Cholecystokinin (CCK) is a major peptide hormone in the gut and a major peptide transmitter in the brain. Its synthesis requires endoproteolytic cleavage of proCCK at several mono- and dibasic sites by prohormone convertases (PCs). Of these, PC1 and PC2 are expressed in cerebral neurons and intestinal endocrine cells. Characteristically, however, the processing of proCCK varies markedly between the brain and the gut. In neurons, CCK-8 is always the predominating form, whereas the endocrine gut cells (I-cells) contain a mixture of small and larger CCK-peptides of which CCK-33 or CCK-22 often predominate. The role of PC1 and PC2 in the processing of proCCK have now been examined by measuring the concentrations of prohormone, processing intermediates and amidated end-products in jejunal and cerebral extracts of PC1 and PC2 deficient mice and corresponding wild type controls. The PC1 null mice revealed a pattern opposite to that of the PC2 null mice, in whom only the cerebral processing of proCCK was affected. Thus PC1 knockouts reveal a severe block in the processing of intestinal proCCK. Accordingly, the intestinal concentration of proCCK was many fold increased, and also the concentrations of different processing intermediates were raised; but the concentration of bioactive, \(\alpha\)-amidated and O-sulfated CCK was reduced to a few percent of normal. The only bioactive CCK peptide in the gut of PC1 deficient mice was CCK-22, and it was present only in trace amounts. The cerebral processing of proCCK was, however, not at all affected by the lack of PC1 - in sharp contrast to the effect of PC2. The results show that the tissue-specific processing of proCCK to a large extent can be explained by prohormone convertases, as PC1 plays a decisive role in the maturation of hormonal CCK in the gut, whereas PC2 governs the processing of brain proCCK.
INTRODUCTION

Cholecystokinin (CCK) is a classical gut hormone produced in endocrine I-cells in the small intestinal mucosa from where CCK is released to circulation to regulate gallbladder emptying, pancreatic enzyme secretion, intestinal motility and gastric acid secretion. In addition, intestinal CCK is a growth factor for the pancreas. The CCK gene is, however, expressed also in large quantities in cerebral and peripheral neurons from where CCK peptides are released as potent neurotransmitters and modulators. Accordingly, cerebral CCK defects have been associated with major neuropsychiatric diseases such as anxiety, schizophrenia and satiety disorders (for reviews, see refs. 1-3). Finally, the CCK gene is also expressed in sarcomas and pituitary tumors (4-6).

The mammalian proCCK sequence harbor several endoproteolytic processing sites of which one dibasic (R<sub>85</sub>R<sub>86</sub>) and four monobasic (R<sub>25</sub>, R<sub>50</sub>, K<sub>61</sub> and R<sub>75</sub>) are known to be cleaved (Fig. 1). The processing is tissue-specific in the sense that the pattern of bioactive CCK peptides in the gut and the brain differs markedly in a characteristic and evolutionary preserved manner. Hence, intestinal I-cells release a mixture of CCK peptides of varying length (CCK-8, CCK-22, CCK-33, CCK-58 and CCK-83) of which CCK-33 and/or CCK-22 predominates in plasma of most mammals (7-10). In contrast, CCK-8 is by far the most abundant neuronal form, and larger forms are present only in trace amounts in the brain (11-14).

The endoproteolytic processing requires prohormone convertases (PCs). Mammals are known to express seven subtilisin-like PCs, of which PC1 and PC2 are assumed to be responsible for most of the endoproteolytic processing of neuroendocrine proproteins (for reviews, see refs. 15-17). PC1 and PC2 typically cleave dibasic sites, although they also are capable of cleaving monobasic sites. Processing of the R<sub>85</sub>R<sub>86</sub> site in proCCK determines the availability of the glycine-extended CCK precursor for the decisive F<sub>83</sub>α-amidation. Cleavage of R<sub>85</sub>R<sub>86</sub> hence establishes the amount of CCK peptides, which can become ligands for the CCK-A and -B receptors. Subsequent cleavage at the C-terminus of R<sub>25</sub>, R<sub>50</sub>, K<sub>61</sub> and R<sub>75</sub> then determines the length of the N-terminal extensions, and hence whether the α-amidated and O-sulfated fragments become CCK-8, CCK-22, CCK-33, CCK-58 or CCK-83.

In order to determine the roles of PC1 and PC2 in the tissue-specific expression of CCK peptides, the cerebral and intestinal processing of proCCK in PC1 and PC2 null mice have been examined, and compared to the processing pattern in corresponding wildtype mice (18 and Rehfeld et al., unpublished results). First, however, it is essential to consider the nomenclature of proCCK and its products.

