Calcitonin, calcitonin gene-related peptide, adrenomedullin and amylin: homologous peptides, separate receptors and overlapping biological actions

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Calcitonin (CT), calcitonin gene-related peptide (CGRP), amylin and adrenomedullin (ADM) have in common N-terminal 6-7 amino acid ring structures and amidated C-termini (Fig. 1) (1-3). Additional amino acids in the middle and C-terminal regions are identical in several peptides. Unique among this family of peptides, ADM has a linear N-terminal extension of the ring structure of 15 and 13 amino acids in human and rat ADM, respectively (2, 4).

Calcitonin is synthesized and secreted from parafollicular or C-cells of the thyroid gland (see Refs. 1 and 5). Calcitonin lowers blood calcium levels through inhibition of osteoclastic bone resorption and stimulation of the urinary calcium excretion. The CT gene has six exons. Exons 1-4 generate messenger RNA encoding the precursor of CT, preproCT, predominantly in thyroid C-cells (6). Procalcitonin is converted at basic amino acid cleavage sites into CT and N- and C-terminal flanking peptides whose biological relevance remains to be recognized. An alternative messenger RNA encoding preproCGRP is formed by exons 1-3 and 5 and 6, mainly in the nervous system. Excision, therefore, of the CT-encoding mRNA corresponding to exon 4 through tissue-specific nuclear splicing mechanisms results in the formation of preproCGRP. The mature products include N-terminal flanking regions in common with proCT and proCGRP. CGRP-I is encoded by exon 5, CGRP-II, which in humans differs in three of the 37 amino acids and in the rat differs in a single amino acid, is encoded by a different gene. An exon 4-like structure with a stop codon in the second CGRP gene reminiscent of CT is not expressed. Calcitonin is the predominant and CGRP a minor product of thyroid C-cells. Calcitonin gene-related peptide, on the other hand, is a neuropeptide synthesized in neurones of the central and peripheral nervous system. The secretion of CT is stimulated at least acutely by increased extracellular calcium, resulting subsequently in classical endocrine fashion in lowered serum calcium levels. Calcitonin gene-related peptide is released from nerve terminals by

| C G N L S T C M L G T Y T Q D F N K H T - - - - - F P Q T A I G V G A - F - N H₂ CT |
| F K N D A T C A W Q R L A N F M V H G S N N F G A I L S S S N N F G S N T - Y - N H₂ Amylin |

Fig. 1. Amino acid sequences of human CT, CGRP-I and -II, ADM and amylin (for references, see text).
voltage-dependent calcium uptake, among other mechanisms. As a result, CGRP acts mainly locally without reaching the general circulation. It is the most potent vasodilator known so far, and shares this effect with the distantly related peptide (about 20% homology) ADM (Fig. 1) (7). The latter was isolated from human phaeochromocytoma and identified in 1993 (2, 4). Amylin isolated from pancreatic islet cells is also related to CGRP (about 40% homology) (3) and much like CT and CGRP, inhibits glycogen synthesis in the isolated soleus muscle (8, 9). This effect is reminiscent of impaired glucose tolerance and insulin resistance of diabetes type II (8, 10).

Calcitonin, CGRP, ADM, and amylin enhance blood flow through relaxation of resistance vessels (5, 7, 11). The vasorelaxant effect of CT and CGRP-II are weaker in humans than those of CGRP-I (13), but CGRP-II inhibits the gastric acid output more potently than CGRP-I (13). Calcitonin gene-related peptide exerts positive inotropic effects on the heart (12). This effect is indirect and brought about through activation of the sympathetic nervous system. Improved contractility of the heart has been achieved in patients with severe heart failure resistant to digoxin (14). Not seen in these patients, but at high concentrations, CGRP has positive chronotropic effects on the heart and, owing to its vasorelaxant properties, lowers the arterial blood pressure (12). In the kidney, CGRP and amylin stimulate renin secretion (10, 15). Calcitonin gene-related peptide is a potent relaxant of mesangial cells, in part responsible for the stimulation of the glomerular filtration rate and of renal blood flow (16, 17). Relaxant effect on the smooth musculature of CGRP are widespread and also encountered in the ureter, uterus and gall bladder (see Ref. 5). Calcitonin, on the other hand, affects renal tubular transport processes, which include stimulation of the urinary flow and the fractional excretion of sodium, chloride, calcium and phosphate through inhibition of tubular reabsorption (17). Osteoclastic bone resorption is suppressed with CT in lower amounts than with CGRP and amylin (18, 19). Together, CT, CGRP, ADM and amylin have overlapping biological effects owing to their related structures and cross-reaction between receptors (Fig. 1 and Table 1).

