

## MOLECULE PAGE

### Caerulein

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Version 1.0, December 22, 2014 [DOI: 10.3998/panc.2014.15]

Gene symbol: [LOC397740](#) (Caerulein *Xenopus Laevis*)

#### 1. General Information

Caerulein is a decapeptide with biological activity on GI smooth muscle contraction and pancreatic and gastric secretion. It was identified based on its pharmacological action to lower blood pressure in extracts of the skin of the Australian Green Tree Frog (**Figure 1**) then known as *Hyla caerulea* (4) (**Note 1**). Most of the early research was centered in Italy and carried out by the research group of V. Erspamer. Subsequently caerulein was identified in other Australian Hylids, in the South American frog, *Leptodactylus*, and in the South African toad, *Xenopus laevis* (3, 10). Sequencing of the peptide showed striking resemblance in amino acid sequence to the carboxyl terminus of cholecystinin (CCK) and gastrin. The amino acid sequence of caerulein and a related peptide, phyllocaerulein, from South American Hylid frogs (2) is shown in **Figure 2**. Caerulein contains a sulfated tyrosine and the C-terminal seven amino acids are identical to CCK octapeptide except for a Thr residue substituting for Met. Both peptides bear a carboxyl terminal amide group but caerulein also has a blocked

amino terminal with pyroglutamine as the initial amino acid. This blocking group reduces susceptibility to inactivation by amino peptidases.



**Figure 1. *Hyla Caerulea* also known as the Australian Green Tree Frog.** Image from [en.wikipedia.org](#).

Pyr—Glu—Asp—Tyr(SO <sub>3</sub> H)—Thr—Gly—Try—Met—Asp—Phe—NH <sub>2</sub>	Caerulein
Pyr—Glu—Tyr(SO <sub>3</sub> H)—Thr—Gly—Trp—Met—Asp—Phe—NH <sub>2</sub>	Phyllocaerulein
Asp—Tyr(SO <sub>3</sub> H)—Met—Gly—Try—Met—Asp—Phe—NH <sub>2</sub>	CCK-OP

**Figure 2. Amino acid comparison of Caerulein, Phyllocaerulein and CCK-OP.** Pyr-Glu is pyroglutamate in which the free amino group of glutamic acid cyclizes to form a lactam.

Subsequent functional studies with purified and synthetic caerulein showed that its biological effects closely resembled that of CCK. Both peptides were powerful stimulants of pancreatic secretion and gallbladder contraction (13). Similar to CCK, these actions required the sulfation of the tyrosine residue (18). In these early in vivo studies, caerulein was about three times as active as CCK purified from pig intestine (8). The early availability of synthetic caerulein, named Caeruletide, for X-ray exam of the gallbladder led to its use in the 1970's for pancreatic function studies in humans (14, 33). Interestingly, cardiovascular effects were minimal at approved dosages suggesting that the prominent effect of skin extracts may have been due to another chemical in skin or usage of a supramaximal dose.

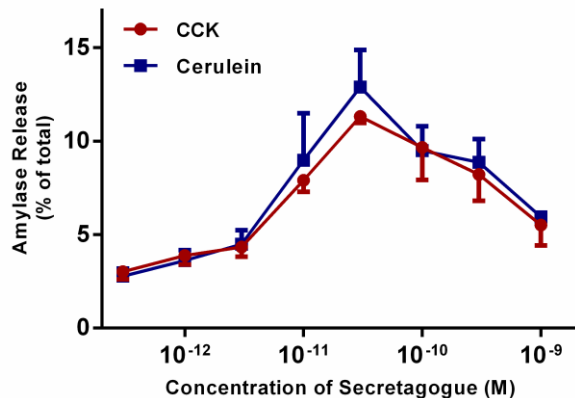
Caerulein precursors were found to be multiply represented in a *Xenopus* skin cDNA library (15, 26). It was concluded that there was a family of precursors from several genes as well as alternative splicing (26). For mature caerulein to be released, multiple processing steps must be involved similar to those involved in the synthesis of GI hormones. In addition to cleaving the precursor into smaller fragments, specific enzymes must process the amino and carboxyl terminals. Ultimately the peptides end up in secretory granules and are released into skin secretions. The physiological role of caerulein and other frog skin peptides is unclear. Most commonly it is speculated to repel predators or to act as an antibacterial agent. How caerulein might have such actions remains unclear.

