Real-time Q-PCR on tyrosine hydroxylase immunocytochemically identified neurons of the human supraoptic nucleus isolated from cryostat sections using laser microdissection: co-expression with GTP cyclohydrolase I

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Our previous studies indicated that tyrosine hydroxylase (TH) -the first and rate-limiting enzyme for catecholamine synthesis - is immunocytochemically (ICC) identified within human neurosecretory cells and co-localizes with vasopressin (AVP) or oxytocin (OXY). Its expression appears to be related to neuronal activation, since increased number of TH immunoreactive (IR) neurons were observed in subjects that suffered from somatic illnesses leading to prolonged osmotic or nonosmotic stimulation of AVP release. GTP cyclohydrolase I (GCH1), however, –the first and limiting enzyme for the synthesis of tetrahydrobiopterin (BH4), cofactor of TH- has not yet been ICC identified in magnocellular neurons of human hypothalamus, making the functional role of TH still questionable. Our purpose was to investigate if GCH1mRNA is co-expressed with THmRNA in AVP synthesizing neurons of human supraoptic (SON) nucleus by using real-time Q-PCR.
We isolated total RNA from TH ICC identified neurons extracted by laser microdissection from 10um frozen sections of the SON of four control subjects with postmortem delay 4-7 hours. We studied the possible co-expression of AVPmRNA and THmRNA with GCH1mRNA using real-time Q-PCR, without prior RNA amplification. Two highly expressed genes -Elongation Factor 1a (EIF1a) and Cytochrome c oxidase (Cox 1)- were also studied in parallel as controls.
Our results showed that GCH1 is co-expressed with TH in AVP synthesizing neurosecretory cells in three out of four cases studied. The case with negative signal for GCH1 had very limited TH-IR neurons in the SON and showed the highest cycle threshold for TH detection. The expression of GCH1mRNA within TH neurons of the SON indicates that TH is functional and might be involved in catecholamine synthesis in AVP synthesizing neurons of the human hypothalamus.
Brain material was obtained from the Netherlands Brain Bank (coordinator R.Ravid)
Supported by Nuffic (Huygens Fellowship 8/2002-5/2003) granted to K.D.

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Speaker at the wednesday NVE teaching session 17