Among this family of well-characterized peptides, the structures of the majority of receptors remain to be identified. So far, the amino acid sequences of porcine, human and rat CT receptors have been obtained through molecular cloning (20–22). A homologous (about 80% identical amino acids) orphan receptor is expressed in the lung and brain, and is linked to a different ligand remaining to be discovered (23, 24). The receptors have in common seven putative transmembrane domains. The CT receptors are coupled to G-proteins linked to adenyllycyclase and phospholipase C activation (25, 26). Osteoclastic activity is inhibited through stimulation of cyclic AMP accumulation and enhanced cytotoxic-free calcium concentrations (19).

Transfection of a human CT receptor into baby hamster kidney cells caused CT to raise cyclic AMP and cytosolic-free calcium concentrations (27). Upon transfection with the CT receptor, the cells became responsive to alterations of extracellular calcium, implying the presence of an extracellular calcium sensor. A porcine CT receptor was transfected into a renal proximal tubular cell line from the American opossum with an endogenous parathyroid hormone receptor linked to inhibition of phosphate transport (28). Here, CT also suppressed phosphate uptake, consistent with its phosphaturic action. Human and rat CT receptors with a 16 amino acid insert in the first putative intracellular domain and a 37 amino acid insert in the second extracellular domain have been identified (21, 22, 29). The 37 amino acid insert CT receptor subtype produced different ligand specificity (22, 31). The porcine CT receptor gene consists of 14 exons and it was mapped to chromosome 9q11-q12 (32).

With all the CT receptors recognized so far, salmon [125I]CT binding was largely irreversible, except for the rat CT receptor with the 37 amino acid insert (31, 33). Consistent with the negligible dissociation of the salmon CT radioligand, cyclic AMP and intracellular free calcium concentrations are raised persistently (34, 35).

In contrast to the continued activation with salmon CT, [125I]CGRP binding to cell membranes is reversible, as expected from a classical receptor (36). In human SK-N-MC neuroblastoma cells, salmon CT inhibits receptor binding at 3000-fold higher concentrations compared to hCGRP-I, and binding displacement with hCT was
negligible (37). The apparent molecular weight of the human CGRP receptor in SK-N-MC cells is 56,000 before and 44,000 after deglycosylation, and is indistinguishable from that in the human cerebellum. Binding displacement of $^{[125]}$IhCGRP-I was obtained with only sevenfold higher concentrations of hADM compared to hCGRP-I (38). Apparently, CGRP and ADM interact with the same receptor linked to cyclic AMP accumulation (38, 39). Interestingly, the corresponding regions of ADM and CGRP are only about 20% homologous. They include the N-terminal ring structure essential for biological activity and certain amino acids in the C-terminus (Fig. 1). But, relaxation of blood vessels, much like the inhibition of receptor binding, was obtained with similar amounts of CGRP, ADM and of N-terminally truncated ADM lacking the N-terminal extension of the ring structure (7, 40).

Amylin has been isolated originally from pancreatic islet cells (3). Inhibition of insulin secretion and, more importantly, inhibition of glucose transport into the skeletal musculature and of gluconeogenesis have been implicated in the pathogenesis of diabetes type II (8, 10, 41). About 40% of the amino acids of amylin and CGRP are identical (Fig. 1), yet inhibition of $^{[125]}$IhCGRP receptor binding to a bona fide CGRP receptor of SK-N-MC neuroblastoma cells has been obtained with 200-fold higher concentrations of amylin compared to hCGRP-I. Conversely, a CT receptor of breast carcinoma T47D cells also recognized amylin at 200-fold higher concentrations compared to salmon CT (37). A third receptor unlike that of CGRP or CT has been recognized in the nucleus accumbens of the rat brain (42). There, closely related displacement of $^{[125]}$Iamylin binding by amylin, CGRP and salmon CT is consistent with the similar action of CGRP, salmon CT and amylin on the inhibition of gluconeogenesis in the skeletal musculature (8, 9, 42).

With CT, CGRP, amylin and ADM, the N-terminal ring structure is required for biological activity (5, 38). Indeed, N-terminally truncated peptides lacking the ring structures are relatively potent antagonists of biological actions. To this end, CGRP(8–37) inhibits vasorelaxation obtained with CGRP and salmon CT (8–32) suppressed the release of plasminogen activator from LLC-PK-1 renal proximal tubule cells (43, 44). An amylin/CT hybrid antagonist has been considered for the treatment of insulin resistance characteristic of diabetes type II (10, 45).

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