxylated proline at the C-terminus, form the binding site with a high affinity to the target calcitonin receptor. In PCT, the N-terminal fragment is preserved, which prevents the forming of this bridge. This explains the current experimental results in which plasmatic PCT does not bind to the calcitonin receptors. A relationship between PCT and the metabolism of calcium and phosphates has not been proved. Even though septic patients often suffer from hypocalcemia, the levels of calcium, phosphates and PCT do not correlate significantly.

To include PCT into the cytokine network would be logical. Cytokines play a crucial role in the regulation of PCT production, and the levels of major proinflammatory cytokines correlate significantly with PCT in septic patients. It is possible that PCT is involved in the modulation of NO synthesis, similarly as other proinflammatory cytokines. However, at present there is no evidence to support such a hypothesis.

A recent study has speculated that the role of PCT in inflammation is that of a "non-steroid analgesic". The analgesic effect of calcitonin has been known for a long time, and despite the fact that its mechanism of action is not clear, it has been widely used in symptomatic relief of pain associated with osteolytic metastases. PCT could exert its effect similarly. *In vitro* experiments have shown a decrease in tromboxane B_2 concentration after adding calcitonin or PCT to the medium. This effect was dose-related and more marked for PCT. A significant decrease in tromboxane B_2 concentration was achieved by PCT concentrations usually achieved in bacterial inflammations in humans. This effect, probably mediated by inhibition of prostaglandin-G,H-synthase, would be similar to the action of non steroid antiinflammatory drugs and does not require the hormone to bind to a specific cellular receptor.

The role of PCT as a "positive" factor in the inflammatory response was brought into doubt by a recent study. It is known that a long-term sustained elevation of PCT in sepsis indicates poor prognosis. Hypothetically, it is possible that PCT not only reflects poor prognosis but also plays a causative role *via* an unknown mechanism. An experiment, in which exogenous PCT was administered to animals in sepsis showed an increase in mortality in comparison with animals in sepsis without exogenous PCT. Similarly, blockade of endogenous PCT production significantly lowered the mortality of septic animals (Nylen *et al.* 1998).

Levels of PCT in various pathological situations

The highest plasma levels of PCT are achieved in acute bacterial infections and sepsis. Plasma levels in infections are enhanced by the presence of a systemic inflammatory response (Nylen *et al.* 1992). Local bacterial infections as well as abscesses do not raise plasma PCT significantly (Eberhard *et al.* 1997). The actual level of PCT is determined by the type and extent of the inflammation (Brunkhorst *et al.* 1999).

PCT is not elevated by viral or autoimmune inflammation, or by the presence of neoplasms (Jarešová *et al.* 1999). Apart from bacterial infections, PCT is elevated in medullar carcinoma of the thyroid and small-cell lung carcinoma, exhibiting paraneoplastic production. PCT levels could also be raised in renal failure. Pharmacological interactions, trauma or actual surgery and extracorporal circulation were excluded (Boeken *et al.* 1998).

PCT can rise as high as 1000 μg/l in severe bacterial infections (Benoist *et al.* 1998). Normal concentrations in the plasma or serum do not exceed 0.5 μg/l in healthy subjects. The decrease of PCT levels at the end of the acute phase of inflammatory response is influenced by the long half-life of this protein in comparison with the much shorter one of the cytokines.

Conclusion

Until 1993, PCT was considered a practically closed chapter of endocrinology. Today, a quarter of a century after its discovery and seven years after its inclusion among inflammatory markers, it has become a

useful tool in clinical practice, although its physiological role still remains unclear. It is a protein playing an undeniable role in the diagnosis of inflammatory states with advantages over other markers. At the same time we do not know where this protein is produced in response to inflammation and how its production is regulated, let alone its physiological and pathological significance.

Acknowledgements

Supported by the grant IGA MZ 4825-3.

