

Sequence analysis of the promoter region of the rat vasopressin gene

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The vasopressin gene is highly transcribed in magnocellular neurons of the supraoptic (SON) and paraventricular nucleus (PVN) in the rat hypothalamus. In order to identify *cis*-acting elements involved in the expression of the vasopressin gene, approximately 1 kb upstream of the transcription start site has been sequenced. Several putative regulatory elements have been detected, including a glucocorticoid response element (GRE), a cAMP response element (CRE), and four AP2 binding sites. In gel shift assays performed with a labelled DNA fragment corresponding to nucleotide residues –214 to –36 and nuclear proteins extracted from SON-derived tissue enriched in magnocellular neurons, three specific protein-DNA complexes have been detected. Complex formation is effectively competed by addition of an excess of unlabelled fragment.

Vasopressin gene expression; Glucocorticoid response element; Binding site, AP2; cyclic AMP response element; Gel shift assay; Nuclear protein fraction

1. INTRODUCTION

To date little is known about the molecular events governing the expression of the gene encoding the precursor for the peptide hormone vasopressin. In the rat and human genome, the vasopressin gene is linked to the closely related oxytocin gene [1,2]. Yet the two genes are transcribed in different subtypes of magnocellular neurons of the supraoptic (SON) and paraventricular nucleus (PVN) of the hypothalamus [3]. Although both hormones control rather different physiological functions in the organism, e.g. water retention in the kidney (vasopressin) and smooth muscle contraction in the uterus (oxytocin), there are several reports that demonstrate a concomitant upregulation in the expression of the vasopressin and oxytocin genes under various physiological conditions. For instance prolonged osmotic stimulation of rats results not only in a 2–3-fold increase of vasopressin message [4], but also of oxytocin-encoding mRNA in magnocellular neurons [5]. A similar rise in vasopressin- and oxytocin-encoding transcripts has also been shown to occur during late gestation and lactation [6]. On the other hand, after systemic administration of vasopressin, a reduction in the two mRNA species has been observed in rat hypothalami [7].

To gain an initial insight into the mechanisms controlling the expression of the vasopressin gene, a DNA

segment upstream of the transcriptional start site of the vasopressin gene has been sequenced and analyzed for regulatory elements.

2. MATERIALS AND METHODS

2.1. Sequence analysis and preparation of nuclear extracts

Nucleotide sequence was determined as reported [3]. Where specified male Wistar rats were treated with 2% saline for seven days. SON were dissected from about 1.5 mm thick brain slices and collected in ice-cold PBS (150 mM NaCl, 10 mM sodium phosphate, pH 7.2). Tissue pools from 5 animals were frozen in liquid nitrogen after removal of PBS. Nuclei from SON tissue or from cerebellum were prepared as published [8]. Nuclear proteins were extracted [9] and their protein concentration determined [10]. Aliquots of nuclear protein were frozen in liquid nitrogen and stored at –80°C.

2.2. Mobility shift assay

Binding reactions contained 3.5 µg nuclear protein, 3 µg poly (dJdC), 20 ng sonicated *E. coli* DNA in 10 mM Hepes, pH 7.9, 10% glycerol, 50 mM KCl, 0.25 mM PMSF, 5 mM DTT, 1 mM EDTA. Prior to the addition of labelled DNA fragment (2.5 fmol/reaction), binding reactions were preincubated for 15 min at 4°C. Labelled DNA and if included, unlabelled competitor DNAs (see legend to fig.3) were added simultaneously. The mixture was incubated at 4°C for 30 min and immediately loaded on 4% prerun acrylamide gels (acrylamide:bisacrylamide 80:1). Electrophoresis was performed for 2 h (20 mA) at room temperature in 6.7 mM Tris-HCl, pH 7.5, 3.3 mM sodium acetate, 1 mM EDTA, with constant buffer circulation. Gels were dried and exposed to X-ray film at –80°C for 15–24 h.

2.3. Labelling of DNA fragments

377 bp (–751 to –374) and 338 bp (–374 to –36) *Pst*I DNA fragments derived from the 5' region of the rat vasopressin gene were subcloned individually into the *Pst*I site of pUC9. DNA fragments were labelled at the unique *Sal*I- or *Hind*III-site of the vector by filling in the 5' cohesive ends with [α -³²P]dCTP (sp. act. 3000 Ci/mmol) and Klenow polymerase according to standard procedures [11]. Subsequently, DNAs were recut with *Aha*II (–561) and *Bal*I (–214) re-

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The nucleotide sequence(s) presented here has (have) been submitted to the EMBL/GenBank database under the accession number no. Y0531

