Molecular cloning and expression of the precursor gene of a D-Trp-containing neuropeptide, NdWFamide, in the ganglia of *Aplysia kurodai.*

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NdWFamide, a cardio-excitatory neuropeptide that contains D-triptophan, is identified in several gastropod molluscs such as *Aplysia kurodai* and *Lymanea stagnalis*. We have cloned two cDNAs that encode different precursor proteins of NdWFamide from cDNA library derived from the central nervous system of *A. kurodai*. Both of the predicted precursors included a single copy of NWFGKR sequence just behind the N-terminal signal peptide. One of them (NWF90) consisted of 90 amino acids, and was identical to the one recorded in the EST database (EY423228). The other precursor consisted of 87 amino acids (NWF87) and had 80% similarity to NWF90 in the amino acids sequence. By whole-mount *in situ* hybridization (ISH) with DIG-labeled cRNA probes specific for the respective precursor, we found that NWF90 was expressed in many neurons in right-upper quadrant of the abdominal ganglia, while NWF87 was expressed in the very limited numbers of neurons in the same region. By contrast, in buccal, cerebral and pleural ganglia, quite a few neurons preferentially expressed NWF90. Sequential ISH and immuno-staining on the same preparation suggested the co-existence of precursor protein and matured NdWFamide peptide. These results indicate that, of the two precursor proteins, NWF90 is the major precursor for NdWFamide.