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A proximal E-box modulates NGF effects on rat PPT-A promoter activity in cultured dorsal root ganglia neurones

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Abstract

The rat preprotachykinin A (rtPPTA) promoter fragment spanning −865 + 92, relative to the major transcriptional start, has previously been demonstrated to be nerve growth factor (NGF) responsive in primary cultures of rat dorsal root ganglion (DRG) neurones [Harrison, P.T., Dalziel, R.G., Ditchfield, N.A., Quinn, J.P., 1999. Neuronal-specific and nerve growth factor-inducible expression directed by the preprotachykinin-A promoter delivered by an adeno-associated virus vector. Neuroscience 94, 997–1003]. In this communication, we demonstrate that an E box element at −60, in part, regulates the activity of this rtPPT-A promoter fragment in DRG neurones in response to NGF. Differential regulation of the promoter is observed in the presence or absence of NGF when the E Box site is present. Under basal conditions binding of proteins to this −60 element may antagonise promoter activity. Hence, in the absence of NGF, mutation of the −60 E box increased reporter gene expression. Further, comparison of levels of reporter gene expression supported by both WT and mutated promoter indicate that in the presence of NGF the −60 E box element also plays a role as an activator domain. This represents a novel mechanism for NGF regulation of rtPPT-A. Similarly, an important role for this signalling pathway was observed in neonate rat DRG neuronal cultures, which require NGF for their survival, namely mutation of the −60 element resulted in higher levels of reporter gene expression.

Keywords: Tachykinins; bHLH; Transcriptional regulation; E box; Repressor; NGF; Transcription factor

1. Introduction

The rat preprotachykinin-A gene (rtPPT-A) encodes the neuropeptides substance P (SP), neurokinin A, neuropeptide K and neuropeptide γ which are derived by alternative splicing of primary RNA transcripts and post-translational processing of the peptide precursors (Carter and Krause, 1990; Bannon et al., 1992). The tachykinins display a significant degree of plasticity in expression levels in vitro and in vivo to various stimuli, e.g., NGF has been shown to upregulate rtPPT-A gene expression and SP concentration, in cultured dorsal root ganglion (DRG) neurones (Lindsay and Harmar, 1989; Vedder et al., 1993; Mulderry, 1994; Jiang and Smith, 1995), and in vivo (Leslie et al., 1995; Amann et al., 1996). In addition, the concentration of SP decreases

Abbreviations: AAV, adeno-associated virus; rAAV, recombinant adeno-associated virus; AP1, activator protein 1; bHLH, basic helix–loop–helix; DRG, dorsal root ganglion; Luc, luciferase; NGF, nerve growth factor; rtPPT-A, rat preprotachykinin-A; NK1, neurokinin receptor 1; USF1, upstream stimulatory factor 1.

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after peripheral nerve transection or injury (Noguchi et al., 1988; Henken et al., 1990; Marchand et al., 1994; Noguchi et al., 1995; Ma and Bisby, 1998). This suggests that the synthesis of SP is downregulated by depletion of NGF, which is caused by the blockade of axonal flow in the peripheral branches of sensory neurones.

The rtPPT-A promoter contains a number of binding sites for transcription factors (Mendelson et al., 1995; Mendelson and Quinn, 1995; Paterson et al., 1995a,c; Fiskerstrand and Quinn, 1996; Quinn, 1996) including those that are induced in response to NGF in a PC12 cell line model such as AP1, CREB, Octamer binding proteins and the bHLH family of proteins (Curran and Morgan, 1985; Curran and Franza, 1988; Quinn, 1991; Mendelson et al., 1992; Wood et al., 1992; Ginty et al., 1994) (Fig. 1).

This study set out to investigate the role of an E box motif (CACGTG) between −60 and −55 base pairs of the proximal rtPPT-A promoter (relative to the major transcriptional start site), hereafter referred to as the −60 element. Previous work addressing the function of this site demonstrated a potential major role for this element in the transcriptional regulation of the rtPPT-A promoter. When this sequence is linked to a heterologous promoter, it has been shown to drive high levels of reporter gene expression in both PC12 and HeLa cell lines and in rat DRG neurones (Paterson et al., 1995a,b; Fiskerstrand and Quinn, 1996). NGF in PC12 cells increased reporter gene expression supported by this E box construct but this induction of reporter gene expression was not observed in microinjected DRG neurones (Paterson et al., 1995b), the latter possibly because of microinjection stress.

