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Research report

Teneurin proteins possess a carboxy terminal sequence with neuromodulatory activity

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Abstract

We have previously shown that a bioactive neuropeptide-like sequence is present at the carboxy-terminus of the teneurin transmembrane proteins. We have subsequently called this peptide 'teneurin C-terminal associated peptide' (TCAP). The sequence encodes a peptide 40 or 41 amino acids long flanked by a cleavage motif on the amino terminus and an amidation motif on the carboxy terminus, characteristic of bioactive peptides. This sequence is highly conserved in all vertebrates. A TCAP-like sequence is encoded by each of the four teneurin genes. We have therefore examined the neurological role TCAP-1 may play in mice and rats. In situ hybridization studies showed that the teneurin-1 mRNA containing the TCAP-1 sequence is expressed in regions of the forebrain and limbic system regulating stress and anxiety. A synthetic version of amidated mouse/rat TCAP-1 was prepared by solid-phase synthesis and used to investigate the in vitro and in vivo activity. TCAP-1 induces a dose-dependent change in cAMP accumulation and MTT activity in immortalized mouse neurons. Administration of synthetic TCAP-1 into the basolateral amygdala significantly increases the acoustic startle response in low-anxiety rats and decreases the response in high-anxiety animals in a dose-dependent manner. When 30 pmol TCAP-1 is administered into the lateral ventricles each day for 5 days, the sensitization of the rats to the acoustic startle response is abolished. These data indicate that TCAP may possess functions that are independent of the teneurin proprotein and together, the teneurins and TCAP, may represent a novel system to regulate neuronal function and emotionality.

Theme: Neurotransmitters, modulators, transporters and receptors
Topic: Peptides: anatomy and physiology

Keywords: Stress; Anxiety; Neuropeptide; Acoustic startle; Evolution; Cell proliferation

1. Introduction

The teneurin (ten-m, odz) genes were originally discovered in *Drosophila* [3,24] and encode a large transmembrane glycoprotein. The ten-m gene is initially activated during the blastoderm stage, and subsequently down-regulated, before being expressed at later stages. The highest levels of ten-m occur in the central nervous system where the protein occurs preferentially on the surface of axons [24,25]. Mutations of the ten-m gene result in embryonic lethality [3,24]. The vertebrate homologues of

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ten-m, the teneurins, are about 2800 amino acids long [32,33,36]. In vertebrates, the teneurin proteins display their C-terminals on the extracellular face of the plasma membrane as a type II transmembrane protein [12,36]. However, the orientation of Ten-m protein in *Drosophila* is not as clear, and some authors suggest it may exist in the opposite polar orientation as a type I transmembrane protein [10].

Currently, the functional roles of the teneurin proteins are not well understood, although a number of studies point to a role in synaptic plasticity and the neuronal stress response. The protein possesses a number of functional domains suggesting that it interacts with elements of the extracellular matrix on the extracellular face and interacts with calcium and SH3-mediated signaling mechanisms on the intracellular component [32,33,36]. Previous studies with teneurins have indicated that the protein may play a role with neurogenesis and neurite outgrowth. Overexpression of teneurin-2 into the mouse neuroblastoma cells (Nb2a) augments the amount of neurite outgrowth and enlarges the growth cones [44]. The number of filamentous actin-containing filopodia are enhanced in the teneurin-2 over-expressing cells [33]. The N-terminal portion of teneurin-2 can be cleaved and translocated to the nucleus where it acts as a transcriptional cofactor [2]. Expression of GADD153 (CHOP), which can occur during periods of cellular stress, leads to an up-regulation of teneurin-4 [59]. Moreover, human teneurin maps to position Xq25 of the X chromosome [4], a region associated with X-linked mental retardation syndromes [15,18,28,40], suggesting that the gene is linked to neurological functioning.