References

ASSICOT M, GENDREL D, CARSIN H, RAYMOND J, GUILBAUD J, BOHUON C: High serum procalcitonin concentrations in patients with sepsis and infection. *Lancet* **341:** 515-518, 1993.

BENOIST J.F, MIMOZ O, ASSICOT M, EDOUARD A: Serum procalcitonin, but not C-reactive protein, identifies sepsis in trauma patients. *Clin Chem* **44:** 1778-1779, 1998.

BOEKEN U, FEINDT P, PETZOLD T, KLEIN M, MICEK M, SEYFERT U, MOHAN E, SCHULTE H, GAMS E: Diagnostic value of procalcitonin: the influence of cardiopulmonary bypass, aprotinin, SIRS, and sepsis. *Thorac Cardiovasc Surg* **46:** 348-351, 1998.

BRUNKHORST FM, EBERHARD OK, BRUNKHORST R: Discrimination of infectious and noninfectious causes of early acute respiratory distress syndrome by procalcitonin.*Crit Care Med* **27:** 2172-2176, 1999.

DANDONA P, NIX D, WILSON MF, ALJADA A, LOVE J, ASSICOT M, BOHUON C: Procalcitonin increase after endotoxin injection in normal subjects. *J Clin Endocrinol Metab* **79:** 1605-1608, 1994.

EBERHARD OK, HAUBITZ M, BRUNKHORST FM, KLIEM V, KOCH KM, BRUNKHORST R: Usefulness of procalcitonin for differentiation between activity of systemic autoimmune and invasive bacterial infection. *Arthritis Rheum* **40:** 1250-1256, 1997.

JAREŠOVÁ M, STŘÍŽ I, ČERMÁKOVÁ J, SEDLÁČEK J, MUDRA K, HÁNA I, VÍTKO S: Serum procalcitonin concentrations in transplant patients with acute rejection and bacterial infections. *Immunol Lett* **69:** 355-358, 1999.

MEISNER M: *Procalcitonin – A New, Innovative Marker for Severe Infection and Sepsis*. Brahms, Berlin 1996.

MONNERET G, LAROCHE B, BIENVENU J: Procalcitonin is not produced by circulating blood cells. *Infection* **7:** 34-35, 1999.

MOYA F, NIETO A, CANDELA JL: Calcitonin biosynthesis: evidence for a precursor. *Eur J Biochem* **55:** 407-413, 1975.

NYLEN ES, O'NEIL W, JORDAN MH, SNIDER RH, MOORE CF, LEWIS M, SILVA OL, BECKER KL: Serum procalcitonin as an index of inhalation injury in burns. *Horm Metab Res* **24:** 439-43, 1992.

NYLEN ES, WHANG KT, SNIDER RH, STEINWALD PM, WHITE JC, BECKER KL: Mortality is increased by procalcitonin and decreased by an antiserum reactive to procalcitonin in experimental sepsis. *Crit Care Med* **26:** 1001-1006, 1998.

OBERHOFFER M, STONANS I, RUSSWURM S, STONANE E, VOGELSANG H, JUNKER U, JAGER L, REINHART K: Procalcitonin expression in human peripheral blood mononuclear cells and its modulation by lipopolysaccharides and sepsis-related cytokines in vitro. *J Lab Clin Med* **134:** 49-55, 1999.

RUSSWURM S, WIEDERHOLD M, OBERHOFFER M, STONANS I, ZIPFEL PF, REINHART K: Molecular aspects and natural source of procalcitonin. *Clin Chem Lab Med* **37:** 789-797, 1999.

SILOMON M, BACH F, ECKER D, GRAETER T, GRUNDMANN U, LARSEN R: Procalcitonin after extracorporal circulation. Synthesis in the hepatosplanchnic region. *Anaesthesist* **48:** 395-398, 1999.

Reprint requests

Dr. P. Maruna, Third Internal Department, First Faculty of Medicine, Charles University, U nemocnice 1, 120 00 Prague 2, Czech Republic, e-mail: maruna@lf1.cuni.cz