Activating transcription factor 4(ATF4)-dependent activation of the human tryptophan hydroxylase 2 gene is mediated through binding to a CCAAT-enhancer-binding protein (CEBP)-ATF composite site in its promoter

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Tryptophan hydroxylase 2(TPH2) plays a critical role in the regulation of 5-HT neurotransmission and is thus a promising therapeutic target for the treatment of neuropsychiatric disorders. Activating transcription factor 4 (ATF4) has been implicated in various neural functions. ATF4 can form a homodimer, and heterodimers with CCAAT-enhancer-binding proteins (CEBPs). ATF4-CEBP heterodimers bind to DNA sequences called CEBP-ATF composite site to regulate target gene expression. Bioinformatics analysis revealed one potential CEBP-ATF composite site near the transcription start site of the hTPH2 gene. In this study, we examined how the hTPH2 promoter activity changes by ATF4 and CEBPs. Promoter activities were assessed by transfections of reporter plasmids containing a 2-kb of the hTPH2 promoter into RN46A cells. Overexpression studies demonstrated that ATF4-mediated activation of the hTPH2 promoter was further enhanced by co-expression of each of the five CEBPs including CEBPG which lacks all known activation domains. The CEBP-ATF composite site mutations negated the effects of ATF4. A dominant negative ATF4 blocked the effects of ATF4. Functional analysis of N-terminal and internal deletion mutants indicated that ATF4 (aa 1-124) is critical for activation. Moreover, co-expression of endogenous inhibitor proteins, Trib3 or TXLNG attenuated the effects of ATF4. Altogether, these results imply that ATF4 plays a pivotal role in regulating the hTPH2 gene expression, and itself undergoes complex regulation at multiple levels.