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Title: Molecular cloning and functional characterization of a type-I neurotensin receptor (NTR) and a novel NTR from the bullfrog brain

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Abstract: Neurotensin (NT) is a tridecapeptide that functions as a neurotransmitter and neuromodulator in the nervous system. To date, three different types of NT receptor (NTR), NTR1, NTR2 and NTR3, have been identified only in mammalian species. In the present study we isolated the cDNAs for an NTR1 and a novel NTR in the bullfrog brain, designated bfNTR1 and bfNTR4 respectively. bfNTR1 and bfNTR4 encode 422- and 399-amino acid residue proteins respectively. bfNTR1 has a 64% amino acid identity with mammalian NTR1, and 34-37% identity with mammalian NTR2. bfNTR4 exhibits 43% and 45-47% identity with mammalian NTR1 and NTR2 respectively. Both receptors are mainly expressed in the brain and pituitary. bfNTR1 triggers both CRE-luc, a protein kinase A (PKA)-specific reporter, and c-fos-luc, a PKC-specific reporter, activities, indicating that bfNTR1 can activate PKA- and PKC-linked signaling pathways. However, bfNTR4 appears to be preferentially coupled to the PKA-linked pathway as it induces a higher CRE-luc activity than c-fos-luc activity. bfNTRs exhibit different pharmacological properties as compared with mammalian NTRs. Mammalian NTR1 but not NTR2 responds to NT, whereas both bfNTR1 and bfNTR4 show a high sensitivity to NT. SR 48692 and SR 142948A, antagonists for mammalian NTR1 but agonists for mammalian NTR2, function as antagonists for both bfNTR1 and bfNTR4. In conclusion, this report provides the first molecular, pharmacological and functional characterization of two NTRs in a non-mammalian vertebrate. These data should help to elucidate the phylogenetic history of the IS protein-coupled NTRs in the vertebrate lineage as well as the structural features that determine their pharmacological properties.

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