PROCCK NOMENCLATURE

ProCCK in mice is a protein of 95 amino acid residues (Fig. 1). The earliest post-translational modification of proCCK appears at our present state of
Fig. 1. Diagram of the co- and posttranslational modifications of preprocholecystokinin. Activation of the CCK amidation site (−FGRR−, sequence 83-86 of proCCK) occurs via a series of carboxyterminal cleavages and modifications. An early modification is as indicated tyrosyl O-sulfation by sulfotransferases of Y_{77}, Y_{91} and Y_{93}. In addition to ensure binding to the CCK-A receptor, tyrosyl sulfation has been shown also to increase endoproteolytic cleavage of the related progastrin (ref. 24). Endoproteolytic cleavage by a prohormone convertase (PC1, PC2 or other PCs) produces the carboxypeptidase E substrate (−FGR−). Carboxypeptidase E then acts to remove the C-terminal arginyl residue yielding glycine-extended CCK by α-amidating monoxygenase (PAM) results in the production of bioactive CCK (F-NH₂). Concomitant N-terminal cleavages by PCs produce bioactive CCKs of varying length.
knowledge to be O-sulfation of Y₇₇, Y₉₁ and Y₉₄ by sulfotransferases in the trans-
Golgi network. Sequence 83-86 (-FGRR-) constitutes the amidation site, which 
requires processing by prohormone convertases, carboxypeptidase E, and the 
amidation enzyme complex (PAM) to release bioactive carboxyamidated CCK 
peptides. The largest bioactive form is CCK-83, which corresponds to the 
carboxyamidated sequence 1-83 of proCCK (19). This sequence is cleaved at 
least at four monobasic sites to release CCK-58, -33, -22 and -8, all of which have 
the same C-terminal heptapeptide amide sequence [-Y(SO₃⁻)MGMDFamide] that 
is also the minimal epitope necessary for CCK-A receptor binding. Binding to 
CCK-A receptors requires that the tyrosyl residue of the heptapeptide amide is O-
sulfated, whereas CCK-B receptors do not discriminate neither sulfated from 
non-sulfated CCK-peptides nor CCK from gastrin peptides (for review, see ref. 
20). In mammals, neuronal CCK is as mentioned by far predominated by 
CCK-8, whereas intestinal endocrine CCK is a mixture of CCK-58, CCK-33, CCK-22, 
and CCK-8 (13, 21).

In studies of proCCK processing, it is essential to know that maximal tissue 
extraction of the small molecular form (CCK-22, CCK-8 and CCK-5) requires 
near neutral pH, whereas the intermediate sized CCKs (CCK-58, CCK-39 and CCK-
33) preferentially are extracted at acidic pH (13). The precise explanation for this 
discrepancy is unknown; but it is generally believed to reflect differences in the 
net charges of the intermediate sized versus the small sized CCKs.

**PROCCK PROCESSING IN PC1 AND PC2 DEFICIENT MICE**

Lack of PC1 grossly changed the processing of proCCK in the small intestine. 
The total amount of the translatable product (proCCK, processing-intermediates 
plus α-amidated end-products) increased nearly threefold in the knockout 
jejunum; but the increase was almost entirely due to an enhanced accumulation 
of proCCK. The concentrations of the processing intermediates (CCK-Gly-Arg 
and CCK-Gly) were also increased, but only moderately. The concentration of the 
bioactive amidated CCK was, however, reduced to a near block of the synthesis. 
In contrast, expression and processing of proCCK was entirely normal in PC2 
knockout mice. Notably, exactly the same extraction techniques and methods of 
measurement were used in the study of PC1 and PC2 null mice (18 and Rehfeld 
et al., unpublished results).

The chromatographic examination of intestinal extracts showed that the trace 
of α-amidated and O-sulfated CCK in the PC1 knockouts nearly all was CCK-22, 
which also was the predominant form of CCK in wild type mice. The high 
amount of precursors in knockout intestines eluted like intact proCCK and a 
fragment containing both the CCK-33 sequence, the amidation site (Gly₈₄, R₈₅, 
R₈₆) and the C-terminal flanking fragment. The precursor pattern in knockouts 
deviated substantially from the wild type controls that expressed only trace 
amounts of proCCK and an intermediate size C-terminally extended CCK.
The lack of PC1 was without effect on the cerebral maturation of proCCK. Hence, there was a remarkable similarity in the concentration of transmitter active, α-amidated CCK and its immediate precursor in PC1 knockout and wild type brains. This similarity contrasts to that of PC2 knockout mice, where the cerebral processing of proCCK is grossly disturbed with a ninefold increase in proCCK concentration and a significant reduction in the concentration of α-amidated CCK (18). The chromatography revealed that apart from a small amount of CCK-83 in the PC1 knockout brains, neuronal amidated CCK was almost entirely of octapeptide size as in normal mice. Lack of PC2 was entirely without effect on the intestinal proCCK processing (18).