## 2. Information on Caerulein and the Pancreas

As indicated above, caerulein appears to have a similar potency in vivo to natural porcine CCK-33 and synthetic CCK-8. Similar to CCK, it primarily stimulates enzyme secretion and synergizes with secretin on fluid secretion in dog (23, 30), human (5, 9, 27), pigs (6), sheep (7). In the rat, similar to CCK, caerulein stimulates both a low bicarbonate fluid secretion and robust digestive enzyme secretions (12). In studies of the perfused rat pancreas, caerulein potentiated glucose stimulated insulin secretion and amylase secretion to levels similar to that of CCK-8 (28). In vitro studies have shown that caerulein directly stimulates amylase release from isolated acinar cells and acini of guinea pig, rat and mouse pancreas (20, 29, 31, 35). When compared, the magnitude of secretion was similar although caerulein was about twice as potent (17, 29). Caerulein increased calcium mobilization and did not affect cAMP levels (11,20). A comparison of the ability of caerulein and CCK-8 to stimulate amylase release from isolated mouse pancreatic acini is shown in **Figure 3**. Note the similar bisphasic effect on secretion.

Caerulein appears to exert all of its biological effects on the pancreas through the CCK receptor. Caerulein displaced <sup>125</sup>I-CCK binding from both acini and pancreatic membranes similar to CCK-8 although in some studies caerulein was more potent and in others less potent than CCK-8 (16, 29). In a study of bullfrog brain and pancreas membranes, caerulein was 2-3 fold less potent than CCK-8 (32). Because errors can result from contained water or other impurities affecting the

potency, it is probably best to conclude that caerulein is comparable to CCK-8 in interacting with the CCK receptor and stimulating amylase secretion.



**Figure 3. Comparison of dose-response curve for caerulein and CCK-8 to release amylase from isolated mouse pancreatic acini.** Caerulein was obtained from Sigma and CCK-8 from Research Plus. Both were prepared as 10<sup>-4</sup> M stock solutions in PBS plus 0.1 mg/ml bovine serum albumin accepting the suppliers' statement of the mass of peptide supplied. Frozen aliquots were thawed and diluted into incubation medium.

The major use of caerulein in pancreatic studies over the last 25 years has been to induce a model of experimental pancreatitis. In 1977 Horst Kern and colleagues showed that a continuous iv infusion of supramaximal caerulein into rats (5 µg/kg per hour) induced edematous pancreatitis with a tenfold increase in plasma amylase, and the appearance of large cytoplasmic vacuoles in pancreatic acinar cells (1, 19). A more complete electron microscopic analysis was carried out by Watanabe et al (34). In 1985, Niederau et al showed that repeated hourly ip injections of caerulein would induce a necrotizing pancreatitis in mice (24). Subsequently several thousand papers have appeared using the caerulein model of acute pancreatitis primarily in rodents where most commonly 50 µg/kg is given in repeated doses. Several reports have also appeared using dogs. Studies in mice have been popular because of the availability of genetic models in

this species. Details of the method to induce acute pancreatitis have been presented elsewhere in the Pancreapedia (21). Subsequently, repeated bouts of acute pancreatitis induced by caerulein have also been used to induce chronic pancreatitis (25) as well as a severe form of the disease (36). One can ask why has caerulein been used so exclusively to induce pancreatitis rather than CCK? It may simply have been that caerulein was more available in Europe at the time of the first studies and then other investigators simply followed suite. By contrast, CCK-8 and caerulein are both used in in vitro studies to induce acinar cell damage. However, caerulein probably has an advantage in vivo in that its blocked amino terminal and replacement of Met residues likely results in a longer half-life in the body which could be important especially with ip injections. One piece of data in support of this was the observation by Mossner and colleagues that infusion of caerulein in humans compared to a derivative of CCK-9 without methionine showed a threefold higher plasma level for comparable doses of caerulein when measured with a bioassay (22). These authors concluded that caerulein was more stable against biological degradation than CCK.

### 3. Tools to study caerulein

#### a. Synthetic Peptide

Caerulein can be obtained from multiple sources including Sigma and Research Plus which we have used.

#### b. Antibodies

Because of similarity to the carboxyl terminal of CCK it would be difficult to measure caerulein by RIA although an antibody directed at the c terminal sulfated tyrosine might react with caerulein as well as CCK. It can be measured by bioassay but the measurement would be the sum of CCK and caerulein. An antibody to the carboxy terminal of CCK should localize caerulein in the frog skin.

## 4. Notes

1. This frog is now placed in the genus *Litoria* and is known as *Litoria caerulea*; at one time it was also called *Rana caerulea*.

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