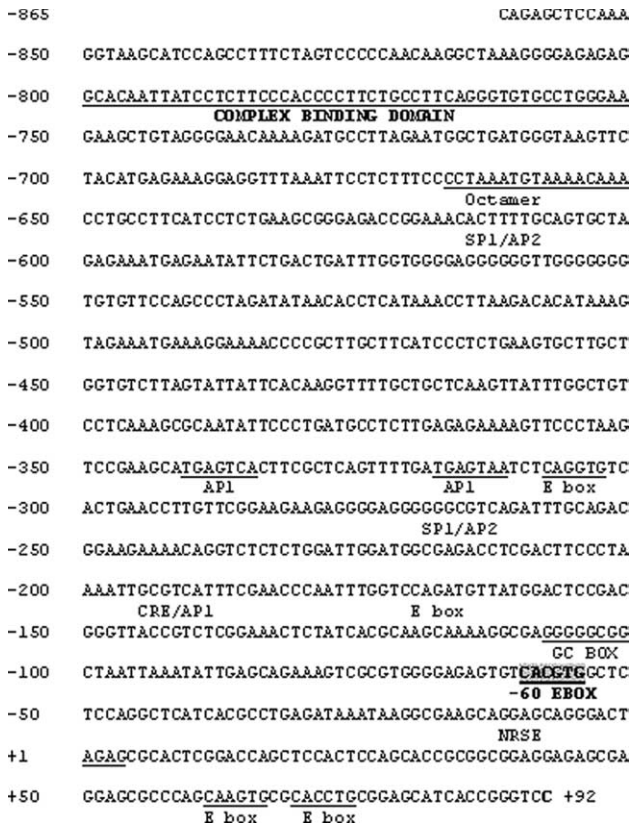

Fig. 1. Sequence of the rat proximal PPTA promoter fragment spanning −865 + 92 base pairs. The positions of transcription factor binding sites are shown as underlined. The proximal rtPPT-A promoter contains a number of transcription factor binding sites including E boxes, an NRSE, CRE or AP1 binding elements, AP2/SP1 binding elements, octamer binding elements and a complex binding element that can bind a number of single and double stranded transcription factors. The E box located between −60 and −55 is shown highlighted in grey.

Our previous analysis of reporter gene constructs in DRG indicated that NGF regulation of the proximal promoter, spanning −865 + 92, could be demonstrated when introduced to the neurone as an AAV vector (Harrison et al., 1999). In contrast, microinjection into primary culture DRG neurones showed that NGF did not influence rtPPT-A promoter activity (Mulderry et al., 1993; Fiskerstrand and Quinn, 1996). We therefore addressed the role of the proximal bHLH binding site at −60 using rAAV that contained a mutant version of the −60 element within the rtPPT-A promoter fragment −865 to +92, driving the luciferase reporter gene.

2. Methods and materials

2.1. Generation of a −60 bHLH mutant in a promoter fragment spanning −865 to +92 of the rtPPT-A promoter

Plasmids containing promoter fragments −865 to +92 and −47 to +92 were as described previously (Harrison et al., 1999). Plasmid pSub201-60mut-LUC was generated as follows. The rtPPT-A promoter fragment spanning base pairs −865 to +92 that contains a mutation at base pair −60 (E box sited disrupted by the insertion of an oligonucleotide containing a *Bam*HI site) was removed from plasmid p22mut (Paterson et al., 1995a) by digestion with *Sal*I and *Hin*dIII. This fragment was ligated to the *Xho*I–*Hin*dIII digested backbone of pGL3basic (Promega) to generate plasmid pGL3-60mut-LUC. The *Mlu*I–*Hin*dIII 2.9 kb fragment from pGL3-60mut-LUC containing the rtPPT-A promoter and luciferase reporter gene was inserted into the *Mlu*I–*Xho*I digested pSnaB1stuffer backbone. This resulting plasmid, termed pSnaB1-60mut-LUC was then digested with *Nhe*I and the 4.65-kb fragment ligated to pSub201-*Xba*I backbone to generate pSub201-60mut-LUC.

2.2. Generation of rAAV using pXX6-80 as helper

A three-plasmid system was used to generate rAAV vectors by transfection of all plasmids into human 293