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GATCAAGAGTTCAATGCCAGCTTTCTGCTATGTAGTAAGGTC AAGGTCAGCCTGGACTAAACGACTGCCTTAGAAACAAACAAATGACTTACCGTCTA -901
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TTCAGTCCCTTGTGTGAAACGAAAGAGTCTGCACACAGACAGACAGACCCCGAGGAGGGTAAAGGAGAGGATTTGTTGATAGTGAAGGTTAGGACGGAATCTA

CTGAGATAGTACCAGCCTCAGGGCATGGGGTCTCCCATAGCTTTTCTCTGTCAGTACTGTGGGCTCACCTAGGACTGTTTCTGAACTATATCCTACCCT -701
GACTCTATCATGGTCGGAGTCCCGTACCCAGAGGGGTATCGAAAAGGAGACGTCATGACACCCGAGTGGATCCTGACAAAGACTTGATATAGGATGGGA

AGCTCTCTACCCTAGAAGGCCTGAAAACACAGAAAATTTCTCTGCTCTGCTTTTCCAATGGCTGGGGTTAAAAGCATGTGTCACAACCTGTCCCTTTTATT -601
TCGAGAGATGGGATCTTCGGACTTTGAGTGTCTTTAAGAGGACGGAGACGAAAGTTACCGACCCCAATTTTCGTACACAGTGTTCACAGGAAAAATAA

CTTTTAATATCGAGACAGGGTCTCACCAAGTTGCCCAAGACGCCACCCACCTGGGACAGGGCAGGCCTTTGGCTCTATGTTTCACTTCTGACTCCATG -501
GAAAATTATAGTCTGTCCAGAGTGGTTCAACGGGGTCTGCGGTGCGTGTGGACCTGTCCCGTCCGGAACCGAGATACAAGTCAGAACTGAGGTAC

ACTGTGGCCGCTAGCCCATGAGGCTGCGCGTGGGAATTTCTTCTGAAAGCTCACCTGGTATCGATGCTTCTCTTATCTACACCACAACAAACAAACC -401
TGACACCGCGGATCGGGTACTCCGACCGCACCCCTAAAGGAAGACTTTCGAGTGGACCATAGCTACGAAGGAGAATAGGATGTGGTGTGATTGTTTGG

TGCCCCACCTCCTGGTCTGACCCCTGCTGCAGACCTGCTAGTCTTGGTGAATGAGACCTGGGGACCCCTCTAGTCTGTTGAGAGCTGTGAAATGTCTA -301
ACGGGGTGGAGGACCAGGACTGGGACGACGCTGGACGATCAGGAACCACTACTCTGGACCCCTGGGGAGATCAGACAACCTCAGCAGCTTACGAGT

ACTATGATTTCCAGGTGACCCCTCAAGTCGGCTCACCTCCCTGATTGCACAGCACCAATCACTGTGGCGGTGGCTCCCGTCCACCGTGGCCAGTGACAGC -201
TGATAAAAAGTCCACTGGGAGTTTCAGCCGAGTGGAGGGGACTAACGCTGCTGTTAGTGTGACACCGCCACCGAGGGCAGTGTGCCACCGGTCACTGTGC

CTGATGGTGGTCCCTCCTCCACCACCTTGCATTGACAGGCCACGTTGTCCTCCAGATGCCTGAATCACTGCTGACAGCTTGGGACCTGTCAGCT -101
GACTACCAGCCGAGGGGAGGAGTGGTGGGAGACGTAACGTGTCGGGTGCACACAGGGGTCTACGGACTTAGTGACGACTGTGCAACCTGGACAGTCTCGA

GTGGGCTCCTGGGGAGCCACTGGGGAGGGGGTTAGCAGCCACGCTGTCGCTCCTAGCCAACACCTGCAGACATAAATAGACAGCCAGCCGCTCAGGC -1
CACCCGAGGACCCCTCGGTGACCCCTCCCCAATCGTCGGTGGCAGCGGAGGATCGGTTGTGGACGCTCTGATTTATCTGTGGGTCCGGCGAGTCCG
    
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* MET +34
 AGCAGAGCAGAGCTGCACGCAGTGCCACCTATG
 TCGTCTCGTCTGCACGTGCGTCAACGGGTGGATAC

