

IAPP/amylin gene transcriptional control region: evidence for negative regulation

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Aberrant expression of the islet amyloid polypeptide (IAPP) gene might be involved in the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM). Here, we report that IAPP promoter-luciferase constructs revealed tissue-specific activity. This activity was not mediated by cAMP. Sequential 5' deletions of the IAPP promoter caused a progressive derepression of the IAPP gene promoter in IAPP-producing cells. Comparison of the nucleotide sequence of the IAPP promoter with that of the insulin promoter (both active in pancreatic β -cells) reveals two sequence elements of putative importance: an insulin enhancer-like sequence and an element which corresponds to a protected domain in rat insulin I gene promoter footprint experiments.

Human IAPP/amylin gene; Promoter activity; Negative regulation; Pancreatic β -cell; Diabetes mellitus type 2

1. INTRODUCTION

Islet amyloid polypeptide (IAPP) or amylin is a novel pancreatic polypeptide hormone which is produced by the β -cells of the islets of Langerhans [1,2] and is co-secreted with insulin in response to glucose [3,4]. Recently, IAPP was detected in human plasma [5,6]. IAPP has been implicated in the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM). IAPP is the formative protein in amyloid deposited in pancreatic islets of NIDDM patients [2,7] and IAPP has been shown to cause insulin-resistance in vitro [8,9] and in vivo [10,11]. Both islet amyloid and insulin-resistance are characteristic pathological features of NIDDM.

We have determined the nucleotide sequence of the human IAPP gene and found no evidence for the assumption that a mutated form of IAPP is present in islet amyloid [12,13]. We now study the regulation of IAPP gene expression, since IAPP may be abnormal in quantity in NIDDM patients which could be due to overexpression of the IAPP gene. Expression of the IAPP gene, like that of the insulin gene, may be limited to pancreatic β -cells and insulinomas [12,14]. In this paper, we report studies on the promoter activity of the 5'-upstream region of the IAPP gene in different cell types using luciferase as a reporter gene. These studies provide evidence for negative regulation of the IAPP gene promoter.

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2. MATERIALS AND METHODS

2.1. Plasmids

Superluc (pSL) was made by replacing the *HindIII-HpaI* fragment of superCAT containing the CAT gene by a *HindIII-BamHI* fragment of pDR101 [15] (gift from Dr P. Oudshoorn, Utrecht, The Netherlands) containing the firefly luciferase gene. pRSVluc contains the LTR of RSV (*AccI-HindIII* fragment) upstream of the luc gene. The IAPP promoter constructs (Fig. 1) are derived from the genomic clone λ h201 [12]. Construct 1 corresponds to a *TaqI-HindIII* fragment. Constructs 2–6 were made by PCR using the following oligonucleotide primers:

–800: 5'-GCATCTAAGCTTCTACAAAGTACTGTGTGAGCT-AA-3'
 –390: 5'-ATTGAGGTCACTTGGGTTCA-3'
 –189: 5'-ACTGTGTATTGCTACGTTA-3'
 +32: 5'-GAGTCCAAGCTTGTATCCACTGGAATA-3'
 ATG: 5'-GGATGCCCATGGCTTCTCAAATTTCTGCAAA-3'
 ATG Δ : 5'-GGATGCCCATGGCTTCTCAAATTTCTTTTAAT-GTTTCAATGTCA-3'

The *HindIII*, *NcoI* (both underlined) or *SmaI* sites were used for cloning PCR-fragments into pSL. In the ATG and ATG Δ constructs a point mutation was introduced in order to create a *NcoI* site (CCATGG, genomic sequence is CAATGG). mRNAs from these constructs contain the same untranslated leader as prepro-IAPP mRNA. All plasmids were purified twice over CsCl-gradients.

2.2. Cell culture, DNA transfections and luciferase assay

RIN AHT2 cells (insulin-secreting subclone of the rat insulinoma cell line RIN-m5F, a gift from Dr B. Roep, Leiden, The Netherlands) were cultured in RPMI 1640, 293 cells (human embryonal kidney cell line) were cultured in DMEM, both media contained 10% fetal calf serum and 0.03% glutamine. On a Northern blot containing RNA from RIN cells, we detected both insulin and IAPP poly A⁺ RNAs (not shown). RIN cells were transfected by electroporation (GenePulser, Biorad). 5 million cells in 0.4 ml serum-free RPMI 1640 were mixed with DNA in gene pulser cuvettes. Following the administra-

tion of current (250 V, 500 μ F, room temperature) cells were allowed to recover for several minutes before transferring them into T-25 flasks with serum-containing medium. 293 cells were transfected by calcium-phosphate coprecipitation. After 4 h cells were exposed to 10% DMSO for 30 s. After 24 h RIN or 293 cells were rinsed once with PBS and the cells were lysed in 250 μ l lysis buffer (25 mM Tris-phosphate (pH 7.8), 8 mM $MgCl_2$, 1 mM DTT, 1% Triton X-100, 15% glycerol).

