1 Abstract

Tissue-type plasminogen activator (*tPA*) is a serine protease which catalyzes the conversion of plasminogen to plasmin within the fibrinolytic pathway to aid with blood clot dissolution. As a protease it is also necessary for processes in development (e. g. embryogenesis) and in cellular migration such as tumor outgrowth and metastasis. Recent studies have implicated *tPA* in neuronal plasticity, synaptic remodelling and neuronal degeneration. *TPA* is widely expressed in the CNS and neuronal aktivity induces the expression of mRNA of *tPA* in pyramidal neurons. Transgenic mice overexpressing *tPA* show an increased and prolonged hippocampal long-term potentiation (LTP) and improved performance in spatial orientation learning tasks while *tPA*-/- mutants exhibit learning deficits, retarded neuronal migration and resistance towards neuronal destruction by injections of excitotoxins. In MS brain *tPA* is thought to be responsible for the final enzyme-mediated process of demyelination. A better untestanding of the role of *tPA* in cognitive processes requires detailed examination of its regulation. No *in vivo* data and only few *in vitro* data could be gained regarding the control of neuronal expression of *tPA* until the beginning of this thesis..

In neuronal cells (SY5Y, SK-N-SH) neurotrophins induced the expression of mRNA of *tPA* in a dose and time dependent manner as shown by Ribonuclease Protection Assays (RPA) and Transactivation Assays. At 2.4 to 2.5 EC_{50} NGF (100 ng/ml) stimulated *tPA* expression 4fold after 48 h whereas BDNF (5 ng/ml) and NT-4 (25 ng/ml) stimulatd *tPA* expression ~5fold after 4 h. NT-3 (5 ng/ml) showed no effect at all. Neither of these effects was based on enhanced mRNA stability nor dependent of *de novo* protein synthesis. *In vivo* genomic analysis of the whole *tPA* gene in neuronal tissue (Kelly, SNB-19, SK-N-SH) exhibited DNaseI HS sites in the promotor region, 1st and 3rd intron. This promotor region was of further interest for this thesis. DNaseI HS sites spanning a region of ~3.6 kb in the promotor were investigated by *in vivo* footprinting and revealed 46 protected responsive sites towards 28 known transcription factors and 27 protected DNA elements of unknown protein factors (all based on matrix and core similarity >90%). Many of these factors are known to play roles in processes of development. One NF-κB responsive element at ~3.1 kb upstream of the transcription start site was further investigated. It bears an almost perfect consensus sequence for the NF-κB/Rel family of protein factors (91-100 % similarity) and shows enhancer properties in Transactivation Assays. Cloning into reporter-gene-vectors and site directed mutagenesis identified this element as being responsible for a very strong response to phorbolester (PMA) of 11-13 fold overnight in neuronal cells.