DISCUSSION

Our studies have shown that the subtilisin-like prohormone convertase, PC1, is of decisive significance for the synthesis of hormonally active CCK in intestinal I-cells. Hence, α-amidated and O-sulfated CCK peptides (the ligands for the CCK-A receptor) constituted only a few percent of the CCK mRNA translation product in the intestine of PC1 null mice, whereas the corresponding fraction in wild type mice was nearly 90%. The significance of PC1 in the intestinal endocrine cells contrast markedly to that in cerebral CCK-neurons, where the synthesis of amidated and sulfated CCK was unaffected by the lack of PC1. The gut specific significance of PC1 in proCCK processing is opposite to that of the other neuroendocrine prohormone convertase, PC2. Hence, PC2 is without effect on the intestinal I-cell processing, but displays major effects on the cerebral processing of proCCK (18). Still, however, cerebral CCK neurons synthesize amidated and transmitter-active CCK peptides in amounts that may be sufficient to ensure most of the synaptic function of CCK neurons in the PC2 knockouts (18). The negligible effect of PC1 on neuronal proCCK processing and the moderate effect of PC2 (18) may suggest that other prohormone convertases contribute to the neuronal synthesis of transmitter-active CCK-8. One such candidate is PC5, that has been reported to be present in cerebral neurons and to cleave proCCK in neuronal cell-lines at Lys_{61} and Arg_{71} (22). But PC5 does apparently not touch the monobasic cleavage site at Arg_{75}, which has to be cleaved in order to release CCK-8 (12). Therefore, additional convertases are necessary to explain the neuronal synthesis of CCK-8.

The grossly deviating CCK patterns in the gut and the brain are difficult to explain in terms of different prohormone convertase expression only. Probably, cell cycle and granule maturation differences also contribute to the difference. Thus, long-lived neurons allow a more complete processing towards the phenotypic endproduct of CCK gene expression (i.e. α-amidated and O-sulfated CCK-8), whereas the short-lived mucosal I-cells in the gut are shed off to the intestinal lumen before the endoproteolytic posttranslational processing has
reached completion. Nevertheless, this apparently incomplete processing of gut-endocrine proCCK may be expedient, because hormonal CCK, i.e. CCK in plasma originate from the gut and not from the brain. Hence, the food-induced intestinal release to blood of a mixture of intermediate-sized CCKs (CCK-58, CCK-33 and CCK-22) with a considerably slower metabolic clearance from plasma than the small CCK-8 peptide ensures adequate stimulation of digestive functions during the entire period (up to hours) required for digestion. Vice versa, it is expedient that the CCK peptide required for rapid synaptic transmission is as short and easily degraded as CCK-8.

The only detectable form of amidated CCK in the gut of PC1 null mice was CCK-22. It is on one hand remarkable, because the synthesis of CCK-22 requires cleavage at the Lys<sub>61</sub>-residue, and Lys-residues are often preferred for cleavage by PC2 rather than PC1 (for reviews, see refs. 15 - 17 and 23). On the other hand, CCK-22 is anyway the most abundant form of CCK in the mouse and rat intestine. It is therefore possible that the small peak of CCK-22 simply reflects the general shut-down of processing, where only a trace of the normally most abundant form becomes detectable. Whatever the mechanism, the tiny amount of CCK in the gut of the PC1 null mice is hardly sufficient to ensure adequate hormonal CCK regulation.

As hopefully illustrated in this review, genetically modified mice short of a processing enzymes emerge as highly useful tools in the study of the often complex biosynthesis of brain-gut peptides. It now remains to be seen whether the mouse results can be extrapolated to other mammals and to human disease.

REFERENCES


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Author's address: Jens F. Rehfeld, M.D., Professor, D.Sc., University Department of Clinical Biochemistry (KB 3014), Rigshospitalet, DK-2100 Copenhagen, Denmark. Phone: +45 3545 3018, Fax: +45 3545 4640
E-mail: rehfeld@rh.dk