The luminometer (Lumac, 3M) injected 100 μ l of 1 mM luciferin (Sigma) and 1 mM ATP in lysis buffer into an appropriate volume of lysate and measured light production. In order to normalize luciferase activities for cell number, the protein content of the lysate was determined [16].

3. RESULTS AND DISCUSSION

In Fig. 1 the IAPP gene promoter-luciferase constructs are schematically represented and the relative luciferase activity in RIN cells (IAPP-producing) and 293 cells (non-IAPP-producing) is shown. Surprisingly, the construct $-800/+32$, which contains a large 5'-upstream region of the IAPP gene, has very low activity in RIN cells. The activity of $-800/ATG$ (containing exon 1, intron 1 and exon 2 up to ATG translation-initiation codon) is 2–3-fold higher. This enhancement is not due to increased mRNA stability, since the 'pre-spliced' construct ($-800/ATG\Delta$, in which intron 1 is specifically removed) shows low luciferase activity. One explanation for the effect of the intron is that the association of pre-mRNAs with the splicing-machinery facilitates mRNA transport to the

cytoplasm [17]. Alternatively, intron 1 sequences may enhance IAPP gene transcription in RIN cells.

The removal of the region -800 to -189 of the IAPP promoter increases promoter activity 5–6-fold. This suggests that elements within this region (especially in the region -390 to -189) are involved in repression of IAPP promoter activity.

In 293 cells the activity of IAPP promoter constructs $-800/ATG$ and $-390/ATG$ is efficiently suppressed when compared to the activities in RIN cells (Fig. 1). Apparently, the IAPP promoter is inactive in non-IAPP-producing cells. This may be due to the absence of tissue-specific *trans*-acting factors which are necessary for IAPP promoter activation or to the presence of transcriptional repressors. The derepression of the IAPP promoter in RIN cells ($-189/ATG$) is also observed to some extent in 293 cells, although in absolute terms the activity is still low. It can be hypothesized that repression of the IAPP promoter is found in many cell types. It may be that this negative regulation is overruled in β -cells by tissue-specific positive *trans*-acting factors, as has been hypothesized for the insulin promoter [18]. For the rat insulin I promoter, negative regulation in non-pancreatic cells has been described, which is thought to be mediated by a *cis*-acting element in the region -219 to -103 [18].

The nucleotide sequence TGATGTCA in the IAPP promoter (-61 to -54) resembles a cAMP-responsive element (CRE with a C to T substitution (underlined)