We have previously reported that the carboxy terminal region of the rainbow trout teneurin-3 possesses a peptide-like sequence that is encoded over a 132-base-pair sequence immediately upstream from the stop codon [41]. We have called this sequence 'teneurin C-terminal associated peptide' (TCAP). A synthetic version of the terminal peptide region of the rainbow trout teneurin-3 paralogue can modulate cAMP concentrations and proliferation in mouse hypothalamic cell lines. Moreover, this synthetic peptide has the capability of regulating the teneurin protein in a dose-dependent manner. Our finding of a bioactive peptide on the carboxy terminus of the teneurins is consistent with the orientation of the protein as a type II transmembrane protein.

The aim of the present study is to establish the bioactivity of one of the TCAP peptides (TCAP-1) in a rat/mouse model in order to take advantage of a number of recently developed immortalized neuronal lines (Gn11, N38) and to corroborate the neuroanatomical distribution of the peptide gene with behavioral models. We now show that the mouse TCAP-1 sequence is also highly effective at modulating cAMP and proliferation in vitro, and when injected directly into the brain can modulate emotionality and anxiety in rats. The exon encoding this region of the teneurins is expressed throughout the limbic region suggesting that the teneurins play a role in regulating behavior in rats.

2. Materials and methods

2.1. Identification and synthesis of mouse TCAP-1

The sequence of the mouse TCAP-1 was determined by examining the carboxy terminal exon region of mouse teneurin-1 (accession number: NM011855). A mouse paralogue of the putative peptide sequence from teneurin-1 was synthesized on an automated peptide synthesizer, Model Novayn Crystal (NovaBiochem, UK Ltd., Nottingham, UK) on PEG-PS resin using continuous flow Fmoc chemistry (Calbiochem-Novabiochem Group, San Diego, CA). Eight times excess diisopropyl ethyl amine (Sigma-Aldrich Canada Ltd.) and four times excess Fmoc-amino acid activated with HATU (*O*-(7-azabenzotriazol)-1-3, 3-tetramethyluronium hexfluorophosphate; Applied Biosystems, Foster City, CA) at a 1:1 (mol/mol) ratio were used during the coupling reaction. The reaction time was 1 h. A solution of 20% piperidine (Sigma-Aldrich Canada Ltd.) in *N*,*N*-dimethylformide (DMF; Caledon Laboratories Ltd., Canada) was used for the deprotection step in the synthesis cycle. The DMF was purified in-house and used fresh each time as a solvent for the synthesis. The cleavage/deprotection of the final peptide was carried out with trifluoroacetic acid (TFA), thioanisole, 1,2 ethandithiol, m-cresole, triisopropylsilane, and bromotrimethyl silane (Sigma-Aldrich Canada Ltd.) at a ratio of 40:10:5:1:1:5. Finally, it was desalted on a Sephadex G-10 column using aqueous 0.1% TFA solution and lyophilized.

2.2. Synthetic peptide characterization

2.2.1. High-performance liquid chromatography (HPLC)

Confirmation of the homogeneity of the synthetic peptide was determined by reverse-phase HPLC. A Beckman model 126 HPLC System Gold (Beckman, Palo Alto, CA), attached to a UV detector module 168 and C_{18} column (3.5 μm particle size; Waters, Inc.) was used to purify the TCAP peptide. A single injection (50 μl) was applied to the column through a 100-μl injection loop and carried to the column at a flow rate of 0.1 ml/min using a dual solvent system (A: 0.05% TFA; B: 80% acetonitrile, 0.05% TFA). The mobile phase B was increased from 0% to 60% over 80 min.

2.2.2. Mass spectrometry (MS)

All samples were dissolved in 5 μl of 1:1 (vol/vol) acetonitrile–water [plus 0.1% (vol/vol) formic acid]. Aliquots of 2–3 μl of each sample were loaded on a glass capillary probe tip and analyzed on a Micromass Q-TOF (hybrid quadruple time of flight) mass spectrometer (Micromass, Manchester, UK). All spectra were acquired under