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Tachykinin peptides and receptors: Putting amphibians into perspective

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Abstract

The tachykinins form one of the largest peptide families in nature. In this review, we describe the comparative features of the tachykinin peptides and their receptors, focusing particularly on amphibians. We also summarize our systematic studies of the localization, characteristics, and actions of bufokinin, a toad substance P-related peptide, in its species of origin. In addition, we discuss the establishment of multiple isoforms of the NK_1-like receptor in the toad, and their structure, pharmacology and tissue distributions. We conclude that tachykinin peptides and receptors are well conserved in terms of their structures, physiological functions and coupling mechanisms during tetrapod evolution.

Keywords: Tachykinins; Bufokinin; Tachykinin receptors; Amphibian; Intestine; Cardiovascular system

1. Overview

1.1. Introduction

The tachykinin family is phylogenetically ancient and has been well conserved throughout evolution. Numerous structurally related peptides have been isolated from mammals, birds, reptiles, amphibia and fish [18,38,42,97] as well as from invertebrates [95]. In this review, we give a brief overview of the tachykinin system, including the comparative features of the tachykinin peptides. We then discuss the distribution, action and structure-activity relationship of tachykinin peptides in amphibians, and the pharmacological and molecular characteristics of the amphibian tachykinin receptors. Our recent work in the intestine of the cane toad *Bufo marinus* has uncovered a novel tachykinin peptide, bufokinin, structurally related to substance P (SP) [21], elucidated the physiological actions of bufokinin in its species of origin [72–74] and cloned three isoforms of bufokinin-preferring tachykinin bNK_1 like receptor in the toad brain and intestine [69]. Emphasis is placed on our own studies with bufokinin, and on others' studies with tachykinins in amphibian systems.

1.2. The tachykinin peptides

Tachykinins are a family of closely related peptides that are actively involved in the central and peripheral nervous systems as well as in the cardiovascular and immune systems of both lower and advanced life forms. Tachykinins have been isolated from virtually all animals ranging from invertebrates up to mammals. Many important early advances were made by studying non-mammalian systems including octopus and amphibia, and the great contributions to this field by Erspamer and colleagues are acknowledged [25,97].

In mammals, tachykinins act as neurotransmitters, paracrine or endocrine factors and neuroimmunomodulators and have roles in the nervous system, gastrointestinal tract and cardiovascular system. Important actions include vasodilatation, plasma extravasation, smooth muscle contraction, secretion, neuronal excitation and processing of sensory information; they also have immune and pro-inflammatory actions [36,85]. For many years, SP, neurokinin A (NKA), neurokinin B (NKB) and two elongated versions of NKA, neuropeptide γ (NPγ) and neuropeptide K (NPK) were

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thought to be the only members of the mammalian tachykinin family. This family is now expanded by the recent identification of new tachykinins, hemokinin 1 and endokinins A–D [58,83,116].

Tachykinin peptides are characterized by a conserved carboxy-terminal pentapeptide amide consisting of -Phe-X-Gly-Leu-Met-NH_2, where X represents an aromatic (Phe or Tyr) or hydrophobic (Val or Ile) residue. Most tachykinins are deca- or undecapeptides but their lengths range between 9 and 42 amino acid residues. The amino acid sequences of tachykinins from mammals, birds and reptiles are fairly similar whereas those from amphibians and fish are quite diverse, possibly reflecting their evolutionary position. The amino termini of the tachykinins are variable, but groups of vertebrate tachykinins with structural similarities have been identified as SP-like, NKA-like and NPγ-like (Tables 1–3). Many of these peptides have been isolated using biochemical techniques and thus represent abundant tachykinins. As yet there is no evidence for non-mammalian counterparts of hemokinin or endokinins, but the existence of these and other less abundantly expressed and/or highly localized tachykinins is likely.