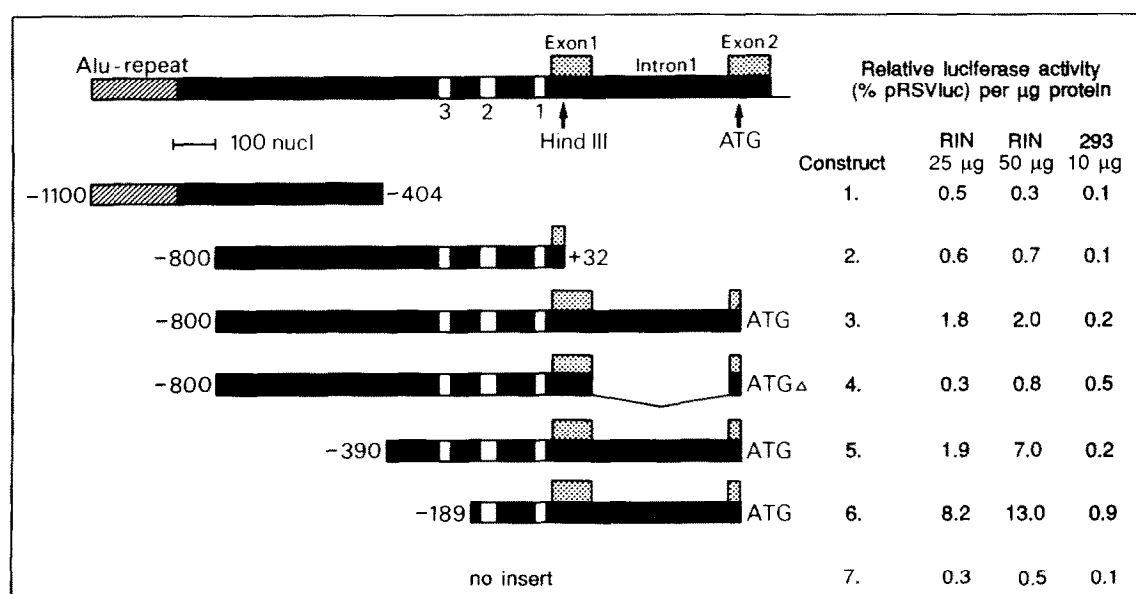


Fig. 1. Schematic representation of 5'-flanking IAPP sequences present in different luciferase constructs and relative luciferase activities of these constructs in RIN cells and 293 cells. Constructs were made as described in section 2. Indicated are (1) TATA-box, (2) E2-domain and (3) insulin enhancer-like sequence. Reporter gene activity is expressed relative to that of pRSVluc and was normalized for cell number (protein content of lysate). Luciferase activities after transfection of 293 cells (two independent duplicate experiments) were very reproducible (SD less than 15%). Indicated are the mean values of one duplicate experiment. Transfections of RIN cells by electroporation was performed 4 times with 25 μ g, 4 times with 50 μ g and 3 times with 100 μ g DNA. Indicated are the mean of representative duplicate experiments with 25 μ g or with 50 μ g DNA. The variability of RIN cell transfections was higher than in 293 cells (SD between 10 and 60%), however, the pattern of responses was very consistent in all experiments.

Table I

Effect of dibutyryl cAMP (1 mM) and forskolin (10^{-6} M) on relative promoter activities of constructs -800/+32, -800/ATG and -189/ATG in RIN cells

	-	db-cAMP (1 mM)	forskolin (10^{-6} M)
-800/+32	0.7	1.5	1.2
-800/ATG	1.8	4.3	2.5
-189/ATG	8.2	7.2	8.1

Luciferase activity is expressed relative to that of pRSVluc and was normalized for the protein content of the lysate

from the consensus sequence [19]). This sequence in the IAPP promoter is probably not a functional CRE because neither the addition of dibutyryl-cAMP nor the activation of adenylate-cyclase with forskolin resulted in a significant increase of promoter activity of the constructs -800/+32, -800/ATG or -189/+32 in RIN cells (Table I).

Ferrier et al. reported expression of the rat IAPP gene in non-pancreatic tissues, e.g. duodenum and stomach [20]. However, the amount of IAPP mRNA in these tissues is very low as compared to the amount of IAPP mRNA in the pancreatic islets. Within the islets IAPP gene expression is limited to the β -cells which in addition selectively express the insulin gene.

A comparison of the IAPP promoter (Fig. 2) with the insulin promoter reveals two conserved sequence elements which might be of importance with respect to transcriptional control. First, the nucleotide sequence AAGATGGC in the IAPP 5'-upstream sequence (-245 to -238, complementary sequence GCCATCTT) resembles the insulin enhancer GCCATCTG [21]. A similar element (GCCATCAG) is

Table II

Comparison of the E2-domain-like sequences in the 5'-upstream regions of the IAPP genes in man (this report) and rat (A.D.M. van Mansfeld et al., Biochim. Biophys. Acta, in press) and in the insulin genes of man, rat (I and II) and mouse (I) [30]

rat insulin 1 gene	<---E2 domain--->			
human IAPP gene	-155	GTTAA	TGTAA	TAATGACCC -137
human insulin gene	-222	-----	GACTC	----- -204
rat insulin 1 gene	-223	-----	-AATC	----- -205
rat insulin 2 gene	-211	C-----	GACTC	-----T- -193
mouse insulin 1 gene	-223	A-----	GA-C-	-----A- -205
rat IAPP gene	-152	-----	-----	-G---G--- -132

Indicated are the position of these elements relative to the transcription-initiation site, and the 18 nucleotide protected domain of the rat insulin I promoter in footprint experiments [26]

present in the β -cell-specific promoter of the rat glucokinase gene [22]. The insulin enhancer activates the insulin promoter in β -cells and is capable of driving a heterologous promoter in a tissue-specific manner [23]. We will refer to this putative IAPP promoter element as the 'IAPP-Far'-box because its position relative to the site of initiation of transcription is similar to that of the Far-box in the rat insulin I gene promoter [24]. It remains to be established whether the IAPP-Far-box is involved in the negative regulation that is attributed to elements within the region -390 to -189. The insulin enhancer in the rat insulin II gene promoter is involved in both positive and negative transcription regulation [25].