B

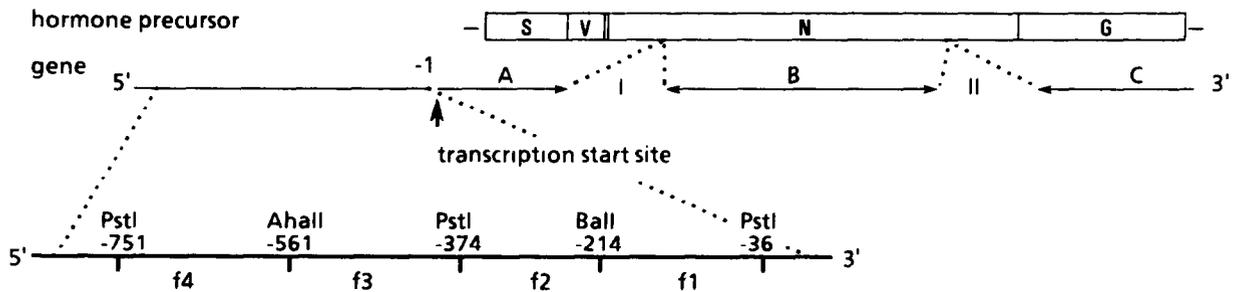


Fig.1. (A) Nucleotide sequence of the 5' region of the rat vasopressin gene. The modified TATA box is underlined. Putative binding sites for glucocorticoid receptor (GR), CCAAT-box binding proteins, cAMP response element binding protein (CREB) and AP2 are indicated by arrows. The protein coding regions and the intron sequences of the vasopressin gene are not shown. The asterisk marks the transcriptional start point. (B) Schematic representation of the rat vasopressin gene, the deduced hormone precursor structure and the derived DNA fragments (f1 to f4) used in mobility shift assays. The numbers indicate the position relative to the transcriptional start point (+1). S, signal peptide; V, vasopressin; N, neurophysin; G, glycopeptide; A, B, C, indicate the three exons of the vasopressin gene, I and II, the two introns.

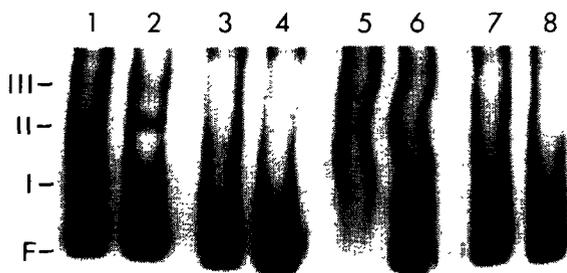


Fig.2. Mobility shift assay. Nuclear protein fraction extracted from rat cerebellum (lanes 1,3,5,7) or SON tissue (lanes 2,4,6,8) was incubated with 2.5 fmol each of ³²P-labelled DNA derived from the 5' region of the rat vasopressin gene. Lanes 1,2: fragment 1 (-214 to -36); lanes 3,4: fragment 2 (-374 to -214); lanes 5,6: fragment 3 (-561 to -374); lanes 7,8: fragment 4 (-751 to -561). F, unbound probe; I, II, III, bound probe.

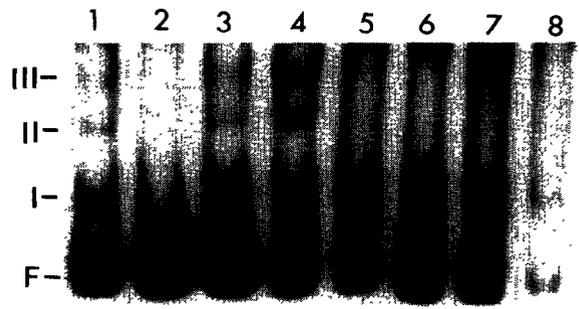


Fig.3. Mobility shift assay. Nuclear protein fraction extracted from rat SON tissue (lanes 1-4) or cerebellum (lanes 5-8) was incubated with 2.5 fmol of ³²P-labelled DNA fragment 1 (-214 to -36) in the absence (lanes 1,5) or presence of various unlabelled competitor DNAs, added in a ~30-fold molar excess. Lanes 2,6: unlabelled fragment 1; lanes 3,7: unlabelled fragment 2; lanes 4,8: unlabelled pUC9 DNA. F, unbound probe; I, II, III, bound probe.

DNA fragment 2 to inhibit or reduce the formation of any complex in the competition experiments described above, as well as the lack of complex formation between DNA fragment 2 and SON nuclear protein. The data may indicate that at least complexes II and III represent cell type-specific protein factor(s) bound to presently unknown enhancer and/or promoter elements within DNA fragment 1. Complex I, on the other hand, seems to contain a protein, which is not restricted to magnocellular neurons, and which might therefore function as a more general transcription factor.

The gel shift data suggest the existence of proteins in magnocellular neurons of the SON that can specifically bind to a DNA fragment, which spans the 5' region from nucleotide -214 to -36. One of the putative *trans*-acting factors is probably also present in cerebellum nuclei, thus representing a more ubiquitous protein. A precise characterization of protein-DNA contact sites by footprint analysis is rendered technically extremely difficult due to the small number of vasopressinergic neurons present in the hypothalamus (approximately 5000 cells per rat brain [17]). Thus linkage of the putative *cis*-acting elements of the promoter region of the AVP gene to a heterologous promoter should help to clarify whether these elements would be responsive to externally added stimuli.

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