Peptides with similarities to SP are widely distributed and are characterized by possession of the N-terminal motif Arg/Lys-Pro-Arg/Lys-Pro-X-Gln (Table 1). SP-like peptides occur in all vertebrates. Interestingly, teleost and elasmobranch SP-related peptides show more identity to mammalian SP than do amphibian SP-related peptides. A Tyr residue at the C-terminal region (corresponding to Phe^8 in SP) appears to be a common feature of all known amphibian SP-related peptides, whereas, most fish SP-like peptides have Phe, Ile or Val at this residue.

The primary structure of NKA has been more strongly conserved under the pressure of evolution than SP (Table 2). NKA from chicken and reptiles is identical to mammalian NKA. Cod and trout NKA are identical and constitute the C-terminal decapeptide of carassin, a goldfish NPγ, though these three species are not closely related phylogenetically [46]. However, *Xenopus* NKA, a duodecapeptide, is unusual in containing three serine residues at the N-terminus [48].

NPγ-related peptides have been isolated from reptiles (python and tortoise), fishes (teleosts, elasmobranches and the bowfin, an ancient ray-finned fish) (Table 3). In goldfish, carassin (together with SP and NKA-like peptides) is the product of the post-translational processing of γ-PPT-A [63,88]. To date, however, NPγ-related peptides have not been detected in amphibian species.

The principal ligand of the NK_3 receptor, NKB, has been isolated from the brain extract of the frog, *Rana ridibunda*, in a molecular form identical to that of mammalian peptide [81]. NKB-related peptides have not been reported in other non-mammalian species, to date.

1.3. Biosynthesis and metabolism of tachykinins

1.3.1. Biosynthesis

Mammalian tachykinins are derived from three preprotachykinin (PPT) genes: the PPT-A (now renamed TAC1) gene that contains four preprotachykinins (α-, β-, γ-, δ-PPT-A) encoding the sequence of SP, NKA and two elongated versions of NKA, neuropeptide γ and neuropeptide K, the PPT-B (TAC3) gene encoding the sequence of NKB [85], and the recently identified PPT-C (TAC4) gene encoding newly described tachykinins, hemokinin 1 and endokinins A–D [58,83,116].

Details on the biosynthesis of amphibian tachykinins remain unknown. However, the γ-PPT gene containing the sequence of SP, NKA and carassin (goldfish NPγ) has been identified in goldfish brain and periphery [63,88]. In the invertebrate, *Drosophila melanogaster*, a tachykinin precursor with sequence homology to mammalian PPT-A gene has

Table 1
A comparison of the primary structures of peptides related to substance P from different vertebrate species

Substance P and related peptides	Sequence	Reference
Mammals	R P K P Q Q F F G L M	[16]
Chicken, *Gallus gallus domesticus*	– – R – – – – – – –	[20]
Alligator, *Alligator mississipiensis*	– – R – – – – – – – –	[107]
Python, *Python molurus*	– – R – – – – Y – – –	[19]
Tortoise, *Gopherus agassizii*	– – R – – – – Y – – –	[108]
Frog, *R. ridibunda* (ranakinin)	K – N – E R – Y – – –	[81]
Frog, *R. catesbeiana* (ranatachykinin A)	K – S – D R – Y – – –	[57]
Toad, *B. marinus* (bufokinin)	**K – R – D – – Y – – –**	[21]
African frog, *X. laevis*	K – R – D – – Y – – –	[48]
Amphiuma tridactylum	D N – S V G – – Y – – –	[110]
Lungfish, *N. forsteri*	K – R – D – – Y – – –	[68]
Goldfish, *Carassius auratus*	K – R – H – – I – – –	[63]
Cod, *Gadus morhua*	K – R – – – – I – – –	[46]
Trout, *Oncorhynchus mykiss*	K – R – H – – – – – –	[46]
Sturgeon, *Scaphirhynchus albus*	K – – – H – – – – – –	[109]
Dogfish, *Scyliorhinus canicula*	K – R – G – – – – – –	[113]
Sea lamprey, *Petromyzon marinus*	R K – H – K E – V – – –	[112]
River lamprey, *Lampetra fluviatilis*	R K – H – K E – V – – –	[110]

–, represents residue identity. The C-terminal methionine residues are amidated.