The second interesting element is a sequence of 19 nucleotides in the human and rat IAPP promoters and in the insulin promoters of man, rat and mouse (Table II). Intriguingly, this conserved element corresponds exactly to a protected region (E2) of the rat insulin I promoter in footprint experiments [26,27]. Ohlsson

-1100	TCGAGACCAG	CCTGACCAAC	ATAGAGAATC	CCCTCTCCAC	TAAAAATACA	AAATTAGCCA	TACATGGTGA	TGCATGCCGT	TAATCCCGAC	TACTCTGTAG
-1000	GCTGAGGCAG	GAGAATCACT	TGAACCCGGG	AGGCACAGGT	TACAGTGAGC	CGAGATCGCA	CCCTGTGACT	CCAGCCTGGG	CAACAAAGAG	TGAAACTTCA
- 900	TCTCAAAAAA	AAAGAAAAAA	AAAGAAAGAG	AGAAAGAAAT	ACAAGAAAAA	GAAAAAGCAA	TTTGAGAGAA	TTTAAAAAGC	AAATCATTTT	AAAAGACAAA
- 800	CTACAAAGTA	CTGTGTGAGC	TAAGCCATTG	ATAATGTTCT	CATGGAGCTA	ATGGGAAACA	AATCTGTTCT	TTCAAAATAA	TTGTAAAAAT	TCTGTGTAAG
- 700	AATGAATGGA	AAAGGTCATC	CAAAACTAC	TTGACTACAT	ATACCTATGG	ATCTCTATCT	AAGTGCTGAA	AAATGAATTT	TTTATTTCAT	TTGGTATAGT
- 600	AGCATTGTGT	CAITCTAATT	TTGTTTTGTT	CCCTGTGCATA	TCTCTGTTAC	CTAGAATAAT	CCCTACCACA	GAGTAGGICT	TCCATTACTC	TTATGCTTTT
- 500	AAATCTCCCC	TCACCTCATT	GTAAATGACT	TTTGATTTCT	CTTTTATGCC	CTTTTATAC	ACCTTTCCTT	TATATCTCCA	TTTATTCCTG	AAGCTTCATG
- 400	GGATTCAGCC	ATTGAGGTCA	CTTGGGTTTA	GATATACCAA	AAGTCTGTGA	TTTCTCTGTT	TGCATATATG	CACATTGTGT	GTTATCCTTA	CCCTTTTCTA
- 300	TCAGTTTCCT	ACCATAACAT	ACACTTAATT	CTTGGAAATT	CACCTATGTC	TTACAAAGAT	GGCAAATTCA	AACTTCTGCT	GTGTATGACA	CACCAITTAAC
- 200	TGCACAAGGA	CACCTGTGAT	TTGCTACGTT	AATATTACT	GATGAGTTAA	TGTAATTAATG	ACCCATCCGC	TTCTGCTGCC	TGTGAGGTAC	TTTCTATCTA
- 100	TAGGGATGGA	AATTAATGAC	AGAGGCTCTC	TGAGCTGCCT	GAITGTCAGAG	CTGAGAAAGG	TGTGAGGGGT	ATATAAGAGC	TGGATTACTA	GTTAGCAAAAT
1	GAGGGGGTAA	ATATTCAGT								

Fig. 2. Nucleotide sequence of the 5'-upstream region (-1100 to +20) of the human IAPP gene. Nucleotides -1100 to -896 represent part of an *Alu*-repetitive sequence. Underlined are the IAPP-Far-box (-245 to -238), the E2-domain-like sequence (-155 to -137), the CRE-like sequence (-61 to -54), the TATA-box (-31 to -25) and the cap-site (+1). The numbering is relative to the mapped 5' end of exon 1 ([29]; J.W.M.H., unpublished results).

and Edlund [26] describe non-identical protection of the E2-domain upon incubation with nuclear extracts from HIT (hamster islet tumor) or BHK (baby hamster kidney) cells, suggesting that different tissue-specific proteins might recognize this element. It may well be that identical or similar *trans*-acting factors involved in β -cell-specific gene transcription can bind to this element in the IAPP and insulin promoters, respectively.

In addition to the sequence elements mentioned above, the IAPP promoter contains a 12 basepair motif with perfect dyad symmetry, TGCATATATGCA (−340 to −329). To our knowledge this sequence does not resemble any known element involved in transcription regulation. Furthermore, the sequence TGCCTGA (−65 to −59) resembles the nucleotide sequence CGCCTGA of the protein binding A-domain of the so-called 'islet cell enhancer' that was recently identified in the glucagon gene promoter [28].

A mutational analysis of these putative transcriptional control elements must reveal whether or not they are true *cis*-acting elements. The conservation of an insulin enhancer-like sequence and an E2-domain-like sequence in the IAPP and insulin promoters justifies further research on their putative role in pancreatic β -cell-specific gene expression.

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